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# Evaluation of Growth Performance of Gamma- Irradiated Cowpea Seeds Challenged with Black Eye Cowpea Mosaic Virus (BICMV)

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## ABSTRACT

*A study was carried out to evaluate disease incidence, severity and growth parameters on gamma - irradiated cowpea seeds inoculated with BICMV in the screen house at the Niger State College of Agriculture, Mokwa. The treatments consisted of dose rates of 0, 300, 500 and 700 Gy inoculated and 0, 300, 500 and 700 Gy un-inoculated. The trail was laid out in complete randomized design (CRD) in three replicates. Results show that 100% disease incidence was obtained at 3WPI for 0 Gy inoculated and 60% incidence was obtained in 300 Gy, while 3.0 disease severity score was obtained in 0 Gy and 2.3 severity was recorded in 300 Gy compared to 1.6 score in 0 Gy un-inoculated at 5WPI. Average plant*

***heights of 33.9 cm at dose rate of 0 Gy in un-inoculated and 30.5 cm at 300 Gy from inoculated cowpea were recorded at 6WPI. Average leaf diameter was 5.5 cm at dose 0 Gy from un-inoculated and 4.5 cm at 300 Gy from inoculated cowpea. There were no significant differences in the number of leaves and branches. Thus, Gamma rays at dosage 300 Gy inoculated cowpea performed better compared to the other doses.***
***Keywords: Disease incidence and severity, dose rate, Gamma rays, growth performance and severity score.***

## INTRODUCTION

Cowpea (*Vigna unguiculata* L Walp) is a popular leguminous crop in Africa which is commonly called 'beans' in Nigeria and 'Neibi' in francophone countries (Dugje *et al*., 2009). The largest production is in the moist and dry Savanna of sub-Saharan Africa (SSA) where it is intensively grown as an intercrop with other cereal crops fallows (Ishoyaku *et al*., 2010, Abdullahi *et al*., 2019). Though it is grown in other parts of the world, Nigeria remains its largest producer and consumer in the world. According to FAO data (2001-2010) (FAO, 2012), Nigeria being the largest producer and consumer accounts for 61 % of the production in Africa and 58 % worldwide. Africa exports, and imports negligible amounts (International Institute of Tropical Agriculture (IITA, 2009). The major cowpea producing areas in Nigeria include Niger, Kwara, Borno and Adamawa States in the Northern part while Oyo, Ogun and Ondo also produce appreciable quantities in the Southern part of the country (Ehlers and Hall, 2009, IITA, 2013).

Cowpea is the major staple food crop in sub-Saharan Africa especially in the dry regions of West Africa (Dugje *et al*., 2009). The seeds are major sources of plant protein and cash income, the young leaves and immature pods are eaten as vegetables (Batiano, 2011). Cowpea plays an important role in providing soil nitrogen to cereal crops such as maize, millet, and sorghum when grown in rotation especially in areas where poor soil fertility is a problem (Timko *et al.*, 2007). Cowpeas also increase soil organic matter content and improve soil structure and after soil incorporation, the soil does not require high rate of nitrogen fertilization (IITA, 2005).

Cowpea plant is attacked by insect pests and bacteria, fungi and viruses pathogens as well as parasitic weeds *striga* and *Alectra*-choke at all stages of the plant growth (Institute of Agricultural Research (IAR), 2008).

Virus diseases are considered to be a major limiting factor for the production and productivity of legumes in the tropical and subtropical countries (Bashir *et al*., 2008). Virus infecting cowpea in Nigeria include *Cucumber mosaic virus* (CMV) genus Cucumovirus, *Cowpea aphid-borne mosaic virus* (CABMV) genus Potyvirus, *Cowpea mottle virus* (CPMoV) genus Carmovirus, *Cowpea mild mottle virus* (CPMMV) genus Carmovirus and *Blackeye cowpea mosaic virus* (BICMV) genus Potyvirus.

