VOLUME 14 (3)

JOURNAL OF AGRICULTURE AND AGRICULTURAL TECHNOLOGY (JAAT)

Journal of Agriculture and Agricultural Technology,
Federal University of Technology, PMB 65, Minna,
Niger State, Nigeria

ISSN 1597-5460

EDITORIAL BOARD

Prof. O.J. Alabi	Editor-in-Chief
Prof. M.T. Salaudeen	Editorial Manager
Dr. Mrs. D. T. Ezekiel- Adewoyin	Business Manager
Prof. A. M. Orire	Member
Prof. M. Ibrahim	Member
Dr. Mrs. K. E. Akande	Member
Dr. K. Tolorunse	Member
Mrs F.B. Femi	Member
Dr. A.B. Mohammed	Member
Mr. M. A. Adavize	Website Manager
Dr. Mrs. O. A. Adediran	Editorial Secretary
Dr. Mrs. A. I. Oseghale	Assistant Editorial Secretary

EDITORIAL ADVISERS

Prof. T.Z. Adama	Federal University of Technology, Minna
Prof. S.L. Lamai	Federal University of Technology Minna
Prof. K.M. Baba	Federal University of Technology Minna
Prof. S.O.E. Sadiku	Federal University of Technology, Minna
Prof. M.G.M. Kolo	Federal University of Technology Minna
Prof. J. N. Nmadu	Federal University of Technology Minna
Prof. B.A. Ayanwale	Federal University of Technology Minna
Prof. O.O. Fagbenro	Federal University of Technology, Akure
Prof. O.O. Odoguwa	Federal University of Agriculture, Abeokuta
Prof. B.A. Raji	University of Ilorin, Ilorin
Prof. O. Bello	Federal University of Agriculture, Makurdi
Prof. I.S.R. Butswat	National Open University of Nigeria
Prof. Lawrence Etim	University of Uyo
Prof. S.O. Ojeniyi	Federal University of Technology, Akure
Prof. B.O. Ogunbamiro	University of Maiduguri
Prof. O. Aworh	University of Ibadan
Prof. I.A. Adeyemi	Ladoke Akintola University of Technology, Ogbomosho

Journal of Agriculture and Agricultural Technology

(JAAT)

Journal of Agriculture and Agricultural Technology 14 (3), 2025

EFFECTS OF NEEM SEEDS SYNTHESIZED SILVER NANOPARTICLES AND GAMMA-IRRADIATION ON CERTAIN MORPHOLOGICAL TRAITS IN SELECTED ACCESSIONS OF PIGEON PEAS [*CAJANUS CAJAN* (L.) MILLSP]

***DAUDU, O. A. Y.[1], ABUBAKAR, A.[1], BELLO, I. M.[1], Abdulsalami, H. [1], BUSARI M. B[2], and ALIYU, M.M [1]**

1. Department of Plant Biology, Federal University of Technology, Minna

2. Department of Biochemistry, Federal University of Technology, Minna

*Corresponding Author's email address and Phone number: dauduoladipupoyusuf@yahoo.com

/+2348062202142

ABSTRACT

Cajanus cajan (L.) Millsp is an essential and nutritious crop plant in Nigeria. This study investigated the combined effects of gamma irradiation and bio-synthesized silver nanoparticles on selected agro-morphological traits of pigeon peas. It is based on this background that two accessions of pigeon peas (NGB05522 and NGB05543) were obtained from the National Centre for Genetic Resources Conservation and Biotechnology, Ibadan. The seeds were irradiated using a gamma irradiation source (Cirus Cobalt- 60 Teletherapy) at various levels. Before sowing, the seeds were primed with different concentrations of biosynthesized Silver Nanoparticles (AgNPs) from neem seeds. The results showed that the combined effects of gamma irradiation and biosynthesized AgNPs stimulate both plant height and the number of leaves per plant. At 12 Weeks after Sowing (WAS), the highest plant height in NGB05543 (115.80cm) was obtained in seeds treated with 400 gray and 100 ppm AgNPs; the value was significantly different ($p < 0.05$) from all the other treatments. In NGB05522, the highest plant height at 12WAS (143.00cm) was observed with 100 gray and 50 ppm AgNPs; this value was significantly different from all the other treatments. In NGB05543, 100 Gray and 100 ppm consistently produced the highest number of leaves throughout the periods under study (36 at 4WAS, 60 at 6WAS, 117 at 8WAS, 210 at 10WAS, and 894 at 12WAS); these values were significantly different ($p<0.05$) from all the other values. It is therefore concluded that gamma irradiation of pigeon pea seeds primed with AgNPs increased plant height and the number of leaves in pigeon peas.

