

Influence of Indole-3-Acetic Acid treated Cowpea (*Vigna unguiculata* L. Walp) Seed on germination, plant development and leaf Chlorophyll Content

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Abstract

Plant growth regulators (PGRs) are natural hormones found in plant or their synthetic hormone analogues. Auxin, a type of PGRs that consist of low molecular weight organic phytohormones which influences cell division, plant growth and development. One of the common auxin types is indole-3-acetic acid (IAA) and was the first plant growth regulator to be discovered with characteristics that involved in the control of various plant growth and development. Auxins can be absorbed in tissues and transport to long distances throughout vascular tissue of a plant. Therefore, this study aimed to investigate the influence of IAA on the treated seeds of cowpea (*Vigna unguiculata* L. Walp) accession TVu-7, TVu-9 and TVu-1945 to 50 % seed germination, seedling growth and development, and leaf chlorophyll content. The various IAA concentration used were 0mg/L, 250mg/L, 500mg/L, 750mg/L and 1000mg/L. The results of this study showed the relative effect of various concentrations of IAA on seed germination, vegetative structure produced and leaf chlorophyll content. For example, increased IAA concentrations of 1000mg/L further extend the number of days to attend 50 % seed germination in the different cowpea accessions. Also, an increased concentration to 1000mg/L significantly increased the leaf chlorophyll content in the different cowpea accessions.

Keywords: cowpea accessions, IAA, growth, chlorophyll

1 Introduction

Cowpea (*Vigna unguiculata*) is one of the important food crop plants with a source of high protein content utilized by many people of sub-Saharan Africa (Lonardiet al., 2019; Singh et al., 2003). In some region of the semi-humid tropics, cowpea provides more than half the protein in the human diet (Dadson et al., 2005). Because cowpea cereal content about 63% carbohydrates, 25% proteins with as low as 1.5% fat content, and the presence of vitamins, mineral, folate, thiamin and riboflavin (Xionget al., 2016). In cowpea both the seeds and leaves are edible and cowpea is an affordable source of high-quality plants protein (Zhang et al., 2004). Despite these numerous benefits of cowpea, several attempts have been made to improve the production of the plants, hence its production is still not adequate to satisfy its demand within Sub-Saharan Africa (Horn & Shimelis (2020). Phytohormones or plant growth regulators (PGRs) are organic substances that control the activity of various plants growth and development. Synthetic auxin a type of phytohormone that is characterized by biological active biochemical molecules with an effect that is more or equal to that of the endogenous hormones (Gaspar et al., 1996). Different studies have shown the influence of PGRs on plant growth and development. One of the important auxin type Indole-3-Acetic Acid (IAA) plays a key role in plant cell division, elongation, fruit development and senescence (Phillips et al., 2011; Ogunkanmi, 2006). IAA, a PGR that shown to be active in most of the bioassay studies using plants at a minuscule amount (Woodward & Bartel, 2005), while plant materials have the potential to store exogenous amount of PGRs as conjugates and released when the plant is in need for normal growth and development (Davies, 2010). Some PGRs such as gibberellic acid (GA3) have shown to induced significant changes in the growth performance, chemical composition, and yield of Mungbean (*Vigna radiate*) and tomato plant (Choudhury et al., 2013; El Karamany et al., 2019). It is important to understand what effect auxin have on the treated seed of cowpea. Because this may help in the knowledge of breeding new varieties with better quality traits.

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Therefore, this investigation aimed to study the effect of Indole -3- Acetic Acid treated cowpea seeds on the plants performance and the chlorophyll content. The objective is to evaluate: (1) The influence of Indole -3- Acetic Acid treated seeds on the germination percentage, (2) Effect of Indole -3- Acetic Acid treated seeds on the seedling development and leaf chlorophyll content.