*Blackeye cowpea mosaic virus* (BICMV) was first reported on cowpea in the U.S in 1955 (Alegbejo, 2015). It was earlier thought to have restricted geographical distribution, but it is now known to occur not only in the US (Langham *et al.,* 2009), but in Kenya and Nigeria (Soyinka *et al.,* 2009). Local symptoms appear as large reddish lesions that spread along the veins, while systemic symptoms appear as severe mottle, mosaic, vein-banding, veinal-chlorosis, distortion and stunting of the plant. Disease symptoms vary with virus strain and host cultivar (Mamman *et al*., 2018). Bashir *et al.,* (2008) reported that transmission is through mechanical inoculation and non-persisting by the *Aphids cracivorer* Koch, *Agossypii* Glover and *Myzus persicae* Sulzer. It is also seed-transmitted (20-50 %) in cowpea. Climatic factors such as temperature and sunshine hours which influence aphid activity, the host of the virus and vectors may give rise to an epiphytotic if these conditions are favourable generally if periods of high rainfall and low relative humidity coincide with periods of low incidence of alate aphids and vice versa. Infection occurs more between four and ten weeks after germination and weather positively affects infectivity of aphids (Balogun *et al.,* 2011). Yield losses due to *Blackeye cowpea mosaic virus* vary from 10-85 % on individually infected plants and vary with time of sowing (Kareem and Taiwo, 2007).

The economical and more effective features of gamma rays due to their high penetration power helps in their wider application for the improvement of various plant species compared to other ionizing radiations. Gamma irradiation has a profound influence on plant growth and development by inducing genetical, cytological, biochemical, physiological and morphogenetic changes in cells and tissues depending on the levels of irradiation (Artk and Peaksen, 2006). The material and energy necessary for initial growth is available in the seed, but some stimulants are required to activate those substances already stored in the cotyledons. Thus, low doses of γ-radiation may increase the enzymatic activation and awakening of the young embryo, which results in stimulating the rate of cell division which enhance not only germination, but also vegetative growth.

The biological effect of gamma radiation is mainly due to the formation of free radicals by the hydrolysis of water, which may result in the modulation of an antioxidative system, accumulation of phenolic compounds and chlorophyll pigments. Gamma rays represent one of the important physical agents used to improve the characteristic and productivity of many plants (Jaywardena and Perris, 2016). Treatment of crop varieties with gamma radiation has been found to alter the germination and accumulation of proline content resulting in the development of stress and disease tolerant varieties, stimulating the rate of cell division, vegetative growth and enhancement in the crop yield. Doses of γ-radiation may increase the enzymatic activation and awakening of the young embryo which might have been infected with the *Blackeye cowpea mosaic virus*. Consequently the present study was, therefore, undertaken to evaluate the growth performance of gamma irradiated seeds challenged with *Blackeye Cowpea Mosiac Virus* (BICMV).

## MATERIALS AND METHODS

### Gamma irradiation

Irradiation of cowpea seeds was performed using a caesium-137 gamma source at the dose rate of 9.6 msv/hr in ambient conditions at the Centre for Energy and Research Training (CERT), Ahmadu Bello University, Samaru, Zaria, Nigeria. The doses of exposure were 300, 500, 700 and 0 Gy. The control cowpea seeds were not irradiated.

### Source of virus inoculum and maintenance

The BICMV inoculum previously identified by DAS-ELISA was obtained from the stock at the Department of Crop Production, Federal University of Technology (FUT), Minna. Cowpea leaf tissues infected with BICMV were preserved on non-absorbent cotton wool over the silica gels in airtight vial bottles. The virus was activated by propagating in 10-day old TVU 76 seedlings. This was accomplished by rubbing the upper leaf surface with virus extract after grinding BICMV-infected leaves in 1:10; w/v with inoculation buffer, at pH 7.2 made of 0.1M sodium phosphate dibasic, 0.1M potassium phosphate monobasic, 0.01M ethylene diamine tetra acetic acid and 0.001M L-cysteine per litre of distilled water as described by Kumar, (2009). Distilled water was applied to the inoculated plants in order to remove excess inoculum as described by Balogun, (2008). The five treatments: 300 GY, 500 Gy, 700 Gy, 0

Gy inoculated and 0 Gy not inoculated were performed under an experimental layout of complete randomized design (CRD) with three replications.

**Crop Establishment and Inoculation**

Seeds were sown directly in the pots arranged in CRD after dressing with Apron plus in mid-July 2017. The seedlings were mechanically inoculated with the virus at 10 days after sowing (DAS). Grinding and inoculation procedure was accomplished as indicated above and weeds in the pots were controlled manually by hand pulling.

**Data collected**

From each pot, randomly tagged plant was used for periodic observations of the vegetative growth as described below:

**Plant height**

Plant height of the randomly selected plants were measured using metre rule from the base of the plant to the apex of the stem at 3 and 6 weeks after post inoculation (WPI).

**Number of leaves per plant**

The number of leaves per plant were counted and recorded from the tagged plants at 3 and 6 WPI.

**Number of branches per plant**

The number of branches per plant was determined from the tagged plants at 6 WPI.

