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Effects of Gamma Rays and Sodium Azide on Yeild Parameters of *Phaseolus Vulgaris* L

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Abstract

The studies of induced mutation in Bokkos red variety of *Phaseolus vulgaris* L was conducted at botanical garden, Department of Biological Sciences, Ahmadu Bello University, Zaria. The healthy and dry seeds of *P. vulgaris* were exposed to gamma rays at different doses of (10krad, 15krad, 20krad, 25krad and 30krad) and the sodium azide at different concentrations (0.10mM, 0.15mM, 0.20mM, 0.25mM and 0.30mM) and combined treatments. The observation were made for number of flower bud, days to flowering, plant height at maturity, pod length, number of pods per plant and number of seeds per pod. The result revealed that 10krad and 25krad had the highest number of flower bud, 0.30mM+30krad had the early days to first flowering, 0.10mM had the highest pod length, 0.30mM+25krad had the highest plant height at maturity, 25krad had the highest number of pods per plant, 0.20mM had the highest number of seeds per pod and the flower with yellow, white and purple was obtained in this studies. Data obtained in this study were statistically significant at 5% level. The results concluded that different treatments of mutagens had different effects on yield parameters of *P. vulgaris*.

Key words: Gamma rays, Sodium azide, combined treatment, *Phaseolus vulgaris* L and yield parameters.

1.0 INTRODUCTION

Common Bean usually refers to food legumes of the genus *Phaseolus*, family *Fabaceae*. The genus *Phaseolus* contains some 50 wild-growing species distributed only in the Americas (Asian *Phaseolus*) have been reclassified as *Vigna*. This species represent a wide range of life histories (annual to perennial), growing habits (bush to climbing), reproductive systems, and adaptations (from cool to warm and dry to wet). The genus also contains five domesticated species: in decreasing order of importance, common bean (*Phaseolus vulgaris* L.*),* Lima bean (*P.lunatus* L.*),* runner bean *(P. coceineus* L.), Tepary bean (*P. acutifolius* A. Gray*)*; and year bean (*P. polyanthus);*while the principal species economically and scientifically is common bean. It originated in Latin America where its wild progenitor *P. vulgaris var.maxicanus* and *var. Aborigineus* has a wide distribution ranging from northern Mexico to north western Argentina (Gepts, 2001).

Common bean (*P. vulgaris)* is the principal grain legume used for direct human consumption worldwide (Broughtan *et al*., 2003) and is increasingly recognized for its high nutritional quality. Common bean (*P. vulgaris)* is a true diploid with 11 chromosomes (Benneth and Leiteh, 2005). Its small genome, with a low incidence of duplications, makes it suitable for sequencing and genomic applications and as a reference for the elucidation of genomes of more complex legume species such as Soya beans (Gepts *et al.*, 2005).

Common bean is the most important legumes worldwide for direct human consumption. The crop is consumed principally for its dry (nature) beans, shell beans (seeds at physiological maturity), and green pods. When consumed as seed, beans constitute an important source of dietary protein (22% of seed weight) that complements cereals for over half a billion people mainly in Latin America (Gepts, 2001).

Annual production of dry beans is around 15 million tonnes and the largest producers of dry beans are Brazil, Mexico, China and the USA. Annual production of green beans is around 4.5 million tones, with the largest production around the Mediterranean and in the USA (Gepts, 2001).

Common bean were introduced to Africa in the 16th century and today are grown at 6.4 million tones mainly by small holders but the crop shows low grain yields ranging from 0.35 to 0.75 g (Katungi *et al.,* 2009). Wortmann *et al.* (1998) indicated that the annual per capita consumption in Africa ranged from 12 to 58kg.

In many sub-saharan African countries, producing enough common bean seed, especially of new varieties remains a big challenge. This has been associated with the failure of the formal seed sector to multiply sufficient quantities of the new varieties and make it available to the farming communities (Rubyogo *et al.*, 2010).

Therefore this study is an attempt to see how through genetic modification (induced mutation) the common bean seeds can be improved in terms of quantity and quality.

Mutation are the tools used to study the nature and function of genes which are the building blocks and basis of plant growth and development, thereby

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providing raw materials for genetic improvement of economic crops (Adamu and Aliyu, 2007).

This study aim to assess the mutagenic effects of Gamma rays and Sodium azide on common bean through yield parameters.