Keywords: Improvement, Silver Nanoparticles, Gamma Irradiation, Plant Height, Leaves

INTRODUCTION

Pigeon pea [Cajanus cajan (L.) Millsp] is one of the most important food legume crops of both tropics and subtropics. Pigeon pea is a drought-tolerant plant and exhibits a large variation in physiological maturity. It primarily grows under marginal and high-risk conditions, but yields are generally poor. Rural communities primarily sow it in low-input, wet locations. The seeds of pigeon peas are enriched with vitamin B, protein, carotene, and ascorbic acid, which is better than other indigenous legumes. Pigeon pea (Cajanus cajan L. Millsp.) is an important grain legume grown in Nigeria. It primarily grows in marginal and poor soil conditions, but yields are generally low (Saxena et al., 2018). The seeds of pigeon peas are enriched with vitamin B, protein, carotene, and ascorbic acid, which is better than other indigenous legumes. There has been an increasing demand for healthy, protein-rich foods and critical minerals over the years. Soybeans and cowpeas, which serve as alternatives to animal protein, have become so expensive and unaffordable to the average Nigerian, hence aggravating the threat to food security.

Despite the nutritional importance of pigeon peas, its detrimental traits, such as a long cooking time (requiring a high cost of cooking energy), have led to low cultivation of the crop and reduced its consumer demand. Due to this, the crop has been branded as an "orphan crop" in many parts of Sub-Saharan Africa, including Nigeria. Mutation has been an essential tool in evolution, and at one time, it was considered the chief source of origin of new species. Mutation has been a crucial tool in evolution, and at one time, it was considered the primary source of new species. Plant breeders over across countries have adopted various strategies for crop improvement, like hybridization, selection, and mutation in pulse crops for generating variability and designing genotypes with desirable traits (Jaganathan et al., 2018). Mutagenesis is a phenomenon in which biological, chemical, or physical stimuli elicit abrupt heritable changes in plant contents that are not mediated by genetic dispersion or integration (Roychowdhury and Tah, 2013). Ionizing radiation has been observed to be useful in improving the overall traits in pigeon pea, including some preferred changes in functional properties of the seed (Bamidele and Akanbi, 2013).

Nanotechnology has permeated several disciplines over the last two decades, including medicine, environmental science, drug development, and biotechnology. To breed pigeon pea varieties with unique genetic architecture and improved agromorphological traits, there is a need to explore the use of mutation breeding through irradiation with gamma rays, as well as priming the irradiated seeds with silver nanoparticles. Nanotechnology has permeated several disciplines over the last two decades, including medicine, environmental science, drug development, and biotechnology (Niharika et al., 2016). Therefore, the objective of this research was to evaluate the effect of gamma irradiation and silver nanoparticles on genetic architecture and the reduction in cooking times of pigeon peas.

METHODOLOGY

Source of Research Materials

Pigeon pea (Cajanus cajan (L.) Millsp.) genotypes (NGB05543 and NGB05522) were obtained from the National Centre for Genetic Resources Conservation and Biotechnology (NACGRAB), Ibadan. Irradiation of the seeds was carried out at the Radiology and Oncology Department, Ahmadu Bello University Teaching Hospital, Shika, Zaria, using a gamma irradiation source (Cirus Cobalt- 60 Teletherapy) from Atomex. The Atomtex is a self-calibrated radiation source with an ambient dose equivalent of 50 nSv – 10 Sv/h. The leakage-free Cobalt-60 machine is a 229.061 TBq (6190.84Ci) model.

