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Gamma Radiosensitivity Study on Snap Bean (*Phaseolus vulgaris*)

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Abstract: Observation on the morphological effects and estimation of LD_{50} for a snap bean (*Phaseolus vulgaris*) upon irradiating snap bean seeds with different doses of gamma rays was done in this study. The seeds were treated with 0, 300, 400, 500, 600 and 800 Gy doses of gamma irradiation by the Malaysian Institute of Nuclear Technology (MINT). These treatments (30 seeds per treatment) were replicated three times and plants monitored by standard procedures. The study started in December 2006 and ended in February 2007. Standard procedures were used to observe and record the variables studied in this research. Increasing dosage of gamma irradiation was accompanied by decrease in height, root length, oven-dry weight of root, shoot and survival of snap bean. It was also found that the LD increased with increasing gamma radiation. Other changes observed in this study were leaflet and chlorophyll mutation. Generally, gamma irradiation greatly induced morphological changes in snap bean.

Key words: Gamma radiation, snap bean, Malaysia, mutation

INTRODUCTION

Since De Vries's concept of mutations as the source of genetic variation and the early ideas about their potential value for plant breeding, his work around the turn of the twentieth century was marked as the starting point of the discipline of plant breeding (Van Harten, 1998). A wide range of characters which have been improved through induced mutation breeding include plant architecture, yield, flowering and maturity duration, quality and tolerance to biotic and abiotic stresses. Most of the mutant varieties (around 89%) have been developed using physical mutagens such as X-rays, gamma rays, thermal and fast neutrons but gamma rays alone account for the development of 60% of the mutant varieties (Kharkwal and Jain, 2004).

Many new cultivars have been directly or indirectly derived through mutation induction and the number of mutant varieties officially released and recorded by FAO/IAEA MVD before the end of the year 2000 was 2,252 (Maluszynski *et al*., 2000). A perusal of the data on specific crops and number of mutant varieties released in the world (till 2000) indicates that the top ten ranks are occupied by some of the most important food and ornamental plant species in agriculture. Snap bean (*Phaseolus vulgaris*) was one of the crops in the top ten ranks with 54 mutant varieties released but to date information of this kind on the local varieties of this plant in Malaysia is lacking although snap bean is an important leguminous crop whose green pod is used as vegetable or dry seeds. In addition, FAO production statistics indicate that the global importance of snap bean production was higher compared to other edible food legumes (FAOSTAT, 1977).

This study was conducted to observe the morphological effects and to estimate the LD_{50} for a snap bean (*Phaseolus vulgaris*) upon irradiating the seeds of this plant with different doses of gamma ray.

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MATERIALS AND METHODS

This research was conducted at the Horticulture Unit of Universiti Putra Malaysia Bintulu Campus (UPMKB). Preparation of sand beds was done 2 days before planting where grasses and debris in the sand beds were removed. The compact sand in the sand beds was loosened to increase porosity, aeration, ease of sowing and maintenance of field capacity. Ridsect (insecticide) was sprayed around and inside the sand beds to ensure the sand beds were free from pests. A blended growth medium with a ratio of 3:2:1 (topsoil: sand: processed chicken dung) was used. This ratio ensured good anchorage as well as adequate retention of nutrients and water.

The local variety used in this study was chosen because of its economic value, availability and good germination (more than 90%). The seeds of this variety were obtained from the Malaysian Agriculture Research and Development Institute (MARDI). The moisture content of the seeds was determined using a moisture analyzer. This analysis was essential because moisture content affects the sensitivity of seeds exposed to gamma radiation. The seeds were irradiated with gamma ray at the Malaysian Institute of Nuclear Technology (MINT). This radiation was used in this study because of its easy application, good penetration, reproducibility, high mutation frequency and less disposal problems (Chahal and Gosal, 2002). Seeds were irradiated with 0, 300, 400, 500, 600, 700 and 800 Gy as lower doses such as 50, 100, 150, 200 and 250 Gy in our previous trial did not yield good results. For each dosage, 30 seeds were used. The irradiated seeds including control (no irradiation) were sown in the sand beds. The planting distance was 2 cm within row and 5 cm between rows. The seeds were sown to a depth of about 1 cm and covered slightly with sand to avoid pests attack and dislodging (when watering). Watering was done manually (when necessary). The temperature of the sand beds was also monitored daily using a thermometer to ensure the temperature was in the normal range. The experimental design was a completely randomized design with 3 replications.

Data on seed germination were collected four times starting from the first day of emergence of the plant's cotyledon from the growth medium surface. Afterwards, 10 plants for each treatment were transferred into polybags in a rain shelter house at UPMKB. Each polybag contained 1.2 kg blended growth medium with top soil, sand and processed chicken dung ratio of 3:2:1.Watering was also done daily and plant growth was monitored until fruiting stage (40 days).