2.0 MATERIALS AND METHODS

2.1 Plant Source and Mutagen Treatment

The Bokkos Red variety of common Bean (*P. vulgaris*) were collected from Bokkos Local Government Areas of Plateau State and were taken to Centre for Energy Research and Training Zaria , Department of Radiography for irradiation with AmBe (Americium-Beryllium) isotopic Sources (^{241}Am/Be). 270 of the seeds were treated with Sodium azide in the laboratory in Biological Sciences Department, Ahmadu Bello University, Zaria. (Altitude 667.88m above sea level, latitude $11^0$4'N and longitude $7^0$42'E).

2.2 Treatments of Seeds with the Mutagens

The matured seeds of common Bean were air-dried and divided into six sets. One set was the control and the remaining five sets of the seeds were treated with physical mutagen at different doses of gamma rays (10krad, 15krad, 20krad, 25krad and 30krad) and chemical mutagen Sodium azide at different concentrations (0.10mM, 0.15mM, 0.20mM, 0.25mM and 0.30mM). Duration of treatments with chemical mutagen: pre-soaking of seed material for four (4) hours in distilled water, soaking of seed material for six (6) hours in Sodium azide and post-soaking of treated seed material for four (4) hours in distilled water. One hundred and eighty (180) of the irradiated seeds were also be soaked in 0.10mM to 0.30mM solution of Sodium azide for four (4) hours respectively (Borkar and More, 2010).

2.3 Sowing of the Seeds

Sowing of the treated seeds with Gamma rays, Sodium azide, combined mutagens and the control seeds was done in botanical garden, Department of Biological Sciences, Ahmadu Bello University Zaria using the 20 X 35mm Polythene bag; one hundred and forty-four Polythene bags was made in the botanical garden in a groups of 36 per treatments and with six replications were used in the experiment. Three (3) seeds were sown in each Polythene bag in a complete randomized design (CRD) lay out.

2.4 Data Collection

Data were collected based on: percentage germination, seedling height and number of leaves following the procedures of Mosisa *et al*. (2014).

2.6 Statistical Analysis

The data collected were subjected to the following statistical methods: Descriptive statistics was performed and one way analysis of variance (ANOVA) was used while Durcan Multiple Range Test (DMRT) was used to determine the level of significant among means at 5% using statistical package for social sciences (SPSS) version 21.0.

3.0

RESULTS

3.1 Yield Parameters

3.1.1 Number of flower bud

Effects of gamma rays and sodium azide on yield parameters are presented in (Table 1). Number of flower bud decreased with an increase in dose/concentration of mutagens. The dose of 25krad of gamma rays exhibited the highest number of flower bud of (12.00) as compared with the control (6.29). In the highest concentration of sodium azide 0.30mM (10.50) and in combined treatments 0.20mM+25krad (7.33) and 0.20mM+30krad (6.50) showed the highest number of flower bud. At 0.30mM+30krad of combined treatment (2.50) showed the lowest number of flower bud. The results revealed that there was significant difference among the treatments at $p \leq 0.05$ (0.029).

3.1.2 Days to flowering

Both the mutagens gamma rays and sodium azide treatments succeeded for inducing the variability in number of days required for first flowering. At the combined treatment of 0.30mM+30krad exhibited earliness of days to flowering of (32.50) as compared with the control (72.13). In the concentration of sodium azide 0.15mM (67.33), gamma rays 15krad (68.00) and in combined treatment 0.25mM+25krad (75.33) showed the lowest days to flowering. At 10krad of gamma rays (81.83) showed the highest days to flowering. The result showed that there was significant difference among the treatments at $p \leq 0.05$ (0.014). The phenotypical population of *P. vulgaris* showed a large number of flower colour mutations, both the physical, chemical and combined mutagens induced the different flower colour mutations. The white flower colours mutants was recorded at 0.10mM, 15krad, 0.25mM+25krad and 0.25mM+30krad while the purple flower colour mutants at 10krad of gamma rays and the yellow flower colour at the control (plate VI-VIII).

3.1.3 Plant height at maturity

Plant height at maturity decreased with an increase in dose/concentration of mutagens. At 0.30mM+25krad of the combined treatment exhibited the highest plant height at maturity of (158.67cm) as compared with the control (135.50cm). In the concentration of sodium azide

0.15mM (138.33cm), gamma rays 20krad (131.67cm) and in combined treatment 0.10mM+30krad (138.33cm) showed the highest plant height at maturity. At 0.30mM+30krad of combined treatment (60.00 cm) showed the lowest plant height at maturity. The result showed that there was no significant difference among the treatments at p>0.05 (0.116).

