

Research Application Summary

Physiological response of cowpea seed to ionising radiation

Nyarko, M. A.¹, Asare, P.A.¹, Adu, M.O.¹, Addy, S.N.T.T.², 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.

²Council for Scientific and Industrial Research, Crops Research Institute, Fumesua, Kumasi,
Ghana

***Corresponding author:** mishaen.nyarko@stu.ucc.edu.gh

Abstract

Fifteen (15) cowpea genotypes were radiated at five (5) doses of gamma radiation (0, 50, 100, 150 and 2000y). The experiment was conducted in the laboratory using the randomised complete block design with four replicates. Data were collected daily on the number of germinated seeds for 14 days and parameters such as first day to germination, germination percentage, coefficient of velocity of germination and others and these were analysed using the analysis of variance procedure in GenStat and means were separated using the least significant differences test at $P = 0.05$. The results showed that significant differences existed between the genotypes in germination parameters such as percentage germination, time to 50% germination, mean germination rate, germination index and coefficient of velocity of germination. However, the dose ranges had no effect on these parameters. Significant interaction effect ($P < 0.01$) observed between the cowpea genotypes and the radiation doses in terms of germination percentage and germination index suggests that there were differential responses to the irradiation doses among the genotypes. Overall, low doses of ionising radiation appear to have little or no effects on both the proportion and time measurements of germination parameters in cowpea genotypes.

Keywords: Germination, ionising radiation, mutation breeding, seed physiology, *Vigna unguiculata*

Resume

Quinze (15) génotypes de niébé ont été irradiés avec cinq (5) différentes doses de rayonnement gamma (0, 50, 100, 150 et 2000y). L'expérience a été menée en laboratoire en blocs complets randomisés avec quatre répétitions. Des données ont été collectées quotidiennement sur le nombre de graines germées pendant 14 jours et des paramètres tels que le premier jour de germination, le pourcentage de germination, le coefficient de vélocité de la germination et tant d'autres, et ceux-ci ont été analysés en procédant à l'analyse de la variance dans GenStat et les moyennes ont été séparées en utilisant le test des différences les moins significatives (LSD) à $p = 0,05$. Les résultats ont montré que des différences significatives existaient entre les génotypes s'agissant des paramètres de germination tels que le pourcentage de germination, le temps jusqu'à 50% de germination, le taux de germination moyen, l'indice de germination et le coefficient de vélocité de la germination. Cependant, les gammes de doses n'ont eu aucun effet sur ces paramètres. L'effet d'interaction entre les génotypes de niébé et les doses de rayonnement en termes de pourcentage de germination et d'indice de germination a été significatif ($p < 0,01$) suggérant qu'il y avait des réponses différentes aux doses d'irradiation parmi les génotypes. Dans l'ensemble, les faibles doses de rayonnement ionisant semblent avoir peu ou pas d'effets sur les mesures à la fois du temps et de la proportion des paramètres de germination chez les génotypes de niébé.

Mots clés : Germination, radiation ionisante, sélection par mutation, physiologie des graines, *Vigna unguiculata*

Introduction

Cowpea (*Vigna unguiculata* (L) Walp) ($2n=2x=22$), a member of the Fabaceae family has been identified to have the potential to feed sub-Saharan Africa's malnourished population with cheap protein source (Boukar *et al.*, 2018). This attribute has contributed greatly to its nickname; the "poor man's crop" (Dube and Fanadzo, 2013). It is a prominent crop in the nutrition of sub-Saharan Africans and its cultivation also improves soil fertility (Yirzagla *et al.*, 2016). This makes cowpea a potential crop for leveraging Africa's basic nutrition especially protein requirements. People consume different parts of the cowpea plant; the immature and young pods, leaves and grains are consumed in most parts of sub-Saharan Africa (Akpan and Mbah, 2016).

Cowpea production is popular in the tropics (Timko and Singh, 2008). There is a vibrant market for cowpea in and out of the West African sub region and this has greatly contributed to employment, poverty alleviation and revenues to governments (Langyintuo *et al.*, 2003). Cowpea is a versatile African crop because of its uses as food, feed and as soil amendment (Mshelmbula *et al.*, 2019) and hence, it can be described as a food security crop (Dube and Fanadzo, 2013) in these times of climate change.