Treatment of Pigeon Pea Seeds with Gamma Radiation

The dried seeds of the pigeon pea [Cajanus cajan (L.) Millsp] were subjected to various doses of gamma rays (control 0,100, 200 and 400 Gy) obtained from a Cobalt-60 (60CO) source with a measured dose rate of 124.5 Gy/min for 8 hours 52 minutes.

Source of Neem Seeds

Fresh and healthy seeds of Neem (Azadirachta indica) were collected from the biological garden at the Federal University of Technology, Minna. The seeds were rinsed thoroughly first under running tap water, followed by deionized water to remove all the dust and unwanted particles. The seeds were then cut into small pieces and dried at room temperature (Niharika et al., 2016).

Preparation of Seed Extracts

Ten grams of the ground seeds were transferred into a 250 ml beaker containing 100 ml of deionized water and stirred on a magnetic stirrer at 80 °C for 20 minutes. The extracts were filtered twice through Whatman filter paper and refrigerated at 4 °C in Erlenmeyer flasks for further experiments. At every step of the experiment, sterile conditions were maintained to ensure effectiveness and accuracy in the results (Niharika et al., 2016).

Biosynthesis of Silver Nanoparticles

A 1 mM aqueous solution of silver nitrate (AgNO3) was prepared in a 250 ml Erlenmeyer flask, and 10% leaf extract was added for the reduction of Ag+ ions. The complete mixture was kept on a magnetic stirrer at 30 oC. Time and colour changes were recorded, along with periodic sampling and scanning using a UVVisible (UV-Vis) spectrophotometer. Suitable controls were maintained throughout the experimental conditions. The Complete reduction of Ag+ ions was confirmed by the change in colour from light or faint to yellowish colloidal brown. The colloidal solution was then left aside for 24 hours to allow for complete bio-reduction, as indicated by UV-Vis spectrophotometric scanning. The solution was sealed and stored properly for further use. The formation of silver nanoparticles will be further confirmed by different spectrophotometric analyses (Niharika et al., 2016).

Seed Treatments

The biosynthesized silver nanoparticles were dissolved at different concentrations (0, 25, 50, 75, and 100 ppm) in deionized water. Irradiated pigeon pea seeds were subjected to priming by soaking in a silver nanoparticle solution for approximately 2 hours. The treated seeds were then dried in the shade before planting (Khalaki et al., 2016).

Field Laid out and Experimental Design

The experiment was arranged in a randomized complete block design with five replicates; seeds of four genotype lines were sown alongside their respective controls. Two seeds were sown per hole, and then thinned to one after five weeks. The inter- and intra-row spacing was 40 × 25 cm. The treatments includes

CONTROLS (for both NGB05543 and NGB05522), W1 (100G/25ppm), W2 200G/25ppm, W3 400G/25PPM, W4 100G/50ppm, W7 100G/100ppm, W8 200G/100ppm, W9 400G/100ppm, 400G/25ppm, 100G/50ppm, 200G/50ppm, 100G/100ppm, R3 400G/25ppm, R4 100G/50ppm,
R5 200G/50ppm, R7 100G/100ppm, R8 200G/100ppm, and R9 400G/100ppm

Data Collection

During the planting phase, data on plant height and the number of leaves per plant were collected using techniques recommended by ICRISAT and IBPGR (1993). The plant height was recorded at 4 weeks after sowing (WAS) to 12 WAS. The length of the shoots (from the base of the stem to the terminal bud) of four randomly selected plants from each treatment, as well as the control, was measured using a meter rule. The number of leaves per plant was taken by directly counting all the leaves attached to the plants from 4 to 12 WAS.

