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MORPHOLOGICAL CHARACTERISTICS OF PIGEON PEA [*Cajanus Cajan* (L.) Millsp.] TREATED WITH SODIUM AZIDE AND GAMMA RADIATION

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ABSTRACT
The aim of this study was to determine the morphological responses of pigeon pea Cajanus cajan (L) Millspaugh to treatments with sodium azide and gamma radiation. This was performed by exposing the seeds of landraces pigeon pea to gamma rays at Centre for Radiotherapy and Oncology Department, ABUTH, Zaria at doses of 0(control), 50, 100, 150 and 200Gy. These seeds were further treated with sodium azide (NaN_3) concentrations at 0.00, 0.01, 0.02, 0.03 and 0.04% SA, giving a total of 25 treatments. The growth parameters were recorded at 4, 8, 12, 16 and 20 Weeks After Planting (WAP). The parameters measured include germination percentage, Leaf and branch number, plant height and root length. The result of these treatments showed a symmetric reduction in germination percentage with respect to most of the mutagenic treatments. There was a higher leaf number with those that received 100Gy + 0.03% SA (41.93 leaves). Also, the branch number of treated plants showed an increase over the R0A0 (control) treatment; Similarly, the mean comparison of the plant height presented showed 150Gy + 0.02% SA (190.93 cm), produced the highest plant height and data recorded on root length indicated that the highest root length due to 100Gy + 0.01% SA (43.13 cm), was significantly higher than those of other treatments. It is, therefore, concluded that the two mutagens affected the pigeon pea plant population morphologically as prominent Tall, High yield, Early flowering and profusely branchingmutants were observed by exposing pigeon pea to single and combined treatments of gamma ray (50Gy – 150Gy) and Sodium azide (0.01% - 0.04%).
Keywords: Pigeon pea, Cajanus cajan, sodium azide, gamma radiation, seed germination, root length, plant height, morphological response, mutagenic treatment.

INTRODUCTION
Pigeon pea belongs to Family Fabaceae, sub family Papilionaceae, tribe Phaseoleae, sub-tribe Cajanae, genus *Cajanus*, species *cajan* (L.) Millspaugh, which also contain soybean (*Glycine max* (L) and Field bean (*Phaseolus vulgaris* (L), an often cross-pollinated diploid 2n = 2x = 22, 44 or 66 chromosomes (Varshney *et al.*, 2012). Pigeon pea originated in India, where the

largest diversity exists and is one of the major grain legume (pulse) crops cultivated in many countries in the Tropics and subtropics. Grain legumes are cherished for their opulence in protein which makes them indispensable along with cereals in daily human diet, being a legume, it contributes towards improving soil fertility by fixing nitrogen in the soil, it has capacity of soil restoration and because of the soil ameliorative properties, have become the most important component of sustainable agriculture. Pigeon pea due, to their high genetic potential to thrive well under varied environmental conditions; it is an important crop in the semi-arid areas (Van der Maesen, 1990).

Pigeon pea is an erect perennial legume shrub often grown as an annual, reaching 91- 366cm in height. Pigeon pea is heat-tolerant, prefers hot moist conditions. It grows in temperature between 18°C and 30°C and the optimum rainfall required for pigeon pea is 600-1000 mm/year. A rain at flowering time has very adverse effect on the seed yield (Edwards, 1981). Pigeon pea does well in low fertility soils with reasonable water-holding capacity and pH 5-7 is favourable for its growth. Pigeon pea does not tolerate shallow soils or water logging. It tolerates a wide range of soils, from sandy to heavy black clays. It is sensitive to salt spray and high salinity. It is a short day plant, flowering is triggered by short days, whilst with long days plant grows vegetative and the leaves have three leaflets that are green and pubescent above and silvery greyish – green with longer hairs on the underside. The flowers are yellow with red to reddish-brown lines or red outside (Centre for New Crops and Plants Products, 2002).

In Africa, Pigeon pea generally produces low yield, due to limiting effects of biotic and a biotic factors. The abiotic factors include water logging, extremes of temperature, injury and salinity. The biotic factors are insect pests (pod borers) and pod fly and diseases (e.g. *Fusarium* wilt). Lack of quality seeds and low use of improved varieties have left the poor farmers with no option but to grow local landraces that are low yielding and late maturing (Mergeai *et al.*, 2001). Mutagenic agents have been employed to induce beneficial mutants for different purposes, such as resistance to pests and diseases, early maturity, improved yield and quality. For any mutation breeding programme, selection of effective and efficient mutagen is very essential to recover higher frequency of desirable mutations (Solanki and Sharma, 2002). A number of chemical and physical mutagens are widely employed to induce genetic variability in plants

(Restaino, 2003). In spite of the huge success that has been recorded using mutagenic agents to induce beneficial mutation, the curtain has not been drawn on this area. Researchers can still explore the use of single or combined mutagenic agents to bring about more desirable mutants useful in achieving plant-breeding goals. It is worthy of note that in spite of numerous works, that have been done on crop plants by induced mutation, in Nigeria such works are inadequate (Odeigah, 1991). Pigeon pea *Cajanus cajan* (L) with all its numerous uses, crop breeders have done very little in this area. The objective of this study was to determine the effect of the different doses/concentrations of gamma irradiation and sodium azide on morphological traits of pigeon pea.