Inducing genetic variability in plants has been made simple with the introduction of mutagenic agents; physical and chemical (Horn *et al.*, 2016). Optimal mutagenic treatment has been used for creating new genetic variability in plant propagules such as seeds, tissues and organs (Horn *et al.*, 2016). Sometimes, lower levels of radiation exposure can only cause morphological aberrations in phenotypes (Amjad and Anjum, 2002). Mutation can be spontaneous or induced artificially to produce large genetic variability in a short time interval. This evolutionary change in plant genetic research is potent for enhancing variability for crop improvement (Girija *et al.*, 2013) and has been a significant breakthrough in genetics. Seed germination characteristics have been reported to be influenced by exposure of seeds to ionising radiation (Amjad and Anjum, 2003).

Germination test is one of the most effective ways of predicting the field establishment and the performance (yield and quality) of farmers' harvests (ISTA, 2011). High quality seeds have faster, better and more uniform establishment on the field (FAO and AfricanSeeds, 2018). In angiosperms, germination is confirmed when the radicle or plumule emerges from the seed coat (Kader, 2005). The germination dynamics (proportions and rates) of a seed lot is influenced by the seed's complex physiological mechanisms as well as environmental conditions (ISTA, 2011). The measurement of germination properties of a seed lot is essential for seed quality assessments (FAO and AfricanSeeds, 2018). The objective of this experiment was to assess the impact of different doses of ionising radiation on seed physiological attributes/parameters of cowpea genotypes.

Methodology

Sampling and radiation. About 2000 seeds of each genotype were sampled. These were further allotted into 400 seeds replicates to be radiated according to genotype. The doses for radiation were 50, 100, 150 and 200Gy including the control, 0Gy. The radiation was carried out at the Biotechnology and Nuclear Agricultural Research Institute (BNARI) of the Ghana Atomic Energy Commission (GAEC). The dose rate for the gamma irradiation was 330Gys⁻¹.

Cowpea genetic materials. Fifteen (15) genotypes of cowpea were used for the experiment (Table 1). These genotypes included three improved varieties from Ghana, three inbred lines from the

International Institute for Tropical Agriculture (IITA, Nigeria) and nine genotypes from Uganda. The genotypes were selected based on their differences in yield, seed and growth habit as well as the source.

Table 1. List of cowpea genotypes and their characteristics

Genotype	Yield(t/ha)	Seed colour	Growth habit	Country	Cultivar type
Asontem	1662.688	Red	semi-erect	Ghana	Improved
Hansadua	1009.413	White	semi-erect	Ghana	Improved
Soronko	1760.524	Red	semi-erect	Ghana	Improved
IT889	1596.249	Mottled	semi-erect	IITA	Inbred line
IT91	1868.884	Brown	semi-erect	IITA	Inbred line
IT97K819	1694.153	Brown	semi-erect	IITA	Inbred line
ACC122W*NE48	1768.275	Brown	semi-erect	Uganda	Inbred line
ACC122W*NE51	1729.894	Brown	semi-erect	Uganda	Inbred line
ACC122W*WC10	1986.81	Brown	semi-erect	Uganda	Inbred line
ACC122W*WC36	2662.519	Black	semi-erect	Uganda	Inbred line
Ebelate	1901.233	Brown	semi-erect	Uganda	Inbred line
F258T2E	1700.164	Brown	Erect	Uganda	Inbred line
H24	2111.407	Black	semi-erect	Uganda	Inbred line
NE21	2356.228	Mottled	semi-erect	Uganda	Landrace
WC10	1539.413	Brown	semi-erect	Uganda	Landrace

Substrate, growth conditions and sowing. Sea sand was conditioned and used as the substrate. The experiment was laid out in a randomised complete block design and data on the number of seeds which germinates daily were taken over a 14 day period (ISTA, 2010) and used to estimate percentage germination and other derivatives of germination.

Data analysis. Data were analysed using the analysis of variance (ANOVA) procedure GenStat Release 12.IDE, Discovery Edition 4, 2016 (VSN International Limited, Rothamsted Experimental Station, Hemel Hempstead, UK). Differences between the treatments were compared by least significant difference (LSD) test at $P = 0.05$.