Data Analyses

Quantitative data on plant height and the number of leaves per plant, obtained at various weeks after sowing, were pooled for analysis. Analysis of variance (ANOVA) was used to determine the level of significance among the treatments, and means were separated using Duncan's Multiple Range Test (DMRT) where necessary. All values were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

The effects of gamma irradiation on pigeon pea seeds primed with silver nanoparticles (AgNPs) are presented in Table 1. The results revealed significant differences ($P < 0.05$) in plant height and the number of leaves per plant throughout the experiment periods. At 4 weeks after sowing (WAS), the highest plant height in the white seed-coated pigeon pea (NGB05543) was observed with 400 gray and 100 ppm AgNPs (20.70 cm); this value was significantly higher than that of all other treatments (Table 1). Similarly, in the red seed-coated pigeon pea (NGB05522), the highest plant height at 4 WAS (25.00 cm) was due to 200 gray and 50 ppm AgNPs treatment; this value was significantly different from all the other treatments, including the controls. At 6 WAS, the highest plant height (36.00 cm) was obtained in NGB05543 treated with 100 Gray gamma rays and 100 ppm AgNPs. Meanwhile, in NGB05522, the highest plant height at 8 WAS (36.00 cm) was due to 200 Gray gamma irradiation and primed with 50 ppm AgNPs. A similar trend was obtained in all the weeks; the treated seeds produced higher plant heights than their controls (Table 1). At 12 WAS, the highest plant height in NGB05543 (115.80 cm) was obtained in seeds treated with 400 gray and 100 ppm AgNPs. In NGB05522, the highest plant height at 12 WAS (143.00 cm) was due to 100 gray and 50 ppm AgNPs (Table 1).

The results obtained for the number of leaves per plant for both pigeon accessions indicated that gamma irradiation and AgNPs enhanced leaf production. In NGB05543, 100 Gray and

100 ppm consistently produced the highest plant heights throughout the periods under study (36 at 4 WAS, 60 at 6 WAS, 117 at 8 WAS, 210 at 10 WAS, and 894 at 12 WAS); these values were significantly different from all the other values (Table 1). In NGB05522, 200G/50PPM consistently produced the highest number of leaves per plant at 4 WAS (27), 8 WAS (84), and 12 WAS (790); these values were significantly different from all the other values (Table 2). Meanwhile, the 100G/50PPM produced the highest number of leaves per plant at 6 WAS (50); however, this value was significantly different from all the other treatments.

Table 1: Combined Effects of Gamma Irradiation and Biosynthesized AgNPs on Plant Height of Selected Pigeon Peas Accessions

Treatments	**PH (cm) 4 WAS**	**PH (cm) 6 WAS**	**PH (cm) 8 WAS**	**PH (cm) 10 WAS**	**PH (cm) 12 WAS**
		NGB05543 (WHITE SEED COAT)			
CONTROL	15.40^{cd}	22.94^{bc}	42.94^{bcd}	59.40^{a}	101.68^{b}
W1 100G/25ppm	16.90^{d}	35.10^{d}	55.60^{de}	75.00^{b}	109.80^{c}
W2 200G/25ppm	13.64^{bc}	18.46^{ab}	35.14^{ab}	62.14^{a}	100.86^{b}
W3 400G/25ppm	11.76^{ab}	26.26^{c}	45.26^{bcd}	71.00^{ab}	107.76^{c}
W4 100G/50ppm	14.00^{bcd}	16.00^{a}	29.00^{a}	55.00^{a}	85.00^{a}
W7 100G/100ppm	11.00^{ab}	36.00^{d}	65.00^{e}	74.00^{b}	108.00^{c}
W8 200G/100ppm	9.14^{a}	16.48^{a}	39.68^{abc}	69.68^{ab}	108.48^{c}
W9 400G/100ppm	20.70^{e}	27.40^{c}	51.60^{cd}	76.40^{b}	115.80^{d}
Standard Errors	0.63	1.34	2.20	2.13	2.21
		NGB05522 (RED SEED COAT)			
CONTROL	14.40^{b}	21.60^{ab}	40.00^{a}	61.20^{a}	106.40^{a}
R3 400G/25ppm	17.20^{b}	29.60^{bc}	57.00^{bc}	83.00^{c}	130.00^{c}
R4 100G/50ppm	19.50^{b}	33.00^{c}	59.00^{bc}	85.00^{c}	143.00^{d}
R5 200G/50ppm	25.00^{c}	36.00^{c}	70.00^{c}	85.00^{c}	122.00^{bc}