2 Materials and methods

2.1 Experimental site, soil collection, and seed material

A pot study was carried out on the research site of the Botanical Garden of the Federal University of Lafia, Nasarawa State, Nigeria. The experimental soil was collected from a soil depth of 0-25cm using a soil shovel at the Botanical Garden of the Federal University of Lafia, Nasarawa State. The texture of the soil was characterized as sand loamy soil. Three (3) different varieties of seeds of cowpea accessions were obtained from the International Institute for Tropical Agriculture (IITA), Ibadan, Nigeria. The accessions include TVu-7, TVu-9 and TVu-1945.

2.2 Plant Growth Regulators (IAA) and Pot Experiment

Indole-3-acetic acid (IAA) concentrations were prepared as follows 250, 500, 750 and 1000mg/L respectively. The various concentrations were dissolved in 1 liter of sterile distilled water while the control used was sterile distilled water only. The three (3) different varieties of seeds of cowpea accessions were soaked for 2 hours in Petri dishes containing the various prepared concentrations of IAA solution. Then three (3) seed of the treated IAA and control were sown directly into each of the 0.7 kg soil filled in polythene bags. The experiment was randomly arranged in the research site of the Botanical Garden of the Federal University of Lafia, Nasarawa State, at a spacing of 60 x 30cm as proposed by Okeleye *et al.* (1999). The polythene bags were labelled based on the presence of treated IAA seed concentrations sown and the control. Thereafter, a water spray bottle was used for daily watering in the morning and evening at nine hours interval till the day of harvest. Plants were sprayed with 0.1mL/L of Kombat E557 Cypermethrin insecticides at 6 weeks after planting to control insect attack. Germination % was examined and expressed as the number of days to 50% germination. Then, plants were assessed for morphological characteristics and development at 8 weeks after germination, and these included: number of leaves, total leaflet area. At plant maturity, the number of pod per plant, and the number of flowers per plant were also examined (Mshelmbula *et al.*, 2012).

2.1.2 Chlorophyll content determination

Leaf samples of four (4) weeks old germinated plant of the Indole-3-acetic acid (IAA) treated seeds and control of the different cowpea accessions used were collected randomly and homogenized in a mortar in acetone. The extract was centrifuged at 5000g for 5 min. The absorbance of the supernatant was recorded at 470 nm, 647 nm and 663 spectrophotometrically (Jenway 6305, England). Chlorophyll (Chl) content was determined following the method of Arnon (1949) and Lichtenthaler (1987) with modification. The Beer-Lambert equations were used to determine the concentrations of Chlorophyll a, total Chlorophyll in the leaf extract as follows:

$$\text{Chlorophyll a } (\mu\text{g/ml}) = 12.25A_{663} - 2.79A_{647} \dots\dots\dots \text{Eqn. 1}$$

$$\text{Chlorophyll b } (\mu\text{g/ml}) = 21.50A_{647} - 5.10A_{663} \dots\dots\dots \text{Eqn. 2}$$

$$\text{Total Chlorophyll} = \text{Chlorophyll a } (\mu\text{g/ml}) + \text{Chlorophyll b } (\mu\text{g/ml}) \dots\dots\dots \text{Eqn. 3}$$

2.1.9 Statistical Analysis

All data obtained were analysed using the SPSS version 26.0 which were subjected to one-way analysis of variance (ANOVA) and the graphs were plotted with GraphPad Prism 9. All the results were presented as mean \pm standard error of mean and their significant differences determined by LSD at ($P < 0.05$).

3 Results and Discussion

The results in this study showed relative variability in the number of days to 50% germination of the Indole-3-acetic acid (IAA) treated cowpea seeds (*Vigna unguiculata*) accession TVu-7, TVu-9 and TVu-1945 (Figure 1). For example, 1000mg/L of IAA treated seed of the various accession showed a delayed to attend 50% germination compared to other treatments and the control (Figure 1). However, a significant difference was not detected among the various accession seeds treated with 1000mg/L of IAA. It was observed that the effect of exogenous application of plant growth regulators (PGRs) including auxin can either be neutral, promote or inhibit germination (Webber, 1987). PGRs have been reported to have influenced plant germination (Webber, 1987). For example, IAA treated seeds of Chinese fir (*Cunninghamialanceolata*) promote a significant germination rate and also germination index at the concentrations of 10^{-4} M and 10^{-5} M IAA (Zhao & Zhong, 2013).