3.1.4 Pod length

Pod length decreased with an increase in dose/concentration of mutagens. The lowest concentration of sodium azide 0.10mM exhibited the highest pod length of (5.80cm) as compared with control (5.61cm). In the dose of gamma rays 25krad (5.62cm) and 0.10mM+30krad (5.53cm) showed the highest pod length. At 0.25mM+30krad of combined treatment (2.45 cm) showed the lowest pod length. The results reveal that there was significant difference among the treatments at p≤0.05 (0.035).

3.1.5 Number of pods per plant

Number of pods per plant decreased with an increase in dose/concentration of mutagens. At the dose of 25krad of gamma rays exhibited the highest number of pods per plant of (7.00) as compared with the control (5.46). At the concentration of sodium azide 0.30mM (6.33) and in combined treatments 0.30mM+25krad (5.17) and 0.10mM+30krad (5.00) showed the highest number of pods per plant. At 0.30mM+30krad of combined treatment (2.50) showed the lowest number of pods per plant. The result showed that there was no significant difference among the treatments at p>0.05 (0.241).

3.1.6 Number of seeds per pod

Number of seeds per pod decreased with an increase in dose/concentration of mutagens. At the concentration of sodium azide 0.20mM exhibited the highest number of seeds per pod of (3.33) as compared with control (2.96). At the dose of gamma rays 20krad (3.00) and in combined treatments 0.30mM+25krad (3.17) and 0.10mM+30krad (2.50) showed the highest number of seeds per pod. At 0.25mM+30krad of combined treatment (1.83) showed the lowest number of seeds per pod. The result showed that there was no significant difference among the treatments at p>0.05 (0.86

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Table 1: Effect of Gamma rays and Sodium azide on Yield Parameters of *Phaseolus vulgaris* L.

Treatment	Number of Flower Bud	Days to Flowering	Plant Height at Maturity	Pod Length	Number of Pods Per Plant	Number of Seeds Per Pod
C_0D_0	6.29±0.71^{abcd}	72.13±3.58^{a}	135.50±10.42^{ab}	5.61±0.30^{a}	5.46±0.45^{abc}	2.96±0.18^{a}
$C_{0.10}$	7.33±2.19^{abcd}	78.83±3.64^{a}	131.67±13.82^{ab}	5.80±0.38^{a}	5.00±0.82^{abc}	3.17±0.48^{a}
$C_{0.15}$	6.00±1.93^{abcd}	67.33±13.83^{a}	138.33±32.11^{ab}	4.72±0.97^{ab}	4.50±1.18^{abc}	2.50±0.62^{a}
$C_{0.20}$	6.83±2.32^{abcd}	78.17±3.49^{a}	119.83±10.99^{abc}	5.45±0.41^{a}	6.00±0.58^{ab}	3.33±0.49^{a}
$C_{0.25}$	8.83±2.30^{abc}	78.33±3.09^{a}	134.67±20.91^{ab}	5.55±0.37^{a}	5.33±0.61^{abc}	2.67±0.33^{a}
$C_{0.30}$	10.50±1.28^{abc}	70.67±3.57^{a}	132.50±16.82^{ab}	5.72±0.40^{a}	6.33±0.21^{ab}	2.83±0.40^{a}
D_{10}	11.83±2.66^{a}	81.83±2.74^{a}	124.00±15.57^{abc}	5.13±0.26^{ab}	6.17±0.95^{ab}	2.50±0.34^{a}
D_{15}	8.17±3.39^{abcd}	68.00±13.91^{a}	129.67±31.26^{ab}	4.55±0.96^{ab}	5.00±1.26^{abc}	2.50±0.62^{a}
D_{20}	6.50±1.78^{abcd}	80.83±3.67^{a}	131.67±13.70^{ab}	5.55±0.37^{a}	5.00±0.73^{abc}	3.00±0.52^{a}
D_{25}	12.00±1.97^{a}	78.17±2.40^{a}	113.33±8.82^{abc}	5.62±0.44^{a}	7.00±0.52^{a}	2.50±0.34^{a}
D_{30}	11.17±2.37^{ab}	74.83±2.52^{a}	112.17±4.25^{abc}	5.47±0.40^{a}	6.17±0.95^{ab}	2.50±0.34^{a}
$C_{0.10}D_{25}$	5.17±1.01^{bcd}	80.00±3.15^{a}	131.00±17.46^{ab}	5.30±0.31^{ab}	5.50±0.85^{abc}	2.83±0.40^{a}
$C_{0.15}D_{25}$	5.33±1.63^{bcd}	81.67±3.47^{a}	123.67±15.68^{abc}	5.18±0.31^{ab}	5.33±0.71^{abc}	2.67±0.33^{a}
$C_{0.20}D_{25}$	7.33±1.23^{abcd}	78.67±3.26^{a}	136.67±19.39^{ab}	5.62±0.44^{a}	5.83±0.31^{ab}	2.83±0.31^{a}
$C_{0.25}D_{25}$	7.17±2.43^{abcd}	75.33±4.30^{a}	135.67±20.15^{ab}	5.52±0.47^{a}	5.17±0.98^{abc}	2.67±0.33^{a}
$C_{0.30}D_{25}$	6.00±1.29^{abcd}	80.50±3.14^{a}	158.67±18.06^{a}	5.53±0.47^{a}	6.00±0.93^{ab}	3.17±0.40^{a}
$C_{0.10}D_{30}$	4.50±1.89^{cd}	75.50±4.13^{a}	138.33±18.64^{ab}	5.53±0.38^{a}	5.00±0.73^{abc}	2.50±0.34^{a}
$C_{0.15}D_{30}$	4.83±1.47^{cd}	70.83±14.50^{a}	95.17±23.85^{abc}	4.62±0.97^{ab}	4.17±1.11^{abc}	2.33±0.61^{a}
$C_{0.20}D_{30}$	6.50±1.75^{abcd}	79.50±4.49^{a}	85.33±26.49^{bc}	5.47±0.31^{a}	4.67±1.33^{abc}	2.33±0.56^{a}
$C_{0.25}D_{30}$	6.00±1.53^{abcd}	68.00±13.91^{a}	68.67±33.63^{bc}	2.45±1.10^{c}	3.33±1.58^{bc}	1.83±0.83^{a}