Data on germination, seedling height, root length, oven-dry weight of root and shoot, survival rate and prediction of dose effects were recorded. Change in leaf shape and chlorophyll deficiency was also observed. Seedling height was measured by the flat method. For every 4 days, seedling height was measured from the surface of the planting medium to the shoot of the plants using a ruler. This measurement was done when the first leaf had stopped growing (FAO/IAEA, 1977). The measurement started from 6 until 18 Days after Planting (DAP). Root length measurement was done at harvest. Roots were separated by washing (gently) away the growth medium with water. Plants were partitioned into root and shoot after which root length was determined using a ruler. Afterwards, these plant parts were bagged in brown paper bags and oven dried at 60°C until constant weight was attained. The plants that survived at harvest were counted. LD_{50} (Lethal Dose that reduces 50% of the total plant population) based on germination, plant height and percentage survival were determined.

Analysis of variance (ANOVA) was used to detect treatment effect and Tukey test was used to compare treatment means ($p = 0.05$). The statistical software used was Statistical Analysis System (SAS) Version 9.1.

RESULTS

The moisture content of the snap bean seed was 8.07%. There was no interaction between gamma-ray dosage and time (Table 1). Compared to control, only the effect of 800 Gy on seed germination was statistically significant. Except for 3, 5 and 6 DAP which were significantly different, time did not have significant effect on germination.

As shown in Table 2, all the doses consistently reduced snap bean height compared to control (6 to 18 DAP). Generally, the higher the dosage, the more pronounced was the effect on the plant height particularly 800 Gy whose effect led to the death of the plants by 18 DAP.

Except for 300 Gy, root length was significantly reduced as dosage increased. 800 Gy had pronounced effect on root length (Table 3). With the exception of 300 Gy, the seeds treated with 400, 500, 600 and 800 Gy significantly reduced shoot dry weight particularly 600 and 800 Gy (Table 4). Similar observation was made for root dry weight except that the effects of 300 and 400 Gy were not different from that of the control. This observation suggests that dry matter production of snap bean reduces with increasing gamma-ray radiation (Table 4).

Compared to control, the survival of snap bean was significantly affected by higher doses particularly when the plants were irradiated above 600 Gy (Table 5). The LD for germination was not determined because the germination percentages of some of the treated seeds were higher than that of the control. For plant height, the LD_{50} was obtained at 300 Gy. The LD_{50} of plant survival was between 500 and 600 Gy. The LD for height and survival increased with increasing dosage (Table 6).

Table 1: Effect of irradiation on germination (%) of snap bean with time

	DAP				
Dose	3	4	5	6	Dose factor
Control	75.56	77.78	80.00	80.00	$78.33^{a''b''}$
300 Gy	82.22	84.44	85.55	88.89	$85.28^{a''}$
400 Gy	66.67	73.33	76.67	76.67	$73.33^{b''}$
500 Gy	74.44	80.00	84.45	84.45	$80.83^{a''b''}$
600 Gy	63.33	71.11	75.56	77.78	$71.94^{b''}$
800 Gy	51.11	58.89	64.44	64.44	$59.72^{c''}$
Time Factor	$68.89^{b'}$	$74.26^{a'b'}$	$77.78^{a'}$	$78.79^{a'}$	

Mean with different letters within column are statistically different between treatments by the Tukey test ($p = 0.05$); Means without letters are statistically not different between treatments by the Tukey test ($p = 0.05$); ’= Time factor, ”= Dose factor; DAP = Days After Planting

Table 2: Effect of irradiation on height (cm) of snap bean with time

	DAP				
Dose	6	10	14	18	Dose factor
Control	14.00^{a}	28.38^{a}	37.82^{a}	50.59^{a}	$32.69^{a''}$
300 Gy	4.95^{b}	8.54^{b}	11.44^{b}	18.73^{b}	$10.92^{b''}$
400 Gy	3.91^{b}	7.43^{b}	9.12^{bc}	15.55^{bc}	$9.00^{c''}$
500 Gy	1.84^{c}	3.75^{c}	7.99^{bc}	12.73^{cd}	$6.58^{d''}$
600 Gy	1.44^{c}	2.87^{c}	5.90^{c}	9.15^{d}	$4.84^{e''}$
800 Gy	0.67^{c}	0.85^{d}	2.17^{d}	0.00^{e}	$0.92^{f''}$
Time Factor	$4.47^{d'}$	$8.64^{c'}$	$12.41^{b'}$	$17.79^{a'}$	