Hypothesis: The two mutagens (gamma irradiation and sodium azide) have no significant effect on the morphology of pigeon pea plant.

MATERIALS AND METHODS

Source of Research Materials

The seeds of pigeon pea (*Cajanus cajan* (L.) Millsp) was obtained from local farmers in Ankpa Local Government Area, latitude 7° 38′E and longitude, 7° 22′N (163m elevation above sea level), Kogi state, Nigeria (Garmin eTrex Venture HC Handheld GPS). The Gamma radiation source (Cirus Cobalt 60 Teletherapy) used for seed treatment was located at the Radiotherapy and Oncology Department, Ahmadu Bello University Teaching Hospital Shika, Zaria. The Sodium azide (SA) used for this research work was manufactured by KEM Light Laboratories P.V.T. Ltd.

Study Area

This research was conducted both in the laboratory and in the Biological Garden of the Department of Biological Sciences, Ahmadu Bello University, Samaru, Zaria (Longitude 07° 39′E and latitude 11° 09′N, 2148 above sea level), Nigeria. (Garmin eTrex Venture HC Handheld GPS). Samaru lies in the Northern Guinea savannah agro-ecological zone of Nigeria with mean annual rainfall of about 1100m. Rainfall in this region is essentially between May and September and dry season between October and April. Hottest months of the year are March and April and the region is with a mean daily temperature of 27°C. The coldest months are November – January (Osuhor *et al.,* 2004).

Treatments

Exposure of Seeds to Gamma Radiation: Uniform healthy dry seeds of *Cajanus cajan* (L.) Millspaugh were exposed to different doses of gamma rays (control 0, 50, 100, 150 and 200Gy), derived from Cobalt-60 (^{60}CO) source with a measured dose rate of 124.5Gy/min which lasted for 8hrs 52mins.

Seeds Treated with Sodium Azide:The radiated healthy and dry (10-12% moisture) seeds of *Cajanus cajan* (L.) Millspaugh were pre- soaked in a phosphate buffer (pH 3.0) to maintain the osmotic content of the cell for six (6) hours and later subjected to the four (4) concentrations (0.01%, 0.02%, 0.03% and 0.04%) of sodium azide [SA] (NaN_3) solutions at room temperature (25°C) for six (6) hours. The seeds were washed thoroughly to remove the residual amount of mutagens and sown immediately.

Collection of Soil Samples

Top soil was collected from uncultivated land within the Botanical Garden in Ahmadu Bello University, Zaria. A sample of the top soil was air dried and taken to the Department of Soil Science, Institute for Agricultural Research, Zaria for physico-chemical analysis.

Pot Preparation and Seed Planting

Top soil was used to fill six hundred and fifty (650) polythene bags and four (4) treated seeds were sown inside each polythene bag during rainy season. The polythene bags were arranged in a complete randomized design (CRD).

Data Collection

Growth parameters

(a) **Germination percentage**: Germination count was taken after 2 weeks i.e. after (15days) sowing and expressed in percentage as follows:

$$Germination\ (\%) = \frac{Number\ of Seeds\ Germinated}{Number\ of Seeds\ Sown} x\ 100$$

(b) **Number of leaves/plants:** The number of leaves selected on three randomly sampled plants was taken at 4, 8, 12, 16 and 20 weeks after planting (WAP) and recorded.

(c) **Number of branches/plants:** The primary branches borne on the main shoot of three randomly sampled plants were counted and recorded.

(d) **Plant height and root length:** The plant heights were determined by measuring the height of three randomly sampled plants per treatments from soil surface of the polythene bag to the tip of the apex using a metre rule. This measurement was taken at 4, 8, 12, 16 and 20 WAP; the average height was calculated and recorded. Similarly, the root were carefully dug from the soil and washed, root length of the three randomly sampled plants per treatment was determined at 4, 8, 12, 16 and 20 WAP. The average length was calculated and recorded.

Statistical Analysis of Data

The data obtained from the parameters of morphological traits were subjected to statistical analysis to assess the extent of induced variations using the analysis of variance (ANOVA) to establish if there was any significant difference between the means of the doses/concentrations of the mutagens using Science Analysis Software (SAS) Version 9.0 2004 software.
Duncan's Multiple Range Test (DMRT) was used to separate and compare means where significance difference was observed using Science Analysis Software (SAS) Version 9.0 2004 software.

RESULTS

Observation on Morphological Traits

Observation on the general morphology of the treated plants shows that they were tall and bushy; foliage was dark green with larger pods compared with the Control. The data obtained on the mutagenic effects of gamma rays and sodium azide on seed germination, number of leaves per plants, number of branches per plants, days to 50% flowering, plant height and root length and some yield parameters are presented in the Tables.

Germination Percentage

The germination percentage was symmetrically reduced in all the mutagenic treatments compared to the R0A0 (Control) treatment as shown in Table 1. The highest seed germination recorded for R0A2(0Gy + 0.02% SA) was not significantly higher than that due to R0A0 (control: 0Gy + 0.00 % SA) and R0A1 (0Gy + 0.01% SA); R0A3 (0Gy + 0.03% SA); R0A4 (0Gy + 0.04% SA).This was followed by those that were treated with R1A0 (50Gy + 0.0% SA) and R1A1 (50Gy +0.0%SA).The lowest seed germination observed with R4A3 (200Gy + 0.03% SA) and R4A4 (200Gy + 0.04% SA) was not significantly lower than that due to R3A4 (150Gy + 0.04% SA), R4A0 (200Gy + 0.00% SA), R4A1 (200Gy + 0.01 % SA) and R4A2 (200Gy + 0.02 % SA) treatments.