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$C_{0.30}D_{30}$	2.50±1.15[d]	32.50±14.53[b]	60.00±27.11[c]	3.33±1.49[bc]	2.50±1.15[c]	2.00±0.89[a]
Total	**7.07±0.40**	**73.67±1.65**	**122.63±4.39**	**5.19±0.14**	**5.24±0.19**	**2.69±0.10**
P value	**0.029***	**0.014***	**0.116ns**	**0.035***	**0.241ns**	**0.863ns**

* - significant at P≤0.05, ns – not significant at P>0.05.

Plate VI: Yellow flower (control)

Plate VII: White flower (0.10Mm, 15krad, 0.20Mm+25krad, 0.25mM+30krad)

Plate VIII: Purple flower (10krad)

4.0 DISCUSSION

Number of flower bud decreased with an increase in dose/concentration of mutagens. Present work revealed that 10 krad and 25 krad of gamma rays had the highest number of flower bud. Gamma rays increased significantly over sodium azide and combined treatments on number of flower bud. This study agreed with the work of Ashish *et al*. (2011).

Days to first flowering were ranged from 67.33 to 78.83 in sodium azide, 68.00 to 81.83 in gamma rays and 75.33 to 81.67 and 32.50 to 79.50 in combined treatments and control 72.13 respectively, among control and treatments. A minimum decrease in days to first flowering (32.50) was recorded in 0.30mM+30 krad when compared to control and other treatments of gamma rays and sodium azide.

Pod length decreased with an increase in dose/concentration of mutagens. Present work revealed that 0.10Mm of sodium azide had the highest pod length. Sodium azide increased significantly over gamma rays and combined treatment on pod length. This study agreed with the work of Elangovan and Pavadai (2015).

Gamma rays and sodium azide at a lower concentration induce hormones responsible for flowering, fruit maturity. Early flowering may be due to the physiological changes caused by gamma irradiation and sodium azide. Both the mutagens at higher concentration caused a delayed flowering might be due to their inhibitory effect. Early flowering was reported by Girhe and Choudhary (2002) and Elangovan and Pavadai (2015). Wani and Khan (2006) found early ripening mutants are competitive with or even superior to their mother varieties with regard to seed production in mungbean.