R7 100G/100ppm	6.00^{a}	17.00^{a}	43.00^{a}	71.00^{ab}	100.00^{a}
R8 200G/100ppm	14.00^{b}	22.50^{ab}	48.00^{ab}	74.00^{bc}	108.00^{a}
R9 400G/100ppm	15.60^{b}	23.90^{ab}	49.00^{ab}	75.00^{bc}	117.40^{b}
Standard Errors	1.12	1.40	2.24	1.91	2.62

Values followed by the same letter(s) do not statistically differ at $p<0.05$, tested with DMRT
N.B: PH is Plant Height, WAS is Week after Sowing, NGB (NACGRAB). PPM (Part per Million)

Table 2: Combined Effects of Gamma Irradiation and Biosynthesized AgNPs on Number of Leaves per Plant of Selected Pigeon Peas Accessions

Treatments	NL 4 WAS	NL 6 WAS	NL 8 WAS	NL 10 WAS	NL 12 WAS
CONTROL	14.34ab	25.34ab	59.58bc	96.66a	379.20bc
W1 (100 g / 25 ppm)	26.60d	39.00c	82.60c	164.00bc	573.00c
W2 (200 g / 25 ppm)	18.88bc	21.20a	31.12a	66.32a	150.40a
W3 (400 g / 25 ppm)	22.50cd	34.76bc	52.26ab	123.50ab	360.76bc
W4 (100 g / 50 ppm)	13.00ab	22.00a	29.00a	72.00a	207.00a
W7 (100 g / 100 ppm)	36.00e	60.00d	117.00d	210.00c	894.00d
W8 (200 g / 100 ppm)	10.14a	20.26a	45.66ab	82.94a	142.38a
W9 (400 g / 100 ppm)	20.00bc	34.00bc	80.80c	161.80bc	530.80c
Standard Error	1.47	2.30	5.17	9.78	44.76
Treatments	NL 4 WAS	NL 6 WAS	NL 8 WAS	NL 10 WAS	NL 12 WAS
CONTROL	18.00bc	24.40b	44.80ab	88.40a	366.00a
R3 (400 g / 25 ppm)	17.60bc	31.80bc	64.60bc	119.60ab	454.00ab

R4 (100 g / 50 ppm)	10.00ab	50.00d	72.00c	193.00d	627.00c
R5 (200 g / 50 ppm)	27.00d	37.00c	84.00c	190.00d	790.00d
R7 (100 g / 100 ppm)	5.00a	14.00a	31.00a	194.00d	504.00abc
R8 (200 g / 100 ppm)	15.00bc	27.00b	68.00bc	149.00c	528.00bc
R9 (400 g / 100 ppm)	19.40c	24.20b	67.60bc	134.80b	478.20ab
Standard Error	1.47	2.04	3.83	8.99	27.06

Values followed by the same letter(s) do not statistically differ at $p<0.05$, tested with DMRT N.B: NL is Number of Leaves

The study has established that gamma irradiation is a potent physical mutagen in inducing variation among pigeon pea accessions. Muhammad et al. (2018) suggested that gamma rays are a potent mutagen, causing variation in the population of crop plants, with effects that can be either stimulatory or inhibitory. In most of the parameters taken, stimulatory effects were established. This observation could be due to the enhancement of certain hormone activities in the crop by AgNPs. It could also be as a result of the interaction of the nanoparticles with the plant hormone (auxin), which is responsible for cell elongation, especially at the tips of a plant. This finding aligns with Latif et al. (2017), who demonstrated that foliar application with various concentrations of silver nanoparticles enhanced the growth parameters of wheat plants. The 50 to 100 ppm of AgNPs could have modulated and reduced the inhibitory effects of the gamma rays in the crop. Najafi and Jamei (2014) found a significant increase in plant height and root fresh weight in mung beans in response to 50 ppm AgNPs. Nanoparticles have been reported to enhance total chlorophyll contents in some plants. Mazhar et al. (2022) reported that seed priming of canola plants with 75 ppm of calcium oxide nanoparticles increased the total chlorophyll content by 28.9% and the number of leaves by 16%. A probable increase in the chlorophyll contents of pigeon pea, coupled with the stimulatory effect of gamma irradiation, could have led to the positive increases in the plant height and number of leaves per plant.