Table 1: Effect of Gamma radiation and Sodium Azide on Germination Percentage of Pigeon pea (*Cajanus cajan*).

Mutagen	Germination Percentage (%)
R0A0	97.2^{ab}
R0A1	97.2^{ab}
R0A2	100.0^{a}
R0A3	91.7^{abc}
R0A4	91.7^{abc}
R1A0	83.3^{abc}
R1A1	83.3^{abc}
R1A2	80.5^{bcd}
R1A3	80.6^{bcd}
R1A4	77.8^{c-f}
R2A0	77.8^{c-f}
R2A1	75.0^{c-f}
R2A2	72.2^{d-g}
R2A3	66.7^{d-h}
R2A4	63.9^{f-i}
R3A0	63.9^{f-i}
R3A1	61.1^{ghi}
R3A2	58.3^{ghi}
R3A3	52.8^{hi}
R3A4	44.5^{jk}
R4A0	41.6^{jk}
R4A1	41.6^{jk}
R4A2	38.9^{jk}
R4A3	36.1^{k}
R4A4	36.1^{k}
Mean± SE	4.30

Means followed by the same letters along column are not significantly different ($P > 0.05$)
Note: R0 = 0Gy, R1 = 50Gy, R2 = 100Gy, R3 = 150Gy, R4 = 200Gy,
A0 = 0.00 % SA, A1 = 0.01% SA, A2 = 0.02% SA, A3 = 0.03% SA, A4 = 0.04% SA,

Number of Leaves Per Plant

The lowest leaf number was due to the control treatment at 16 and 20 WAP (Table 2). At 4 WAP, with the exception of the significantly higher leaf number observed in plants under R1A3 (50Gy + 0.03 %SA)(22 leaves) and R3A0 (150Gy + 0.00 %SA)(20.3 leaves), other treatments produced leaf number that were not significantly different from the control, R0A0 (20.0 leaves). At 8 WAP, there were no significant differences between the treatments compared with the control ($P \geq 0.05$). At 12 WAP, most of the treatment with 100Gy – 200Gy produced significantly higher leaf number than the other treatments. The highest leaf number in pigeon pea observed with treatment 150Gy + 0.03%SA (R3A3)(35.3 leaves) was comparable some of the other treatments in that category. Other treatments resulted in lower leaf number with the lowest from R1A2 (50Gy + 0.02 % SA)(24.7 leaves) treatment. At 16 WAP, treatment 100Gy + 0.02%SA (R2A2)(62.3 leaves) and 0.03SA (R2A3)(61.7 leaves) produced the highest leaf number per plant. This was followed by that due to most treatments with 100Gy – 200Gy in combination with SA concentrations. Lowest leaf number at 16 WAP was observed with treatments that received 0 – 50Gy in combination with SA concentration with the lowest due to R0A0 (0Gy +0.00%SA)(44.0 leaves) (Table 2). Similarly, at 20 WAP, treatments R2A3, (100Gy+ 0.03%SA)(75.3 leaves) had the highest leaf number. The lowest mean leaf number was observed in the control treatment (R0A0)(52.7 leaves). The combined ANOVA of data on leaf number showed that, the highest leaf number due to R2A3 (100Gy + 0.03%SA)(41.93 leaves) was only comparable with that due to R2A2 (100Gy + 0.02%SA)(40.80 leaves) treatment. This was followed by that due to plants that received 100Gy in combination with SA concentrations. The other treatments resulted in lower leaf number with the lowest due to the Control (R0A0)(33.47 leaves) treatment (Table 2).

Table 2: Effect of Gamma Radiation and Sodium Azide on Leaf Number Per Plant of Pigeon Pea at growth stages