Mean with different letters within column are statistically different between treatments by the Tukey test ($p = 0.05$); ’= Time factor, ”= Dose factor; DAP = Days After Planting

Table 3: Effect of irradiation on root length of snap bean 45 days after planting

Treatments	Root length (cm)
Control	12.75^{a}
300 Gy	8.26^{ab}
400 Gy	6.03^{bc}
500 Gy	5.37^{bc}
600 Gy	1.16^{cd}
800 Gy	0^{d}

Mean with different letters are statistically different between treatments by the Tukey test ($p = 0.05$)

Table 4: Effect of irradiation on oven-dry weight of roots and shoot of snap bean 45 days after planting

Treatment	Root (g)	Shoot (g)
Control	0.06^{a}	$0.49^{a'}$
300 Gy	0.05^{ab}	$0.38^{a'b'}$
400 Gy	0.04^{ab}	$0.26^{b'c'}$
500 Gy	0.03^{bc}	$0.20^{c'}$
600 Gy	0.01^{cd}	$0.04^{d'}$
800 Gy	0.00^{d}	$0.00^{d'}$

Mean with different letters are statistically different between treatments by the Tukey test (p = 0.05); ’ = Time factor

Table 5: Effect of irradiation on survival of snap bean 45 days after planting

Treatment	Survival (%)
Control	66.67^{a}
300 Gy	46.67^{a}
400 Gy	43.33^{a}
500 Gy	36.67^{ab}
600 Gy	3.33^{bc}
800 Gy	0.00^{c}

Mean with different letters are statistically different between treatments by the Tukey test (p = 0.05)

Table 6: Effect of lethal dose on height and survival of snap bean

Dose	Height (cm)	Survival (%)
300 Gy	67	30
400 Gy	72	35
500 Gy	80	45
600 Gy	85	95
800 Gy	97	100

DISCUSSION

The seed moisture content was not consistent with the standard range of 12 and 14% but the low moisture content did not affect the radiosensitivity of treatments. This was evident in the morphological variables studied. The absence of interaction between time and dose suggest that the 2 factors were not interdependent. The lower percentage germination at 800 Gy might have caused injury to the seeds and this may have prevented them from germinating well. The differences in germination due to different treatments was expected as many of the metabolic events that are known to occur during germination differ in their timing, both among the various organs of a particular seed and among seeds of different species (Mayer and Poljakoff-Mayber, 1989; Bewley and Black, 1982; Hegarty, 1978).

The height reduction caused by the doses was in agreement with the findings of Kharkwal and Jain (2004) who reported that reduction of plant heights belongs to the most frequently arising types in mutation experiment. Kharkwal (2000) also got dwarf or shorter plant height in their study on snap bean. The reduction in root length with increasing dosage also partly explains the reduction in plant height as these two variables are interrelated. The reduction in root length observed in this study was consistent with findings of Szarejko and Maluszynki (1980), Savaskan and Toker (1991) and Feix *et al*. (1997). The low dry matter production (root and shoot) in this study with increasing dosage of gamma radiation relates to the reduction in plant height, shoot and root length. For instance, good rooting system is characterized by efficient use of water and nutrients which consequently lead to good plant growth and development (Lavender, 1992).

The fact that 600 and 800 Gy significantly affected the survival of the snap bean plants compared to the plants of the other treatments including control suggests that snap beans may not survive with time if their seeds are exposed to high gamma radiations. The survival of the snap bean was not much affected by environmental factors since it is a cold temperature tolerant crops and more resistant to disease attack.

The LD_{50} for germination could not be determined in this study and this finding was in agreement with the study of Ciftci *et al*. (2004) who stated that it was not possible to determine LD_{50} on the basis of germination because of no significant difference in treatments effect. The LD_{50} of plant height showed growth reduction of seedling height. The LD_{50} on the basis of plant survival was between 400 and 500 Gy.

Other physical damages that occurred on the leaves seem to fit the description of Chontira *et al*. (2005). According to their description a mutated leaf has round-cuneat leaflet with a short petiole and does not set pod. They also explained that wrinkled leaflet is that leaf that has a wrinkled character.

CONCLUSIONS

Exposing seeds of snap bean to higher gamma ray dosages particularly 600 and 800 Gy cause reduction in height, root length, oven-dry weight of root, shoot and survival rate. The lethal dosages for these variables increase with increasing dosage of gamma ray. Gamma irradiation also causes mutation of the leaflets and chlorophyll deficiency in snap bean. From this study, it can be generally concluded that irradiating seeds of snap bean with higher amounts of gamma rays greatly induce morphological changes.

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