

Research Application Summary

Radio-sensitivity test of five cowpea genotypes

Lumorh, D. P.¹, Asare, P.A.¹, Adu, M.O.¹, Amenorpe, G.², Addy, S.N.T.T.³, Danquah, O.A.¹ & Afutu, E.¹

¹Department of Crop Science, School of Agriculture, College of Agriculture and Natural Sciences, University of Cape Coast, Cape Coast, Ghana.

²Nuclear Agriculture Research Centre (NARC), Biotechnology and Nuclear Agricultural Research Institute (BNARI), Ghana Atomic Energy Commission (GAEC), P.O. Box LG80, Legon, Accra, Ghana.

³Council for Scientific and Industrial Research, Crops Research Institute, Fumesua, Kumasi, Ghana

***Corresponding author:** dieudonne2k@gmail.com

Abstract

The productivity of cowpea is much less, when compared to other pulse crops because new varieties do not satisfy the consumer needs and the expected yield of farmers. Mutagenesis is one of the effective methods of crop improvement. To effectively administer an acute dose for mass irradiation, differential sensitivity of cowpea genotypes to gamma rays is required. This is a prerequisite for further improvement using mutagenesis. The objective of this study is to determine LD50 and RD50 of various genotypes to gamma rays (differential sensitivity to gamma rays). Five cowpea genotypes (Hansadua, WC-36, ACC122WxWC- IO, IT97K-819 and WC-10) were irradiated with gamma rays from 60Co source at Ghana Atomic Energy Commission (GAEC) at thirteen doses of from 0 - 1,200 Gy at 100 Gy intervals. Data were collected on germination, plant height, whole seedling weight, root weight, root length and shoot weight. The data were analyzed using Genstat version-12 to determine the LD50, RD50 and ANOVA. The lowest LD50 value was 531 Gy for Hansadua (most sensitive to gamma rays) and the highest of 903 Gy for ACC122WxWC-10 (most tolerant to gamma rays). The RD50 showed a positive relationship between the seedling height and gamma rays administered. It was observed that the response of cowpea genotypes to gamma rays differ.

Key words: Breeding, cowpeas, Ghana, mutagenesis

Resume

La productivité du niébé est beaucoup plus faible que celle des autres légumineuses parce que les nouvelles variétés ne répondent pas aux besoins des consommateurs et au rendement attendu par les agriculteurs. La mutagenèse est l'une des méthodes efficaces d'amélioration des cultures. Pour administrer efficacement une dose aiguë pour l'irradiation de masse, une sensibilité différentielle des génotypes de niébé aux rayons gamma est nécessaire. Il s'agit d'une condition préalable à toute amélioration ultérieure par mutagenèse. L'objectif de cette étude est de déterminer la DL50 et la RD50 de divers génotypes aux rayons gamma (sensibilité différentielle aux rayons gamma). Cinq génotypes de niébé (Hansadua, WC-36, ACC122WxWC- IO, IT97K-819 et WC-10) ont été irradiés avec des rayons gamma provenant d'une source de 60Co à la Commission de l'énergie atomique du Ghana (GAEC) à treize doses de 0 à 1 200 Gy à des intervalles de 100 Gy. Les données ont été recueillies sur la germination, la hauteur des plantes, le poids des semis entiers, le poids des

racines, la longueur des racines et le poids des pousses. Les données ont été analysées à l'aide de Genstat version-12 pour déterminer la DL50, la RD50 et l'ANOVA. La plus faible valeur de LD50 était de 531 Gy pour Hansadua (la plus sensible aux rayons gamma) et la plus élevée de 903 Gy pour ACC122WxWC-10 (la plus tolérante aux rayons gamma). Le RD50 a montré une relation positive entre la hauteur des semis et les rayons gamma administrés. Il a été observé que la réponse des génotypes de niébé aux rayons gamma est différente.

Mots clés : Panification, niébé, Ghana, mutagenèse.