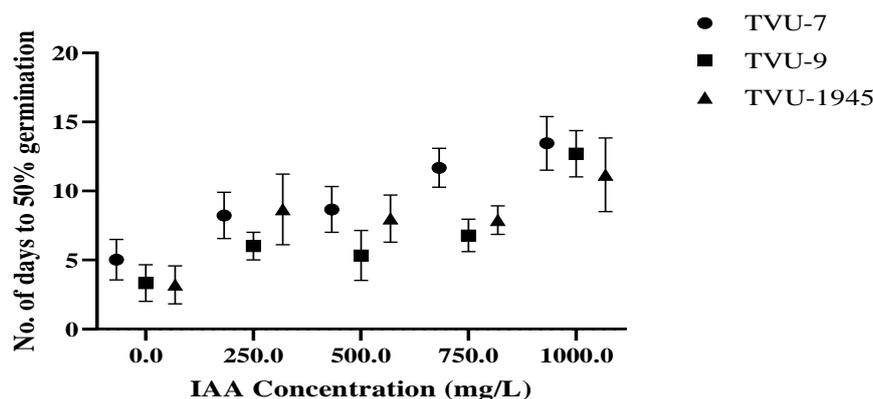


Figure 1 Number of days to 50% germination of the Indole-3-acetic acid (IAA) treated seeds of cowpea (*Vigna unguiculata*) accession TVu-7, TVu-9 and TVu-1945.

Table 1 Comparison of chlorophyll a, chlorophyll b and total chlorophyll content in the four 4 weeks old cowpea Plant (*Vigna unguiculata*) accession TVu-7, TVu-9 and TVu-1945 germinated from the various concentrations of

Treatment (mg/L)	TVU-1945			TVU-7			TVU-9		
	Chl. a (µg/ml)	Chl. b (µg/ml)	Total Chl. (µg/ml)	Chl. a (µg/ml)	Chl. b (µg/ml)	Total Chl. (µg/ml)	Chl. a (µg/ml)	Chl. b (µg/ml)	Total Chl. (µg/ml)
0	22 ± 0.65 ^a	18 ± 1.1 ^{bc}	39 ± 1.8 ^{bce}	12 ± 0.63 ^d	11 ± 1.1 ^c	24 ± 0.58 ^{fg}	9.9 ± 0.45 ^f	12 ± 0.54 ^{bc}	22 ± 0.91 ^g
250	14 ± 1.2 ^{ef}	15 ± 0.62 ^{bc}	30 ± 0.88 ^{eg}	15 ± 1.1 ^{acf}	20 ± 0.74 ^{bc}	35 ± 0.59 ^{bcef}	12 ± 1.4 ^{def}	14 ± 1.8 ^{bc}	27 ± 2.2 ^{eg}
500	12 ± 0.16 ^{ef}	12 ± 1.7 ^{bc}	24 ± 1.5 ^{fg}	16 ± 2.8 ^{acf}	22 ± 6.3 ^{ac}	37 ± 6.7 ^{bce}	18 ± 0.66 ^{ace}	19 ± 0.28 ^{bc}	36 ± 0.59 ^{bcef}
750	19 ± 1.8 ^{acd}	16 ± 1.1 ^{bc}	35 ± 2.7 ^{bcef}	19 ± 0.91 ^{ac}	25 ± 3.7 ^{ab}	44 ± 3.2 ^{ab}	15 ± 0.76 ^{bef}	15 ± 0.2 ^{bc}	30 ± 0.66 ^{deg}
1000	21 ± 1.3 ^{ab}	20 ± 3.3 ^{bc}	42 ± 2.1 ^{acd}	19 ± 0.47 ^{ac}	33 ± 2.3 ^a	53 ± 1.9 ^a	18 ± 0.54 ^{ace}	25 ± 0.48 ^{ab}	43 ± 0.11 ^{ac}

Indole-3-acetic acid (IAA) treated seeds.