Results and discussions

The days to first germination for most of the genotypes were on the 3rd day after sowing. There were no significant differences in the effect of the doses on the days to first germination among the genotypes (Table 2). On the other hand, there were significant differences ($P < 0.01$) between the genotypes for percentage germination, time to 50% germination, mean germination time, mean germination rate, coefficient of velocity of germination and germination index (Table 3). This could be attributed to the differences in plant qualities and provenance effects ((Basra, 1995). These differences could also be attributed to the differences in the physical attributes such as seed size and seed coat thickness of the genotype. Seed size and seed coat thickness have been reported to influence imbibition in seeds and subsequently the elongation of the embryonic axes (radicle or plumule) to complete the germination process (Liu *et al*, 2013). The doses ($P > 0.05$) did not differ significantly ($P > 0.05$) in influencing day to the first germination and last day to germination. Day to first or final germination give an indication of how slow or fast the germination process took

between the treatments (Kader, 2005). The percentage germination was not significant ($P > 0.05$) among the doses but was significant ($P < 0.01$) for days to 50% germination between the genotypes. This suggests that the range of radiation used was too low to be able to kill the embryo but could affect the rate and trend of germination in cowpea (Bind and Dwivedi, 2014; Gwata *et al.*, 2016; Dhanavel and Girija, 2019). The significant interaction ($P < 0.01$) between the cowpea genotypes and the radiation doses of the germination percentage and germination index implies that there were differential responses to the irradiation doses among the genotypes but this was influenced by the radiation dose, as reported by Gwata *et al.* (2016).



Figure 1. Seed trays showing how drills were made and seeds sown



Figure 2. Seed trays covered with polythene sheet to minimize evaporation.

Table 2. Germination parameters taken on cowpea genotypes radiated at five different doses

Dose	Parameters								
	Days to First Germination (day)	Days to Last Germination (day)	Time Spread of Germination (day)	Time to 50% Germination (%)	Germination Percentage (%)	Mean Germination Time (day)	Mean Germination Rate	Coefficient of Velocity of Germination (%)	Germination Index (day)
0	3.04	5.8	2.76	3.25	78.00	3.85	0.26	26.21	10.67
50	2.98	5.98	3.00	3.31	80.00	3.89	0.26	25.86	10.87
100	3.02	6.33	3.31	3.31	79.00	3.92	0.26	25.76	10.60
150	3.04	6.29	3.24	3.39	79.00	3.97	0.26	25.52	10.62
200	3.00	6.00	3.00	3.32	80.00	3.92	0.26	25.79	10.78
L.s.d.	0.673	0.4857	0.3646	0.07	3.80	0.133	0.0077	0.766	0.602

Data are the means of four replicates

Table 3. ANOVA for the cowpea genotypes and radiation doses

Source of variation	Parameters								
	Days to First Germination (day)	Days to Last Germination (day)	Time Spread of Germination (day)	Time to 50% Germination (%)	Germination Percentage (%)	Mean Germination Time (day)	Mean Germination Rate	Coefficient of Velocity of Germination (%)	Germination Index (day)
Genotype	0.117	0.841	0.815	0.238*	6.60**	0.23	.0133**	1.326**	1.042**
Dose	0.673	0.4857	0.3646	0.07	3.80	0.133	0.0077	0.766	0.602
Genotype × Dose	0.261	1.881	1.823	0.53	14.80**	0.514	0.0297	2.966	2.33*

* shows significance treatment efforts at $P = 0.05$.

Conclusions

In a suitable medium for cowpea seed, lower dose of ionizing radiation (<200Gy) has no effect on days to first germination, days to last germination, germination percentage, time to 50% germination, germination index, mean germination time, mean germination rate and coefficient of velocity of germination. However, cowpea genotype has significant influence on the germination percentage, time to 50% germination, mean germination rate, coefficient of velocity of germination and germination index. The genotype-dose interaction significantly affects germination percentage and germination index.

Acknowledgment

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 Program (RU/2018/POST DOC RTP/01) offered to the last author. This paper is a contribution to the Fifteenth RUFORUM Annual General Meeting held 2-6 December 2019 in Cape Coast, Ghana.