Introduction

Cowpea (*Vigna unguiculata* L. Walp.) is a major grain legume widely cultivated in most tropical regions around the world, especially in a wide range of agro-ecological zones across Africa. It is a leguminous crop used as food, forage, and vegetable mainly in the tropics (Steele, 1972). It is an annual legume which is commonly referred to in Ghana as 'beans', ayi, adua, atedua, waakye, yoor, Ekye. The green leaves, green pods and grains provide dietary source of protein (Ghaly and Alkoaik, 2010). The cowpea grain contains 23% protein and 57% carbohydrate, and the leaves contain 27- 34% of proteins. Cowpea production remains one of the most prominent activities by farmers in most sub-Saharan African countries. The main reason for this prominence being the natural ability of the crop to withstand moderate levels of drought and its adaptation to nutrient limited soils and fixation of atmospheric nitrogen.

The productivity of cowpea is much less, when compared to other pulse crops. This is because most of the present-day varieties do not meet consumer satisfaction and expected yield of farmers. The crop is self-pollinated and the process of fertilization in most instances is complete before flower opens. Therefore, the inherent variability in this crop is very low. The available yields of cowpea cultivars in Ghana are considerably low. The average yield is 1.41 metric tons per hectare and the potential yield is 2.5 metric tons (MoFA, 2016). In addition to the need for high yielding varieties, there is also a need for lines which are resistant to major diseases like Fusarium wilt, Cercospora leaf spot, anthracnose and Pythium root rot. Artificial induction of genetic variation has been proposed as one of the quick and cheap methods of increasing genetic variability in crop plants (Gregory, 1955). This study was therefore conducted in a quest to evaluate the responses of some selected cowpea lines to different doses of gamma rays radiation.

Methodology

The study was conducted at the Biotechnology and Nuclear Agriculture Research Institute (BNARI) of Ghana Atomic Energy Commission. The study involved five cowpea genotypes consisting three introduced lines from Uganda (WC-10, WC-36 and ACC I 22Wx WC-10), one from IITA (IT97K-819) and an improved variety from Ghana (Hansadua).

Determination of optimum irradiation dose (LD50). Radio-sensitivity test was carried-out following the methods described by Mba et al. (2010) and Tshilenge-Lukanda et al. (2012) with some modification. Three hundred and thirty (330) seeds including control for each of the five genotypes were gamma irradiated making thirty (30) seeds per treatment for each genotype. Thirteen (13) irradiation doses of (0 Gy, 100 Gy, 200 Gy, 300 Gy, 400 Gy, 500 Gy, 600 Gy, 700 Gy, 800 Gy, 900 Gy, 1000 Gy, 1100 Gy and 1200 Gy) were used with three replications. The seeds were packed in separate zip-lock bags and placed in a desiccator for three days to attain the desired moisture level of about 8%.

The irradiated seeds were sown in 500 ml polybags filled with well mixed loamy soil under standard

cultural practice using a Completely Randomized Design. Data were collected on germination, seedling height, fresh whole plant weight, fresh root weight, root length and fresh shoot weight. Data on germination percentage and seedling height were analyzed using Genstat version-12 to generate linear equations for the five genotypes which led to the determination of the optimal lethal dose (LD50) for germination and emergence reduction (RD50) and analysis of variance (ANOVA) for the various traits measured.

Results and discussions

Figures 1, 2, 3, 4 and 5 show the radio-sensitivity curves for Hansadua, ACC122WxWC-10, WC-36, IT97K-819 and WC-10, respectively. Figure 6 shows the emergence reduction curve for the five varieties. The results indicated great differential responses of the five cowpea genotypes to gamma rays doses. There were significant gamma ray dose effects on the different parameters assessed in the study.

Table 1 shows LD50 values of the five cowpea genotypes. In decreasing order of values, LD50 of 903, 858.7, 762, 705 and 531 Gy were observed for ACC122WxWC-10, WC-36, IT97K-819, WC-10 and Hansadua, respectively.