Values are means ± SEM, n = 3 per treatment group. Means in a column without a common superscript letter differ ($P < 0.05$) as analyzed by one-way ANOVA and the LSD test. Chl. a = Chlorophyll-a; Chl. b = Chlorophyll-b; Total Chl. = Total chlorophyll.

In Table 1, there were variations in the content of chlorophyll a, chlorophyll b and total chlorophyll among the different cowpea accession plants obtained from the various concentrations of IAA germinated treated seed. The results showed that the cowpea accession TVU-1945 from the control treatment has the highest chlorophyll a content of 22 µg/ml while for chlorophyll b content there is no significant difference detected among the IAA treated plants. At 500 mg/L IAA of the TVU-1945 accession plant, recorded the least response to total chlorophyll content compared to others treatment. When exposed to (500 mg/l) IAA concentration. The coefficient of variations of Chl a, Chl b, and total Chl a+b at 1000 mg/L of TVU-7 accession, were 19, 33 and 53, respectively (Table 1). Generally, the coefficient of variations of Chl a, Chl b, and total Chl a+b among IAA treatments of the different plants accessions were relatively smaller, but all the 1000 mg/L IAA treatments have recorded higher coefficient values of Chlorophylls. The variations on the leaves chlorophyll contents suggest the possible effect of IAA treatments concentrations in the different accessions of cowpea plants. Chlorophyll is an important pigment for photosynthesis that bring about plant growth and development. Chl a and Chl b plays a significant role to absorb sunlight at different wavelengths, leading to a direct influence of the total amount of leaf chlorophyll content (Chl a+b) to contribute to the photosynthetic capacity of plants (Croft *et al.*, 2017; Li *et al.*, 2018). This may have suggested the changed variations in morphological vegetative structures in the different cowpea accessions of various IAA treatments concentrations.

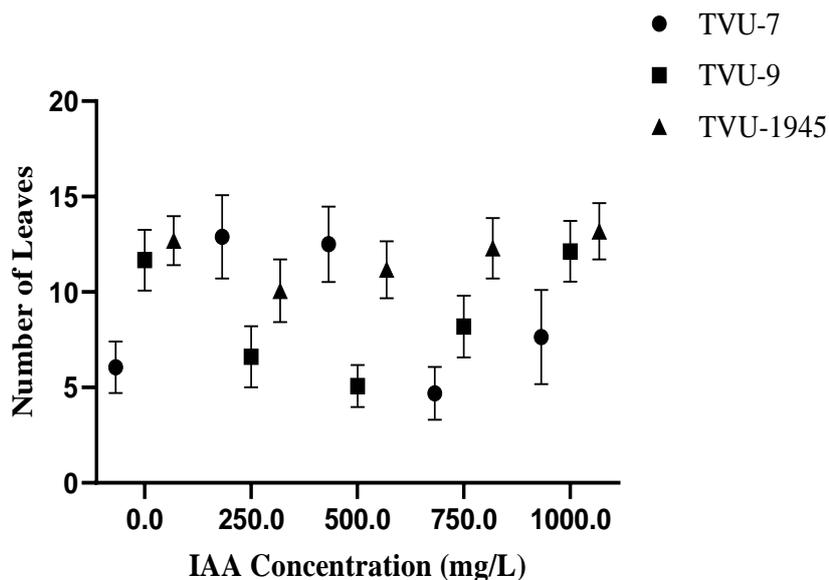


Figure 2 Number of leaves of the cowpea plant (*Vigna unguiculata*) accession TVu-7, TVu-9 and TVu-1945 germinated from the various concentrations of Indole-3-acetic acid (IAA) treated seed and control.