Mutagen	Age of plant (WAP)					Mean
	4	8	12	16	20	
R0A0	20.0^{abc}	22.0^{a}	28.7^{de}	44.0^{h}	52.7^{f}	33.47^{i}
R0A1	15.3^{bc}	20.3^{a}	27.3^{efg}	54.3^{cde}	66.3^{bcd}	36.73^{e-h}
R0A2	18.0^{abc}	21.7^{a}	26.7^{efg}	47.7^{gh}	64.7^{bcd}	35.73^{gh}
R0A3	15.0^{c}	23.0^{a}	25.0^{fg}	49.0^{fg}	61.7^{cde}	34.73^{hi}
R0A4	18.3^{abc}	22.0^{a}	25.7^{efg}	52.0^{efg}	60.0^{cde}	35.60^{ghi}
R1A0	15.7^{bc}	20.7^{a}	25.3^{efg}	54.0^{def}	63.3^{b-e}	35.80^{gh}
R1A1	20.0^{abc}	23.7^{a}	25.7^{efg}	48.0^{gh}	56.3^{ef}	34.73^{hi}
R1A2	18.7^{abc}	21.7^{a}	24.7^{g}	50.7^{efg}	61.3^{cde}	35.40^{ghi}
R1A3	22.0^{a}	24.3^{a}	25.7^{efg}	54.3^{cde}	59.7^{de}	37.20^{d-g}
R1A4	18.7^{abc}	21.7^{a}	25.3^{efg}	58.0^{a-d}	67.0^{bc}	38.13^{c-f}
R2A0	17.0^{abc}	23.0^{a}	27.3^{efg}	55.3^{b-e}	61.3^{cde}	36.80^{e-h}
R2A1	18.3^{abc}	23.0^{a}	28.3^{def}	54.3^{cde}	61.0^{cde}	37.00^{d-h}
R2A2	16.7^{bc}	23.7^{a}	31.3^{cd}	62.3^{a}	70.0^{ab}	40.80^{ab}
R2A3	18.0^{abc}	24.0^{a}	30.7^{cd}	61.7^{a}	75.3^{a}	41.93^{a}
R2A4	19.3^{abc}	23.7^{a}	32.7^{abc}	59.0^{a-d}	62.7^{cde}	39.47^{bc}
R3A0	20.3^{ab}	23.0^{a}	25.3^{efg}	54.7^{cde}	60.0^{cde}	36.67^{e-h}
R3A1	19.0^{abc}	24.3^{a}	32.0^{bc}	58.3^{a-d}	64.0^{bcd}	39.53^{bc}
R3A2	17.0^{abc}	22.3^{a}	33.7^{abc}	59.3^{a-d}	63.3^{b-e}	39.13^{bcd}
R3A3	18.0^{abc}	19.3^{a}	35.3^{a}	60.3^{ab}	64.7^{bcd}	39.53^{bc}
R3A4	18.3^{abc}	22.0^{a}	32.3^{abc}	58.0^{a-d}	62.7^{cde}	38.67^{b-e}
R4A0	15.7^{bc}	21.3^{a}	32.0^{bc}	56.0^{b-e}	63.3^{b-e}	37.67^{c-g}
R4A1	16.3^{bc}	22.3^{a}	34.7^{ab}	59.0^{a-d}	63.0^{b-e}	39.07^{bcd}
R4A2	16.0^{bc}	21.7^{a}	27.0^{efg}	52.3^{efg}	59.7^{de}	35.33^{ghi}
R4A3	15.3^{bc}	20.0^{a}	33.3^{abc}	59.7^{abc}	64.7^{bcd}	38.60^{b-f}
R4A4	17.3^{abc}	22.0^{a}	30.7^{cd}	51.3^{efg}	60.3^{cde}	36.33^{fgh}
Mean± SE	1.41	1.11	1.00	1.30	2.10	0.14

Means followed by the same letters along column are not significantly different ($P > 0.05$)

Note: R0 = 0Gy, R1 = 50Gy, R2 = 100Gy, R3 = 150Gy, R4 = 200Gy, A0 = 0.00 % SA, A1 = 0.01% SA, A2 = 0.02% SA, A3 = 0.03% SA, A4 = 0.04% SA,

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Number of Branches Per Plant

A comparison of the mean values of the branch number in the 4, 8, 12, 16 and 20 WAP is shown in Table 3. The data revealed that, there was no branch observed on the plants at 4 WAP. At 8 WAP, the highest branch number observed due to R0A1 (0Gy + 0.01 % SA)(3.3 branches) was comparable with that due to other treatments that received R0A0 (3.0 branches) and that of R1A0, R1A1, R1A2 and R2A1 treatments (Table 3). The lowest branch number due to R4A3 and R4A4 (0.3 branch) was comparable with that due to all treatments that involved R3 and R4 (except R3A0 with 2.0 branches) combined with various SA concentrations. However, at 12 WAP, the lowest branch number due to R3A0 (3.0 branches) was significantly lower than that due to R3 and R4 combined with SA concentrations. At 16 WAP, the lowest branch number due to R1A4 (4.3 branches) was only significantly lower than that due to R2A2, R2A3, R2A4, R3A1, R3A2, R3A3 and R4A1 treatments. The highest leaf number was due to R3A2 treatment at 16 (15.0 branches) and 20 (27.0 branches) WAP and also in the combined ANOVA of the overall data. At 20 WAP, R0A4 treatment resulted in the lowest branch number which was comparable with that due to treatments that consisted of R0, R1 and R2A0 and R2A1 treatments. However, other treatments of R2 and most of those consisting of R3 and R4 produced higher leaf numbers (Table 3). The combined ANOVA of the data on branch number in pigeon pea showed that the highest number of branches due to R3A2 (10.47 branches) was only comparable with that due to R2A2, R2A3, R2A4, R3A1 and R3A3 treatments. The lowest branch number was due to R4A4 (4.27 branches) (Table 3).

Table 3: Effect of Gamma Radiation and Sodium Azide on Number of Branches Per Plant of Pigeon Pea at Growth Stages.