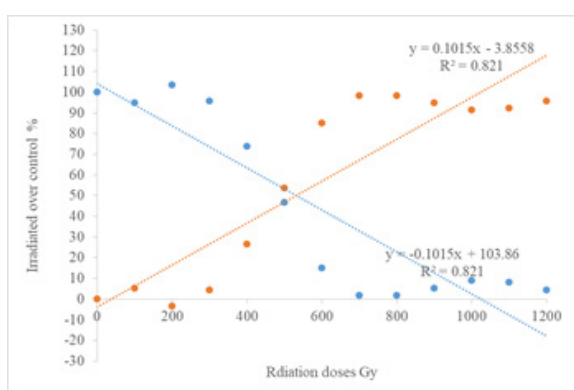


Figure 1. Radio-sensitivity curve of Hansadua

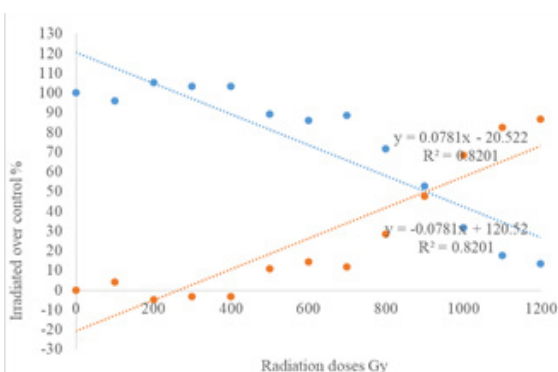


Figure 2. Radio-sensitivity curve of ACC:122WxWC-10

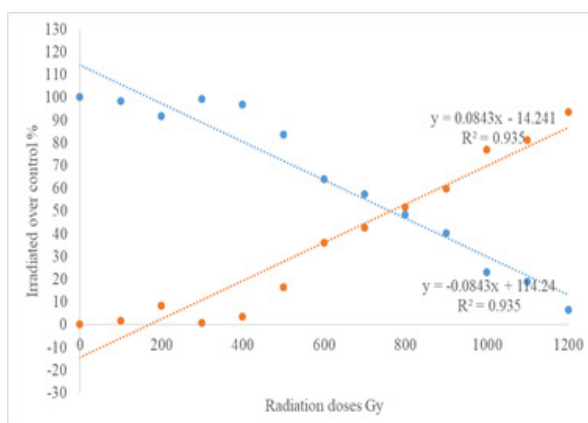


Figure 3. Radio-sensitivity curve of WC-36

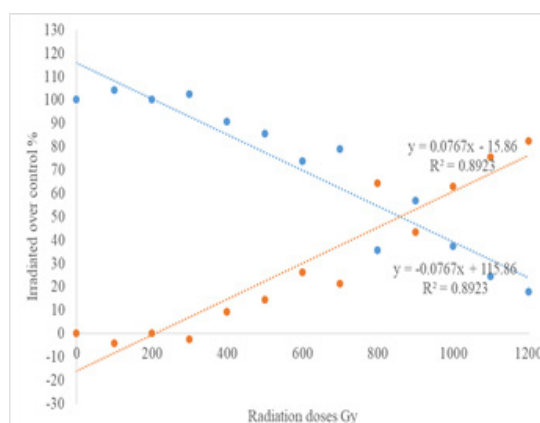


Figure 4. Radio-sensitivity curve of IT97K-819

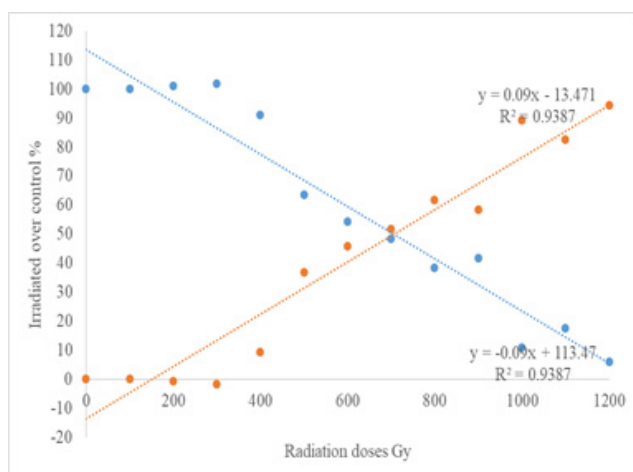


Figure 5: Radio-sensitivity curve of WC-10

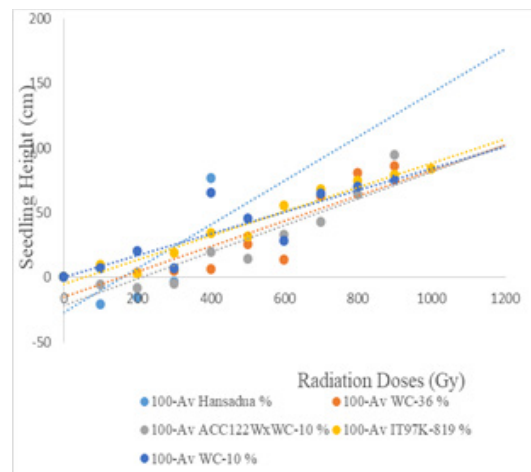


Figure 6: Emergence Reduction curve of five cowpea genotypes

The LD50 values observed were based on the seedling population during germination count, which later reduced during plant establishment. The reduction in population was due to radiation effect that led to reduction in growth and death of some of the induced plants days after germination. This corroborates earlier reports by Rajput *et al.* (2001) and Valli (2017) who also observed that higher radiation caused reduction in survival and growth of induced plants. This therefore, suggests the need to increase the number of seeds for mutagenesis to increase the chances of obtaining higher number of seeds surviving after the process of mutagenesis, as more seeds survive, the greater the chance of obtaining wider variability in the germplasm (FAO/IAEA, 2018) or better still the breeder should collect other forms of data such as epicotyl, hypocotyl, and seedling weight to help determine the actual percentage level to apply. The results of RD50 on the other hand showed a positive relationship between dose rates and seedling height.

Table 1. Values of LD50 of the five cowpea genotypes

Genotype/Optimum doses	LD50
Hansadua	531 Gy
WC-36	858.7 Gy
ACC122WxWC-10	903 Gy
IT97K-819	762 Gy
WC-10	705 Gy