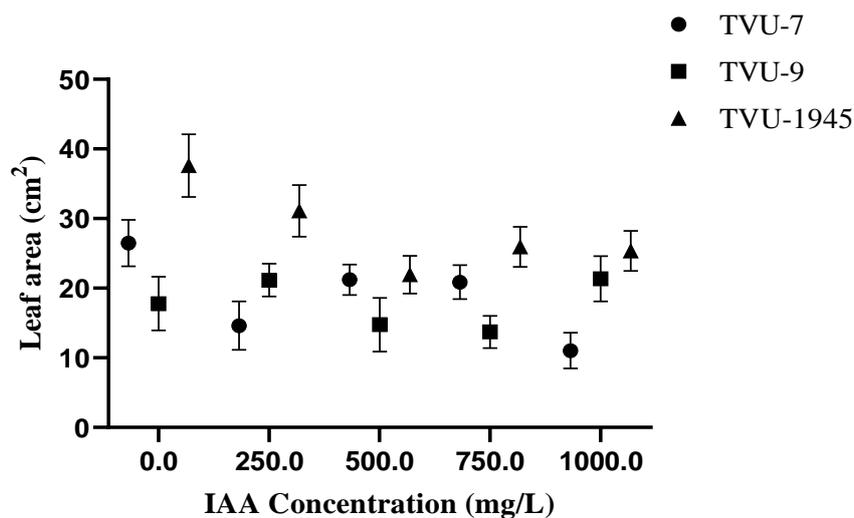


Figure 3 Leaf area (cm²) of the cowpea plant (*Vigna unguiculata*) accession TVu-7, TVu-9 and TVu-1945 germinated from the various concentrations of Indole-3-acetic acid (IAA) treated seed and control.

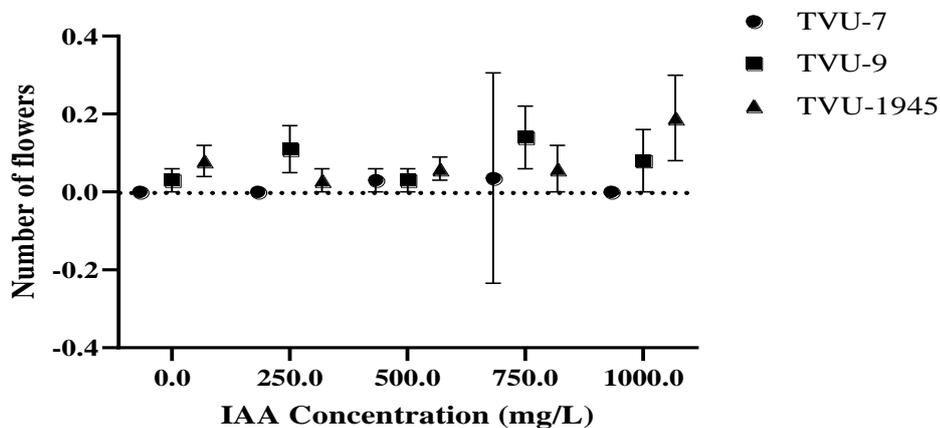


Figure 4

Number of flowers of the cowpea plant (*Vigna unguiculata*) accession TVu-7, TVu-9 and TVu-1945 germinated from the various concentrations of Indole-3-acetic acid (IAA) treated seed and control.