**Leaf diameter per plant**

The leaf diameter of five leaves from the tagged plants was done by measuring the length of and breath of each leaf at 6 WPI and multiplying by a factor and the figure recorded

**Disease incidence:** Disease incidence was assessed as percentage of inoculated plants showing virus disease symptoms for the first three weeks after inoculation (WAI) as follows:

Disease incidence = $\frac{Number\ of\ infected\ plants\ in\ a\ plot}{Total\ number\ of\ plants\ in\ a\ plot}\ X\ 100$

**Disease severity:** Disease severity was also recorded based on 1 – 5 visual scale as described by Arif and Hassan (2002). On the scale: 1 = no symptoms or apparently healthy plants; 2 = slightly mosaic leaves (10 – 30 %); 3 = mosaic (31 – 50 %) and leaf distortion; 4 = severe mosaic (51 – 70 %), leaf distortion and stunting; 5 = severe mosaic (>70 %), stunting and death of plants.

**Data Analysis**

Data collected was subjected to analysis of variance (ANOVA) and treatment means separated using Duncan Multiple Range Test (DMRT) at 5 % level of probability.

## RESULTS AND DISCUSSION

**Disease incidence and severity assessment of gamma irradiated seeds challenged with BICMV**

Table 1 shows the incidence and severity of BICMV disease of gamma irradiated cowpea seeds. The result show that at week 1 WPI, cowpea seeds irradiated with 300 and 700 Gy recorded the highest percentage incidence of 60.0%, while the lowest disease incidence was in 0 Gy un-inoculated with 31.0%. In week 2, the lowest incidence was in 0Gy un-inoculated with 40.3% and the highest disease incidence recorded on 0 Gy inoculated was 80.0%. Similarly, at week 3 post inoculation 0 Gy inoculated had higher disease incidence of 100% and 0 Gy un-inoculated recorded the least disease incidence of 47.6%.

**Table 1. Disease incidence of gamma irradiated seeds challenged with BICMV at different periods.**

| Rates of gamma irradiation | | | |
|---|---|---|---|
| 300 inoculated<br>500 inoculated | 60.0[a]<br>46.6[ab] | 73.3[ab]<br>66.6[ab] | 60.0[b]<br>66.6[b] |
| 700 inoculated | 60.0[a] | 60.0[b] | 53.3[b] |
| 0 gy inoculated | 53.3[ab] | 80.0[a] | 100[a] |
| 0 gy un-inoculated | 31.0[b] | 40.3[c] | 47.6[b] |
| SE ± | 8.29 | 4.75 | 8.34 |

Means within column that share the same letter are non-significant at p ≤0.05
using Duncan's Multiple Range Test.

WPI=weeks after inoculation, DI= disease incidence, WPI= weeks post inoculation

**Disease severity**

At week three, there was no significant difference among the treatment except 0 Gy un-inoculated with 1.0 score and at week five there is significant different with 0 Gy inoculated recording the highest score of 3.0 and 0 Gy inoculated recording the lowest score of 1.6 but there were no significant differences within the treatment combinations.

**Table 2. Disease severity of gamma irradiated seeds challenged with BICMV at different periods.**

| Rates of gamma irradiation | 1WPI | DS 2WPI | DS 3WPI | DS 4WPI | DS 5WPI |
|---|---|---|---|---|---|
| 300 Inoculated | 1.6[bc] | 2.5[a] | 2.5[a] | 2.3[b] | 2.3[b] |
| 500 inoculated | 1.4[c] | 2.6[a] | 2.6[a] | 2.4[b] | 2.3[b] |
| 700 inoculated | 1.8[a] | 2.5[a] | 2.8[a] | 2.3[b] | 2.4[b] |
| 0 gy inoculated | 1.7[ba] | 2.7[a] | 3.0[a] | 2.9[a] | 3.0[a] |
| 0 gy un-inoculated | 1.0[d] | 1.5[b] | 1.6[b] | 1.6[c] | 1.6[c] |
| SE ± | 0.07 | 0.10 | 0.15 | 0.09 | 0.04 |

Means within column that share the same letter are non-significant at p = 0.05 Using Duncan's Multiple Range Test.
DS= disease severity, WPI= weeks post inoculation

**Plant height (cm)**

The result showed that there was significant difference among the treatments. It showed significantly in which 0 Gy un-inoculated at 3 and 6 WPI as the higher plant heights with 17.6 and 30.5 cm as compared to 500 Gy inoculated with 13.4 and 22.4 cm which were the shorter plant heights among the treatments combination.

**Number of leaves**

There was no significant difference in the number of leaves at 6 WPI, but at 3 WPI showed significance in which higher number of leaves of 12.6 among treatments combination was recorded with 0 Gy un-inoculated (Table 3).