References

- Akpan, A.U. and Mbah, E.U. 2016. Growth and yield of vegetable cowpea varieties (*Vigna unguiculata* (L) Walp.) as influenced by potassium fertilizer rates in Umudike, Nigeria. *Nig. J. Agric. Food Environ* 12: 121-126.
- Amjad, M. and Anjum, M.A. 2002. Effect of gamma radiation on onion seed viability, germination potential seedling growth and morphology. *Pakistan Journal of Agricultural Sciences* 39: 202-206.
- Amjad, M. and Anjum, M.A. 2003. Effect of post-irradiation storage on the radiation-induced damage in onion seeds. *Asian Journal of Plant Sciences* 2 (9): 702-707. <https://doi.org/10.3923/ajps.2003.702.707>.
- Basra, A. S. 1995. Seed quality: basic mechanisms and agricultural implications. Food Products Press. Retrieved from <http://agris.fao.org/agris-search/search.do?recordID=US9551094>.
- Bind, D. and Dwivedi, V. K. 2014. Effect of mutagenesis on germination, plant survival and pollen sterility in M1 generation of cowpea [*Vigna unguiculata* (L.) Walp] *Indian Journal of Agricultural Research* 48 (5): 398-401. <https://doi.org/10.5958/0976-058X.2014.01322.5>.
- Boukar, O., Belko, N., Chamarthi, S., Togola, A., Batieno, J., Owusu, E., Haruna, M., Diallo, S., Umar, M.L., Olufajo, O. and Fatokun, C. 2019. Cowpea (*Vigna unguiculata*): Genetics, genomics and breeding. *Plant Breeding* 138 (4): 415-424. <https://doi.org/10.1111/pbr.12589>.
- Boukar, O., Togola, A., Chamarthi, S., Belko, N., Ishikawa, H., Suzuki, K. and Fatokun, C. 2019. Cowpea [*Vigna unguiculata* (L.) Walp.]. *Advances in Plant Breeding Strategies: Legumes* 7: 201-228.
- Dube, E. and Fanadzo, M. 2013. Maximising yield benefits from dual-purpose cowpea. *Food Security* 5 (6): 769-779. <https://doi.org/10.1007/s12571-013-0307-3>.
- FAO & African Seeds. 2018. Seeds toolkit (Seed quality assurance). Bahri, H. and Mendez, G. D. (Eds.). Rome: The Food and Agriculture Organization of the United Nations and AfricaSeeds.
- Girija, M., Dhanavel, D. and Gnanamurthy, S. 2013. Gamma rays and EMS induced flower color and seed mutants in cowpea (*Vigna unguiculata* L. Walp). *Advances in Applied Science Research* 4 (2): 134-139.
- Gwata, E.T., Shimelis, H. and Matove, P. 2016. Potential of improving agronomic attributes in tropical legumes using two mutation breeding techniques in southern Africa. pp.71-85. In: *Alternative Crops and Cropping Systems*. Retrieved from <http://dx.doi.org/10.5772/62262>.
- Horn, L.N., Ghebrehiwot, H.M. and Shimelis, H. 2016. Selection of novel cowpea genotypes derived through gamma irradiation. *Frontiers in Plant Science* 7: 262-275. <https://doi.org/10.3389/fpls.2016.00262>.

- org/10.33891 fpls.2016.00262.
- ISTA. 2010. International Rules for Seed Testing. 1st ed. Bassersdorf: The International Seed Testing Association (ISTA).
- ISTA. 2011. ISTA Annual Meeting Conclusion. ISTA.
- Kader, M. A. 2005. A comparison of seed germination calculation formulae and the associated interpretation of resulting data. *Journal & Proceedings of the Royal Society of New South Wales* 138: 65-75.
- Langyintuo, A.S., Lowenberg-DeBoer, J., Faye, M., Lambert, D., Ibro, G., Moussa, B., Kergna, A., Kushwaha, S., Musk S. and Ntoukam, G. 2003. Cowpea supply and demand in West and Central Africa. *Field Crop Research* 82 (2-3): 215-231. [https://doi.org/10.1016/S0378-4290\(03\)00039-X](https://doi.org/10.1016/S0378-4290(03)00039-X).
- Liu, B., Liu, X.B., Li, Y.S. and Herbert, S.J. 2013. Effects of enhanced UV-B radiation on seed growth characteristics and yield components in soybean. *Field Crop Research* 154: 158-163. <https://doi.org/10.1016/j.fcr.2013.08.006>.
- Mshelmbula, B. P., Akomolafe, G. F., Thomas, T. L. and Ologundudu, F. A. 2019. Effect of varying uv irradiation on germination and growth of cowpea (*Vigna unguiculata* (L.) Walp). *FUDMA Journal of Sciences* 3 (2): 206-209.
- Timko, M. P. and Singh, B. 13. 2008. Cowpea, a Multifunctional Legume. *Genomics of Tropical Crop Plants* 1: 227-258. https://doi.org/10.1007/978-0-387-71219-2_10.
- Yirzagla, J., Atokple, I.D.K., Haruna, M., Kusi, F., Suguri, I. and Muntari, A. 2016. Scaling out cowpea production in Northern Ghana: Community seed production scheme. In Pan African Grain Legume and World Cowpea Conference. Livingstone, Zambia.