These findings showed that gamma rays and sodium azide can change the days to flowering. Some workers reported earlier that gamma ray can change flowering in either positive or negative direction (Karim *et al*., 2008). Mahala *et al*. (1990) found that mutagenesis could widen variability to either positive or negative direction which resulted in a sufficient variability in the treated population that could be utilized for selection of early or late flowering plants.

According to Karim *et al*. (2008), early flowering chickpea varieties by gamma rays treatments are required to minimize the cropping period which will increase the cropping intensity. Early flowering was found in gamma treated (76 Gy) M_2 plants of niger (*Guizotia abyssinica* Cass.) cultivar N-71 by Naik and Murthy (2009). According to Dhanavel *et al*. (2012) reported that 20kR and 25kR gamma rays treated plants showed early maturity in *Vigna unguiculata*. Early flowering was observed in gamma rays treated *Cajanas cajan* (Ravikesavan *et al*., 2001) and *Cicer arietinum* (Wani and Anis, 2001). Gamma rays and EMS at a lower concentration induced the hormones responsible for early flowering and fruit maturity in *Jatropha curcas* (Dhakshanamoorthy *et al*., 2010). According to them, early flowering and fruit maturity may be due to the physiological changes caused by gamma irradiation and EMS at low doses/concentration. They also found that both the mutagens at higher concentrations caused a delayed flowering and fruiting probably due to their inhibitory effect. Sodium azide had induced early flowering character in M_2 and M_3 generations in chickpea (*Cicer arietinum* L.) var. Akash (Kulthe and Kothekar, 2011).

The induction of flower colour mutations observed in *P. vulgaris* the physical, chemical and combined mutagens succeeded in including the different colour mutations. The different flower colour mutations showed broad range of colour like white, purple and yellow in the control. The relative percentage of white flower mutants was highest followed by yellow and purple. The induced mutation for flower colour had been reported by Borkar and More (2010) in *P. vulgaris*.

Plant height at maturity decreased with an increase in dose/concentration of mutagens. This work revealed significant increase in 0.30mM+25krad 158.67 of combined treatment as compared with the control 135.50. 0.30mM+30krad of combined treatment had the less plant height at maturity of 60.00. Effects of mutagenic treatments by radiation on plant height at maturity have been reported by Jamil and Khan (2002) found that radiation doses 5 and 10 kR have slightly reduced the plant height at maturity. Reduction in plant height at maturity by mutagens was observed in *Vigna radiata* L. (Das *et al.,* 2004) and *Capsicum annuum* (Omar *et al.,* 2008). Linear reduction in plant height at maturity was also observed in *Oryza sativa* (rice) after the exposure of low UV-B radiations (Mohammed and Tarpley, 2013).

Number of pods per plant is an important seeds yield component of *P. vulgaris*. Variations in pods number among treatments were significantly greater for both sodium azide, gamma rays and combined treatments average 6.33, 7.00, and 6.00, 5.00 pods per plant, respectively compared to control 5.46 pods per plant. This increase in pods number might be due to mutagenic effect controlling floral induction, and production of floral buds (Seligman *et al.*, 2008).

The number of pods per plant increased in most of the treatments, however, a significant reduction was observed at highest dose treatments of all the mutagens. Increase and decrease in number of pods per plant have been reported by many workers (Waghmare and Mehra, 2000; Kozgar *et al*., 2011) after treatments with physical and chemical mutagens. Reduction of mean in mutagenic populations might be due to induction of more mutations in negative direction and increase in mean could be attributed to induction of more positive mutations in the polygenes governing the character (Sarada *et al*., 2015).

The number of seeds per pod reflects a number of fertilized ovules which grow to seeds. This work showed that sodium azide, gamma rays and combined treatments revealed significant increase in number of seeds per pod 3.33, 3.00 and 3.17, respectively compared to less value of seeds number 0.25mM+30krad 1.83. In order to increase the number of seeds per pod, frequency of ovules fertilization should be increased; mutagens increased the number of ovules per pod and reduced the number of seed abortion (Attiya *et al.*, 1998). No significant differences between treatments were observed; sodium azide had highest average number of seeds per pod 3.33.

5.0 CONCLUSION

This studies concluded that different treatments of mutagens had different effects on yield parameters of *P. vulgaris*.

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