Table 2 presents means of average germination percentages of different cowpea genotypes under different radiation doses. The results showed significant differences ($P < 0.001$) among radiation doses, genotypes and their interactions. Doses 0, 100, 200 and 300 Gy were observed to produce similar effects on germination. Germination in these treatments was significantly higher than doses 400, 500 and 600 Gy. Radiation doses at 700, 800, 900 and 1000 Gy significantly lowered germination values compared with the other doses suggesting that higher radiation doses reduced the germination of the cowpea genotypes used in the study.

This agreed with the results of the study done by Olasupo *et al.* (2016) on mutagenic effects of gamma radiation on eight accessions of cowpea. The response of genotypes to mutagen depends on biotype of plants (Ogbonnaya *et al.*, 2003). Dose rate and genotype interactions were significant ($P < 0.001$). The range of germination was 0.8 — 19.8% and 5.64 — 13.55% for dose rates and genotypes, respectively.

Highly significant ($P < 0.001$) differences were observed among genotypes on the basis of average germination percentage. Germination percentage of WC-36, ACC122WxWC-10 and IT97K-819 were similar but significantly higher than for the rest. Germination percentage of WC-10 was also significantly higher than for Hansadua. This agreed with the work of Abdel-Hady et al. (2008) who reported highly significant increase in seed germination percentage in *Atropa belladonna* due to the effect of gamma ray application.

Dose rates depend on genotypes. Dose rate and genotype interactions were highly significant ($P < 0.001$). The range of germination was 0.8 — 19.8% and 5.64 — 13.55% for dose rates and genotypes, respectively.

Table 2. Mean germination percentages for the different genotypes and radiation doses

Dose Rates (Gy)	Hansadua	WC-36	ACC122WXWC-10	IT97K-819	WC-10	Germination (%)
0	9	21	20	23	21	18.8 a
100	18	19	21	21	20	19.8 a
200	17	20	21	22	17	19.4 a
300	15	20	21	19	20	19 a
400	3	20	16	15	7	12.2 b
500	0	16	17	16	12	12.2 b
600	0	18	13	10	15	11.2 b
700	0	8	11	7	8	6.8 c
800	0	4	7	6	6	4.6 d
900	0	3	1	5	5	2.8 e
1000	0	0	0	4	0	0.8 f
Mean	5.64 c	13.55 a	13.45 a	13.45 a	11.91b	

LSD ($P = 0.05$) of germination means (1%) for dose rate, genotype and dose rate and genotype interaction were 1.4, 1.0 and 3.2 respectively.