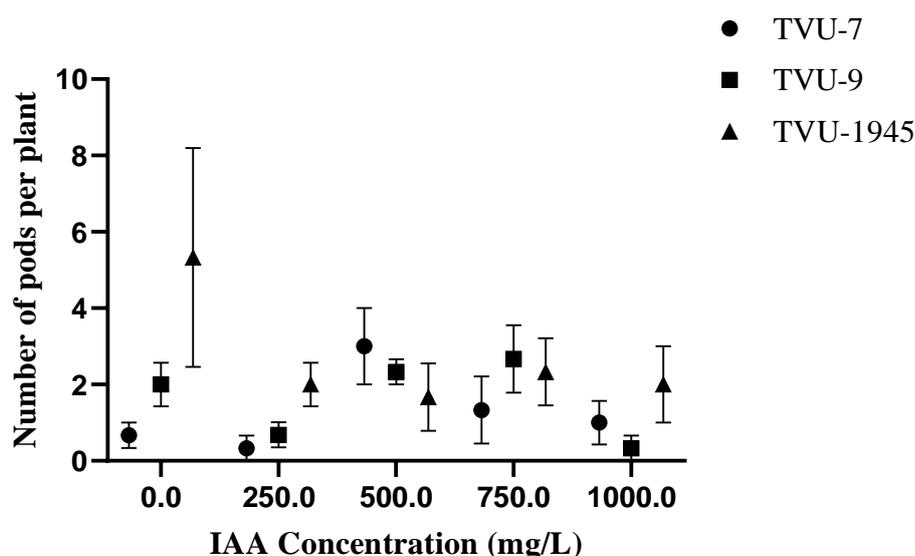


Figure 5 Number of pods of the cowpea plant (*Vigna munguiculata*) accession TVu-7, TVu-9 and TVu-1945 germinated from the various concentrations of Indole-3-acetic acid (IAA) treated seed and control.

Here in this study, the number of leaves, leaf area, number of flowers and number of pods produced from the different accession TVu-7, TVu-9 and TVu-1945 cowpea plant germinated from the IAA treated seeds showed significant difference (Figure 2-5). For example, the number of leaves produced in the cowpea plant accession TVu-7 germinated from 250 and 500 mg/L IAA treated seeds recorded higher numbers compared to their treatment control (Figure 2). Though results from plant accession TVu-9 recorded low numbers of leaves in the IAA treatment 250, 500 and 750 mg/L compared to the control treatment (Figure 2). A different study by El-Saeid *et al.* (2010) reported that a higher concentration of 1000 mg/L IAA increases the number of leaves in cowpea. This is also similar to the case of plant accession TVu-1945 which showed an increase in the number of leaves with increased concentrations of IAA treatment (Figure 2). While the leaves area of plant accession TVu-1945 decreases with increasing concentrations of IAA treatments (Figure 3). Although another study revealed that cowpea varieties responded differently to different IAA concentrations with evidence of variations in their vegetative structures (Mshelbula *et al.*, 2019). El-Saeid *et al.* (2010) reported that an increase in the leaf area of cowpea with increasing concentrations of IAA treatments. Also in another study by Lakshmi *et al.* (2017) demonstrated that IAA at 100 ppm which was a moderately used concentration significantly increased the leaf area of cashew plant.

Here in this study, the result in Figure 4 showed that there was no significant difference detected in number of flowers produced among the different cowpea accessions at various concentrations of IAA treatments. This is contrary to the report of Ud-Deen (2009) that the percentage of flowers were increased with an increase in the concentration of Gibberellic acid (GA₃) up to 150 ppm 90 days after planting. The number of pods produced also showed no significant difference detected among the different cowpea accessions at the concentrations of 500, 750 and 1000 mg/L IAA treatments (Figure 5). This also quite agrees with the report of Mshelbula *et al.* (2019). However, another study reported that 25 mg/L and 50 mg/L treatments showed a significant increase in the number of pods produced but higher concentrations of IAA treatments showed no effect of a significant increase in the number of pods (El-Saeid *et al.*, 2010).

4 Conclusion

The effect of IAA a type of plant growth regulator has now been evaluated on seed germination, seedling development and leaf chlorophyll content of the different cowpea accession TVu-7, TVu-9 and TVu-1945. Although, the present results did not explain the complex interaction that may have occurred between plant growth regulator IAA and the biochemical responses of the cowpea germinated seedlings. But more importantly, the objectives of this study have been addressed. Which include relatively variations to attend 50 % seed germination, number of leaves produced, total leaflet area, the number of produced pod per plant, and the number of flowers per plant. It is important to mention that increased IAA concentrations to 1000 mg/L further extend the number of days to attend 50 % seed germination in the different cowpea accessions.

Although, an increased concentration of 1000 mg/L significantly increased leaf chlorophyll content of the different cowpea accessions.

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