	Age of plant(WAP)					
Mutagen	4	8	12	16	20	Mean
R0A0	0.0	3.0^{ab}	5.7^{c-g}	9.3^{a-g}	13.7^{ef}	6.33^{f-i}
R0A1	0.0	3.3^{a}	4.3^{e-h}	6.0^{d-g}	19.3^{cde}	6.60^{e-i}
R0A2	0.0	2.7^{abc}	3.3^{gh}	10.0^{a-g}	17.0^{c-f}	6.60^{e-i}
R0A3	0.0	2.3^{a-d}	4.7^{d-h}	5.3^{fg}	13.7^{ef}	5.20^{ij}
R0A4	0.0	2.7^{abc}	4.7^{d-h}	9.0^{a-g}	12.3^{f}	5.73^{hij}
R1A0	0.0	2.3^{a-d}	4.7^{d-h}	5.7^{efg}	17.3^{c-f}	6.00^{g-j}
R1A1	0.0	2.7^{abc}	9.0^{abc}	10.7^{a-f}	19.3^{cde}	8.33^{b-e}
R1A2	0.0	2.7^{abc}	7.3^{b-f}	9.7^{a-g}	17.7^{c-f}	7.47^{d-h}
R1A3	0.0	1.7^{cd}	6.0^{c-g}	7.7^{c-g}	15.0^{ef}	6.07^{g-j}
R1A4	0.0	1.7^{cd}	4.0^{fgh}	4.3^{g}	18.0^{c-f}	5.60^{hij}
R2A0	0.0	1.3^{cde}	5.7^{c-g}	8.3^{b-g}	16.7^{def}	6.40^{f-i}
R2A1	0.0	2.3^{a-d}	4.0^{fgh}	5.3^{fg}	15.0^{ef}	5.33^{ij}
R2A2	0.0	1.7^{cd}	8.3^{a-d}	14.0^{ab}	20.0^{b-e}	8.80^{a-d}
R2A3	0.0	1.7^{cd}	7.7^{b-f}	12.0^{a-d}	22.3^{a-d}	8.73^{a-d}
R2A4	0.0	1.7^{cd}	8.0^{b-e}	10.7^{a-f}	25.7^{ab}	9.20^{a-d}
R3A0	0.0	2.0^{bcd}	3.0^{h}	5.3^{fg}	18.7^{c-f}	5.80^{hij}
R3A1	0.0	1.3^{cde}	10.3^{ab}	13.0^{abc}	23.0^{a-d}	9.53^{abc}
R3A2	0.0	1.3^{cde}	9.0^{abc}	15.0^{a}	27.0^{a}	10.47^{a}
R3A3	0.0	1.3^{cde}	11.7^{a}	12.0^{a-d}	23.3^{abc}	9.67^{ab}
R3A4	0.0	1.7^{cd}	9.0^{abc}	$8.7b^{c-g}$	19.3^{cde}	7.73^{c-g}
R4A0	0.0	1.3^{cde}	7.0^{b-f}	10.0^{a-g}	22.3^{a-d}	8.13^{b-f}
R4A1	0.0	1.3^{cde}	8.7^{abc}	11.7^{a-e}	20.0^{b-e}	8.33^{b-e}
R4A2	0.0	1.0^{de}	10.3^{ab}	10.0^{a-g}	17.3^{c-f}	7.73^{c-g}
R4A3	0.0	0.3^{e}	6.3^{c-g}	6.0^{d-g}	15.7^{ef}	5.67^{hij}
R4A4	0.0	0.3^{e}	4.3^{e-h}	4.3^{g}	12.3^{f}	4.27^{j}
Mean±SE	0.0	0.38	1.11	1.71	1.71	0.11

Means followed by the same letters along column are not significantly different ($P > 0.05$)

Note: R0 = 0 Gy, R1 = 50Gy, R2 = 100Gy, R3 = 150Gy, R4 = 200Gy,
A0 = 0.00 % SA, A1 = 0.01% SA, A2 = 0.02% SA, A3 = 0.03% SA, A4 = 0.04% SA,

Plant Height

The mean comparison of plant height presented in Table 4 showed that plant heights in the various sampling dates differed significantly ($P \leq 0.05$). The plant heights were observed to fluctuate among treatments. Treatment R_3A_2 (150Gy+0.02%SA) produced the highest plant heights from 4 to 16 WAP and also when data was combined. Also, R_4A_2 had the lowest plant height from 4 to 16 WAP and in the combined ANOVA. At 4 WAP, the highest plant height observed due to R_3A_2 (150Gy + 0.02 % SA)(81.0 cm) was comparable with that due to other treatments that received R_0A_0 (0Gy + 0.00 % SA)(75.7 cm) and that of R_0A_2, R_3A_4 and R_3A_3 (Table 4). The lowest plant height due to R_4A_2 (200Gy + 0.02 % SA)(61.3 cm) was comparable with that due to all treatments except that of R_0A_0, R_0A_2, R_2A_1, R_2A_4, R_3A_2, R_3A_3, R_3A_4, R_4A_0 and R_4A_1 treatments. Similarly at 8 WAP the lowest plant height due to R_4A_2 (200Gy + 0.02 % SA)(118.7 cm) was significantly lower than that due to most treatments involving R_2 and R_3 combined with SA concentration (Table 4).

At 12 WAP, the lowest plant height due to R_4A_2 (200Gy + 0.02 % SA)(129.0 cm) and R_0A_1 (0Gy + 0.01 % SA)(129.3 cm) was significantly lower than that due to R_1, R_2 and R_3 combined with various concentrations of SA. At 16 WAP, the lowest plant height due to R_0A_3 (0Gy + 0.03 % SA)(191.3 cm) was not significantly lower than those due to most R_0 (0Gy) treatments and that due to R_1A_2 (50Gy + 0.02% SA)(204.0 cm). Most treatments that consisted of R_1, R_2 and R_3 in combination with various concentrations of SA showed higher plant height than other treatments. At 20 WAP, R_4A_4 treatment (200Gy + 0.04 % SA)(215.0 cm) resulted in the lowest plant height which was comparable with that due to treatments that consisted of R_0A_0, R_0A_1 and R_4A_2 treatments. The highest plant height due to R_3A_3 was comparable with that due to R_0A_2, R_0A_3, R_2A_4, R_3A_0, R_3A_2 and R_4A_0 treatments. The combined ANOVA of the data on plant height showed that, the highest plant height due to R_3A_2 (150Gy + 0.02 % SA)(190.93 cm) was only comparable with that due to R_2A_1, R_2A_4 and R_4A_0 treatments. This was followed by that due to most treatments that consisted of R_2 and R_3 in combination with various concentrations of SA. The lowest plant height due to R_4A_2 was comparable with that due to R_0A_1 and R_4A_4 (Table 4).