Conclusion

The results of the study indicated marginal differences among the responses of the genotypes to gamma rays. This makes it necessary to carry out radio-sensitivity test on biotypes before embarking on mass radiation of the same. Hansadua was the most sensitive among the five cowpea genotypes while the most tolerant to gamma rays was ACC122WxWC-10. The germination percentage dropped from 100% (at 0 Gy, control treatment) to 2% at 700 Gy in Hansadua, 18% at 1000 Gy in WC-36, 13% at 1000 Gy in ACC122WxWC-10, 7% at 1000 Gy in IT97K-819 and 6% at 1000 Gy in WC-10.

Acknowledgments

We are grateful to the Carnegie Corporation of New York and the Regional Universities Forum for Capacity Building in Agriculture (RUFORUM) for funding this research through the Carnegie Post-Doctoral Fellowship Regional Training Programme (RU/2018/POST DOC RTP/01) offered to Dr. E. Afutu. This paper is a contribution to the Fifteenth RUFORUM Annual General Meeting held 2-6 December 2019 in Cape Coast, Ghana.

References

- Abdel-Hady, M. S., Okasha, E. M., Soliman, S. S. A. and Talaat, M. 2008. Effect of gamma radiation and gibberellic acid on germination and alkaloid production in *Atropa belladonna* L. *Australian Journal of Basic and Applied Sciences* 2 (3): 401-405.
- FAO/IAEA. 2018. Manual on Mutation Breeding - Third edition. Spencer-Lopes, M.M., Forster, B.P. and Jankuloski, L. (Eds.), Food and Agriculture Organization of the United Nations. Rome, Italy.
- FAOSTAT, F. A. O. 2010. Agriculture. 2006 [2010-01-18]. http://www.fao.org/inclex_zh.htm.
- Gregory, W. C. 1955. X-Ray breeding of peanuts (*Arachis hypogaea* L.) 1. *Agronomy Journal* 47 (9): 396-399.
- Mba, C., Afza, R., Bado, S. and Jain, S. M. 2010. Induced mutagenesis in plants using physical and chemical agents. *Plant Cell Culture: Essential Methods* 20: 111-130.
- Ministry of Food and Agriculture. Ghana. 2016. Facts and Figures 2016. Ministry of Food and Agriculture, Accra, Ghana.
- Olasupo, F. O., Ilori, C. O., Forster, B. P. and Bado, S. 2016. Mutagenic effects of gamma radiation on eight accessions of cowpea (*Vigna unguiculata* [L.] Walp.). *American Journal of Plant Sciences* 7 (2): 339-351
- Ogbonnaya, C. I., Sarr, B., Brou, C., Diouf, O., Diop, N. N. and Roy-Macauley, H. 2003. Selection of cowpea genotypes in hydroponics, pots, and field for drought tolerance. *Crop Science* 43 (3): 1114-1120
- Rajput, M. A., Khan, Z. H., Jafri, K. A. and Fazal, A. 2001. Genetic improvement of Sesamum indicum through induced mutations (No. IAEA-TECDOC-1195).
- Tshilenge-Lukanda, L., Funny-Biola, C., Tshiyoyi-Mpunga, A., Mudibu, J., Ngoie-Lubwika, M., Mukendi-Tshibingu, R. and Kalonji-Mbuyi, A. 2012. Radio-sensitivity of some groundnut (*Arachis hypogaea* L.) genotypes to gamma irradiation: indices for use as improvement. *Biotechnology Journal International* 2 (3): 169-178.
- Valli, F. 2017. Physical mutagenesis in giant reed (*Arundo donax* L.) and phenotypic and genomic characterization of mutagenized clones. Doctoral Dissertation. University of Bologna, Italy.
- van Harten, A. M. 1998. Mutation Breeding: Theory And Practical Applications. Cambridge University Press.