CONCLUSION

In conclusion, gamma irradiation of pigeon pea seeds primed with silver Nanoparticles increased plant height and the number of leaves per plant. Where 400 Gray at 100 ppm and 100 Gray at 100 ppm tend to be the optimum dose in NGB05543 (White Coated seeds) that enhanced plant height and number of leaves per plant respectively, in NGB05522 (Red Coated seeds) the optimum doses tend to be 100 Gray at 50 ppm (for plant height) and 200 Gray at 50 ppm for number of leaves per plant.

ACKNOWLEDGEMENTS

The authors wish to acknowledge the Tertiary Education Trust Fund for sponsoring this study through theTETFUND Institutional-Based Research Intervention (IBRI) fund, 2024. RefNo. TETEFUND/FUTMINNA/2024/071.

REFERENCES

Bamidele, O. P. and Akanbi, C. T. (2013). Influence of gamma irradiation on the nutritional and functional properties of pigeon pea (*Cajanus cajan*) flour. *African Journal of Food Science*, 7(9): 285-290.

International Board for Plant Genetic Resources & International Crops Research Institute for the Semi-arid Tropics. (1993). *Descriptors for Pigeon pea (Cajanus cajan*(L.) Millsp.).Bioversity International.

Jaganathan, D., Ramasamy, K., Sellamuthu, G., Jayabalan, S., and Venkataraman, G. (2018). CRISPR for crop improvement: an update review. *Frontiers in Plant Science*, 9: 985.

Khalaki, M.A., Ghorbani, A. andMoameri, M. (2016). Effects of silica and silver nanoparticles on seed germination traits of *Thymus Kotschyanus*in laboratory conditions. *Journal of Rangeland Sciences*, 6(3): 221-230.

Latif, H. H., Ghareib, M., and Abu Tahon, M. (2017). Phytosynthesis of silver nanoparticles using leaf extracts from *Ocimum basilicum*and *Mangifira indica* and their effect on some biochemical attributes of *Triticum aestivum*. *Gesunde Pflanzen*, 69 (1): 39–46.

Mazhar, M. W., Ishtiaq, M., Maqbool, M., and Akram, R. (2022). Seed priming with calcium oxide nanoparticles improves germination, biomass, antioxidant defence and yield traits of canola plants under drought stress. *South African Journal of Botany*, 151 (1): 889–899.

Muhammad, M.L, Falusi, O. A., Daudu, O.A Y., Abubakar, A., Gado, A. A., Kolo, J. T. (2018). *Improvements of Some Crop Plants in Nigeria through Gamma Ray*. In: Status of Mutation Breeding of Crop Plants in Nigeria. Muhammad M.L (Ed).LAP Lambert Academic Publishing, Mauritius. Pp 18-38. http://repository.futminna.edu.ng:8080/jspui/handle/123456789/720.

Najafi, S., and Jamei, R. (2014). ‘Effect of silver nanoparticles and Pb (NO3)2 on the yield and chemical composition of mung bean (*Vigna radiata*)’. *Journal of Stress Physiology and Biochemistry*, 10: 316-325.

Niharika, N., Shikha, J., Pranav, K., and Vijay, D. (2016). Synthesis and characterization of silver nanoparticles via green route. *Korean Journal of Chemical Engineering*, 33 (10): 29902997.

Roychowdhury, R., and Tah, J. (2013). Mutagenesis—A potential approach for crop improvement. In *Crop Improvement*. Springer, Boston, MA. Pp 149-187.

Saxena, K.B., Choudhary, A.K., Saxena, R.K., and Varshney, R.K. (2018). Breeding pigeon pea cultivars for intercropping: synthesis and strategies. *Breeding Science*, 68(2): 159-167.