Table 4: Effect of Gamma Radiation and Sodium Azide on Plant height (cm) of Pigeon Pea at Growth Stages.

Mutagen	Age of Plant (WAP)					
	4	8	12	16	20	Mean
RoAo	75.7^{abc}	143.3^{a-e}	162.0^{b-e}	225.3^{f}	228.0^{f-j}	166.87^{e-i}
RoA1	64.0^{cd}	122.3^{def}	129.3^{f}	202.3^{h}	216.7^{hi}	146.93^{j}
RoA2	75.7^{abc}	140.3^{b-f}	160.7^{b-e}	239.0^{b-f}	257.7^{ab}	174.67^{c-f}
RoA3	69.7^{a-d}	131.0^{c-f}	148.0^{def}	191.3^{h}	253.3^{abc}	158.67^{i}
RoA4	69.0^{a-d}	132.3^{c-f}	164.7^{a-d}	193.3^{h}	245.3^{b-e}	160.93^{hi}
R1Ao	64.7^{bcd}	136.0^{c-f}	163.3^{a-e}	225.0^{f}	245.7^{b-e}	166.93^{e-i}
R1A1	64.7^{bcd}	126.0^{def}	152.7^{c-f}	228.3^{ef}	248.3^{b-e}	164.00^{ghi}
R1A2	69.0^{a-d}	141.0^{b-f}	176.3^{abc}	204.0^{h}	238.7^{c-g}	165.80^{f-i}
R1A3	65.3^{bcd}	132.7^{c-f}	183.0^{ab}	226.7^{f}	232.7^{e-h}	168.07^{e-h}
R1A4	70.3^{a-d}	145.7^{a-e}	157.7^{b-e}	231.3^{def}	236.0^{c-g}	168.20^{e-h}
R2Ao	72.0^{a-d}	151.3^{abc}	178.3^{abc}	248.7^{a-d}	237.0^{c-g}	177.47^{bcd}
R2A1	79.0^{a}	161.0^{ab}	190.3^{a}	245.7^{a-e}	246.7^{b-e}	184.53^{ab}
R2A2	73.0^{a-d}	143.0^{b-f}	167.7^{a-d}	247.7^{a-d}	246.7^{b-e}	175.60^{b-e}
R2A3	72.0^{a-d}	146.3^{a-d}	175.3^{abc}	226.7^{f}	253.3^{b-e}	174.73^{c-f}
R2A4	75.3^{abc}	151.7^{abc}	180.3^{ab}	256.7^{ab}	253.7^{abc}	183.53^{abc}
R3Ao	72.0^{a-d}	144.3^{a-e}	168.3^{a-d}	250.0^{a-d}	251.3^{a-d}	177.20^{bcd}
R3A1	69.0^{a-d}	153.3^{abc}	181.0^{ab}	252.0^{abc}	244.7^{b-f}	180.00^{bcd}
R3A2	81.0^{a}	166.7^{a}	190.0^{a}	260.0^{a}	257.0^{ab}	190.93^{a}
R3A3	74.3^{abc}	145.7^{a-e}	171.3^{a-d}	240.0^{b-f}	265.7^{a}	179.40^{bcd}
R3A4	77.3^{ab}	152.3^{abc}	176.7^{abc}	247.3^{a-d}	247.3^{b-e}	180.20^{bcd}
R4Ao	69.7^{a-d}	153.3^{abc}	181.7^{ab}	257.7^{ab}	256.7^{ab}	183.80^{ab}
R4A1	73.3^{a-d}	146.0^{a-e}	168.0^{a-d}	236.7^{c-f}	237.7^{c-g}	172.33^{d-g}
R4A2	61.3^{d}	118.7^{f}	129.0^{f}	196.3^{h}	226.7^{ghi}	146.40^{j}
R4A3	65.0^{bcd}	136.3^{b-f}	163.3^{a-e}	221.7^{ef}	234.3^{d-g}	164.13^{ghi}
R4A4	64.0^{cd}	121.3^{ef}	138.0^{ef}	206.7^{gh}	215.0^{i}	149.00^{j}
Mean± SE	3.80	7.40	8.12	5.70	5.30	0.60

Means followed by the same letters along column are not significantly different ($P > 0.05$)

Note: Ro = 0Gy, R1 = 50Gy, R2 = 100Gy, R3 = 150Gy, R4 = 200Gy, Ao = 0.00 % SA, A1 = 0.01% SA, A2 = 0.02% SA, A3 = 0.03% SA, A4 = 0.04% SA,

Root length:

Data recorded on root length is presented in Table 5. The root length differed significantly with treatments and particularly at 12 WAP showed that most treatments consisting of R2, R3 and R4 produced longer root length. At 4 WAP and 12 WAP, the highest root length was that due to R2A1 (100Gy + 0.01% SA) with 16.3 cm and 40.0 cm respectively. While the least root length at 4 WAP was due to R4A3 (200Gy + 0.003% SA)(5.7 cm) compared to R0A0, R0A1, R0A2, R0A3, R0A4, R1A0, R1A1, R1A2, R1A4, R3A0, R3A1, R4A2 and R4A4. At 8 WAP, the highest root length due to R2A0 (100Gy + 0.0% SA)(42.3 cm) was comparable to R1A2 and R1A4. The lowest root length recorded was due to R4A4 (200Gy + 0.004% SA)(22.3 cm) which was comparable with all the treatment that received R0 in combination with SA apart from other treatments. In the 12 WAP, R0A0, R2A1, R2A4 and R4A4recorded the highest root length which was not significantly different from those due to R0A1, R1A4, R2A0, R2A2, R2A3, R3A0, R3A1 and R4A3. The least root length was that due to R1A0 which is comparable to R0A2, R0A3, R0A4, R1A1, R1A2, R1A3, R3A3 and R3A4.

The highest root length recorded at 16 WAP was that due to R4A4 (200 + 0.004% SA)(61.7 cm) and this was comparable to those of R2A1, R2A3 and R0A0 .While the least root length was that due to R0A2 (0Gy + 0.02% SA)(35.7 cm). At 20 WAP, the highest root length due to R4A4 (200Gy + 0.04% SA)(67.0 cm) was not significantly different from those of R0A0, R1A3, R1A4, R2A1, R2A2, R2A3, R2A4 and R3A0. The combined ANOVA of data on root length in Pigeon pea showed that the highest root length due to R2A1 (100Gy + 0.01% SA)(43.13 cm) was significantly higher than those of other treatments. The lowest mean value was that due to R0A2 (0Gy + 0.02% SA)(28.13 cm).

Table 5: Effect of Gamma Radiation and Sodium Azide on Root Length (cm) of Pigeon Pea at Growth Stages

	Age of Plant(WAP)					
Mutagen	4	8	12	16	20	Mean
R0A0	6.0^{f}	24.3^{ij}	40.0^{a}	56.7^{abc}	59.3^{a-d}	37.27^{b-f}
R0A1	9.7^{b-f}	25.0^{hij}	37.7^{ab}	48.0^{d-g}	54.0^{b-g}	34.87^{f-i}
R0A2	7.3^{def}	26.0^{g-j}	29.3^{d-h}	35.7^{k}	42.3^{h}	28.13^{n}
R0A3	7.3^{def}	28.7^{d-j}	25.3^{gh}	47.7^{d-g}	53.3^{b-g}	32.47^{h-l}
R0A4	8.3^{b-f}	26.0^{g-j}	28.3^{e-h}	39.7^{h-k}	45.0^{gh}	29.47^{mn}
R1A0	7.3^{def}	32.3^{b-g}	24.3^{h}	37.0^{ij}	46.7^{fgh}	29.53^{mn}
R1A1	8.0^{c-f}	34.0^{b-e}	27.7^{fgh}	44.7^{e-i}	56.0^{b-f}	34.07^{g-j}
R1A2	7.0^{ef}	37.7^{ab}	25.3^{gh}	37.7^{jk}	53.3^{b-g}	32.20^{i-m}
R1A3	10.7^{b-e}	35.0^{bcd}	27.3^{fgh}	50.0^{c-f}	61.0^{ab}	36.80^{c-g}
R1A4	8.0^{c-f}	37.7^{ab}	34.0^{a-e}	50.3^{c-f}	58.0^{a-e}	37.60^{b-f}
R2A0	10.0^{b-f}	42.3^{a}	34.7^{a-d}	47.3^{d-g}	55.7^{b-f}	38.00^{b-e}
R2A1	16.3^{a}	41.3^{a}	40.0^{a}	55.3^{abc}	62.7^{ab}	43.13^{a}
R2A2	11.7^{bcd}	27.0^{f-j}	37.3^{ab}	51.3^{b-e}	60.7^{abc}	37.60^{b-f}
R2A3	12.0^{bc}	25.0^{hij}	36.3^{abc}	57.3^{ab}	60.0^{a-d}	38.13^{bcd}
R2A4	12.7^{b}	29.7^{c-i}	40.0^{a}	51.0^{b-e}	62.0^{ab}	39.07^{bc}
R3A0	9.0^{b-f}	27.7^{e-j}	38.7^{ab}	53.0^{bcd}	58.7^{a-f}	37.40^{b-f}
R3A1	9.0^{b-f}	32.7^{b-f}	38.3^{ab}	46.7^{d-h}	50.7^{d-h}	35.47^{d-g}
R3A2	11.0^{b-e}	31.3^{b-h}	32.3^{b-f}	44.7^{e-i}	56.0^{b-f}	35.07^{e-i}
R3A3	12.0^{bc}	28.7^{d-j}	27.7^{fgh}	38.3^{ijk}	49.0^{e-h}	31.13^{klm}
R3A4	11.3^{b-e}	35.3^{bc}	33.3^{b-f}	41.0^{g-k}	53.3^{b-g}	34.87^{f-i}
R4A0	12.3^{bc}	33.3^{b-f}	31.0^{c-g}	42.0^{g-k}	51.0^{c-h}	33.93^{g-k}
R4A1	11.7^{bcd}	34.0^{b-e}	35.0^{a-d}	40.0^{h-k}	55.3^{b-f}	35.20^{d-h}
R4A2	9.0^{b-f}	27.3^{f-j}	32.3^{b-f}	41.7^{g-k}	46.3^{fgh}	31.33^{j-m}
R4A3	5.7^{f}	23.7^{ij}	34.3^{a-e}	43.3^{f-j}	47.7^{fgh}	30.93^{lm}
R4A4	8.3^{b-f}	22.3^{i}	40.0^{a}	61.7^{a}	67.0^{a}	39.87^{b}
Mean± SE	1.30	1.89	1.90	1.88	2.83	0.18