**Number of branches and leaf diameter**

The result presented in Table 3 also shows that the number of branches did not differ significantly at 6WPI, while there were significant differences in leaf diameter at 6WPI among the treatment combinations with 0 Gy un-inoculated recording the highest leaf diameter of 5.5 cm as compared to 700 Gy inoculated which recorded the least leaf diameter of 3.8 cm.

**Table 3. Plant height, number of leaves, leaf diameter and number of branches per plant as influenced by Gamma Irradiated cowpea seed at 3WPI and 6WPI inoculated with BICMV.**

| Rates of gamma irradiation | Plant height at 3 WPI | Plant height at 6 WPI | Number of leaves at 3WPI | Number of leaves at 6 WPI | Leaf diameter at 6 WPI | Number of branches at 6WPI | Number of Branches at 6WPI |
|---|---|---|---|---|---|---|---|
| 300 Inoculated | 17.6$^{ab}$ | 30.5$^{ab}$ | 10.0$^{b}$ | 20.3$^{a}$ | 4.0$^{bc}$ | 7.0$^{a}$ | |
| 500 inoculated | 13.4$^{c}$ | 22.4$^{c}$ | 10.0$^{b}$ | 19.6$^{a}$ | 3.8$^{c}$ | 6.6$^{a}$ | |
| 700 inoculated | 15.0$^{bc}$ | 27.4$^{b}$ | 9.0$^{b}$ | 18.3$^{a}$ | 4.5$^{b}$ | 6.0$^{a}$ | |
| 0 gy inoculated | 16.3$^{b}$ | 29.2$^{a}$ | 9.0$^{b}$ | 22.0$^{a}$ | 5.5$^{a}$ | 7.6$^{a}$ | |
| 0 gy un-inoculated | 20.1$^{a}$ | 33.9$^{a}$ | 12.6$^{a}$ | 22.0$^{a}$ | 7.0$^{a}$ | 7.3$^{a}$ | |
| SE ± | 0.84 | 1.50 | 0.53 | 1.67 | 0.16 | 0.62 | |

Means within column that share the same letter are non-significant at p ≤0.05 using Duncan's Multiple Range Test.
WPI= weeks post inoculation.

The response of gamma irradiated cowpea seed (*V. unguiclata*) inoculated with BICMV at 10 DAS on disease incidence and severity and growth parameter shown in Tables 1- 4 indicate that one hundred percent (100%) incidence was obtained at 3WPI in un-irradiated plants but inoculated. The irradiated plant recorded least virus incidence with no significant difference among the doses at 3 WPI. Gamma irradiation, particularly from cobalt-60 is used as an effective non-chemical treatment to sterilise agricultural commodities in order to manage losses elicited by various diseases (Chu *et al*., 2015; Hallman, 2011; Mostafavi *et al*., 2010). During irradiation, high energy photons are emitted from an isotope source like cobalt-60 throughout the object (Choi and Lim, 2015; Mostafavi *et al*., 2011).

The result shows that gamma irradiation significantly impacted on the severity of BICMV infected cowpea plants. There were no significant differences at 1WPI and 2WPI also at 4WPI and 5WPI which may be due to the response of the plants to the environmental condition. However, in between the treatments there were significant differences at 1 WPI 700 Gy with severity score of 1.8 and at 5WPI 0 Gy inoculated recorded severity score of 3.0. This was the highest disease severity at that period, while the 0 Gy un-inoculated plants recorded the lowest disease severity throughout the period of the study.

The result of the growth parameters showed that plant height differed significantly at 3WPI and 6WPI. The control recorded taller plants of 20.1 cm and 33.9cm followed by 300 Gy inoculated plants with the heights of 17.6 cm and 30.5 cm at 3 and 6WPI respectively. At 3WPI the number of leaves per plant differed significantly among the irradiated treatments with the 0 Gy un-inoculated recording the highest leaf number of 12.6, at 6 WPI. There were no significant differences among the treatment combinations for the number of branches and leaf diameter at 6WPI 0 Gy un-inoculated plants recording 5.5 cm leaf diameter which was the widest. These results support the work of Horn and Shimelis (2013), who reported that low gamma radiation treatment can be used to stimulate vegetative growth and plant vigour of cowpea. Thus, the reduction in leaf diameter, leaf number and plant height of the test cowpea plants as compared to the control in the irradiated seed treatments can be due to the infection by BICMV. The present study has, therefore, provided information that BICMV infected cowpea treated with low doses of gamma irradiation can be savaged and planters of such seeds can still get good harvest.

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