Means followed by the same letters along column are not significantly different ($P > 0.05$)

Note: R0 = 0Gy, R1 = 50Gy, R2 = 100Gy, R3 = 150Gy, R4 = 200Gy, A0 = 0.00 % SA, A1 = 0.01% SA, A2 = 0.02% SA, A3 = 0.03% SA, A4 = 0.04% SA,

DISCUSSION

The seed germination percentage decreased with increasing doses of Gamma rays with the various concentrations of sodium azide in Pigeon pea. This clearly indicates that the mutagen had an inhibitory effect on seed germination. Similar inhibitory effects on seed germination by the mutagen

have also been reported earlier by Apparao (2005) in cowpea, Khan and Wani (2006) in Mungbean.
The reduction in germination might be due to genetic and physiological processes inhibited by the mutagens. Decrease in percent seed germination in pigeon pea caused by Gamma Rays and Sodium azide could be attributed to physiological perturbations and partly to the chromosomal damages (Mensah *et al.,* 2007), Gaiward and Kothekar (2004) observed that with increase in concentration/doses, there was corresponding increase in damage to chromosomes in lentil after EI, NEU, EMS, SA and gamma rays treatments. According to Kumar and Yadav (2010), delayed and reduced seed germination could be due to delay or inhibition in physiological and biological process necessary for seed germination which includes enzyme activity. Reduced seed germination due to mutagenic treatments may be the result of damage of cell constituents at molecular level or altered enzyme activity (Khan and Goyal, 2009).

The increase in leaf number of plants under mutagens treatments with those receiving R2 and R3 with various concentrations of SA might be due to chromosomal aberrations that tend to produce an increase in certain morphological traits such as leaf number. Similar observation was reported by Khan *et al.* (2000) in Soya beans using ionizing radiation, with effect been dose-independent. The stimulatory effect might be due to an activation of growth hormones, e.g., auxin (Zaka *et al.*, 2004). There was increased branch number in most treatments (particularly, those consisting of R2 and R3) except in the mutagenic treatments with higher SA concentration R0A4 (0Gy+ 0.04% SA) and R4A4 (200Gy + 0.04% SA). This showed that the gamma ray at R4A4 (200Gy + 0.04%SA) exerted inhibitory effect on branches per plant, while lower treatment of SA showed profuse effect on branches per plant. Such mutations can be considered evolutionary conversion of the plant habit gene carrying substantial polygenic significance. Similar results were obtained by Chandirakala and Subbaraman (2010) who reported high magnitude of primary branches in pigeon pea. There was increase in plant height with increasing gamma ray doses and sodium azide concentrations (especially, those consisting of R2 and R3 in combination with SA) compared to the Control except with mutagenic treatments R0A1 (0Gy + 0.01% SA) and R4A4 (200Gy + 0.04% SA), where there was reduction in plant height.

The increase in plant height by chemical and physical mutagens was ascribed to different factors resulting from the combined effect of the mutagens. Earlier studies by Markeen *et al.* (2007) reported reduction in plant height with higher doses of gamma rays, which was attributed to gross injury caused at cellular level. The mean increase in plant height at maturity in the present study might be due to the alteration of their genome integrated by environmental signals as reported by Uno *et al.* (2001); probably by increasing the rate of cellular division and expansion at their meristematic regions. This is also in agreement with the findings of Hoballah (1999) who reported increase in plant height of Sesame due to radiation mutagenesis.The reduction in root lengths in plants under R0, R1 and R4 in combination with SA concentrations was possibly due to the delay in root elongation. On the other hand, plants from seeds treated with 100Gy (R2) with its sodium azide concentrations showed increases in root length compared with other doses of radiation. This could be attributed to the reduced chromosomal disturbance during cell division and elongation.

CONCLUSION

The two mutagens affected the pigeon pea plant population morphologically as prominent tall, early flowering and profusely branching mutants were observed in the present experimental work.

RECOMMENDATION

Mutants isolated in this investigation should be utilized in cultivation of pigeon pea. The overall results suggest that low doses/concentrations in both gamma rays and sodium azide gave superior performance in pigeon pea [*Cajanus cajan* (L) millspaugh]. Increasing population and rising per capita incomes are fuelling growth in demand for food and feed. Diversification is an option that is waiting to be explored as agriculture is increasingly becoming vulnerable and sensitive to limiting factors such as growth frequency, so further work should be employed using lower doses/concentrations of gamma rays and sodium azide to determine the effects in inducing resistance against diseases and cooking time in pigeon pea. Given the crop's versatility, it could substantially contribute to people and environment.

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