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COWPEA (VIGNA UNGUICULATA) SEED GERMINATION AND INDOLE-3-ACETIC ACID: UNRAVELING GROWTH MECHANISMS

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Abstract:
Cowpea (Vigna unguiculata) is a vital food crop in sub-Saharan Africa, known for its high protein content and nutritional value. Despite its importance, cowpea production has struggled to meet the growing demand in the region. Plant growth regulators (PGRs) such as auxins, specifically Indole-3-Acetic Acid (IAA), play a crucial role in controlling plant growth and development. IAA is involved in processes like cell division, elongation, and fruit development. This study investigates the impact of IAA, a synthetic auxin, on cowpea seed development. While previous research has demonstrated the influence of PGRs on the growth and yield of other plants like mungbean and tomatoes, its specific effects on cowpea remain relatively unexplored. Understanding how auxins affect cowpea seeds is essential for breeding new varieties with improved traits. The research examines whether IAA treatment can enhance cowpea seed quality, growth performance, and chemical composition. By shedding light on the interaction between auxins and cowpea seeds, this study aims to contribute valuable insights into optimizing cowpea production and meeting the region's nutritional needs.

Keywords: Cowpea, Vigna unguiculata, Indole-3-Acetic Acid (IAA), plant growth regulators, seed development, nutritional value.

1 Introduction

Cowpea (*Vignaunguiculata*) is one of the important food crop plants with a source of high protein content utilized by many people of sub-Sahara African (Lonardi*et al.,* 2019; Singh *et al.,* 2003). In some region of the semihumid 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 (Xiong*et 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-Sahara African (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 bybiological active biochemical moleculeswith an effect that is more or equal to that of the endogenous hormones (Gaspar *et al.,* 1996).Different studies have shown the influenceof 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 exogenously amount of PGRs as conjugates and released whenthe 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.

Therefore, this investigation aimed to study the effect of Indole -3- Acetic Acidtreated 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 ofIndole -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.

2.2Plant Growth Regulators (IAA) and Pot Experiment

Indole-3-acetic acid (IAA) concentrations were preparedas follows 250,500, 750 and 1000mg/Lrespectively. The various concentrations were dissolved in 1liter 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 the0.7 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 plantingto 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).

2.1.2Chlorophyll content determination

Leaf samples of four (4) weeks old germinated plant of the Indole-3-acetic acid (IAA) treatedseeds and control of the different cowpea accessions usedwere 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:

Chlorophyll a (μg/ml) = $12.25A_{663} - 2.79A_{647}$ …………………………………Eqn. 1

Chlorophyll b (μg/ml) = $21.50A_{647} - 5.10A_{663}$ ………………………………..Eqn. 2

Total Chlorophyll = Chlorophyll a (μg/ml) + Chlorophyll b (μg/ml)..............Eqn. 3

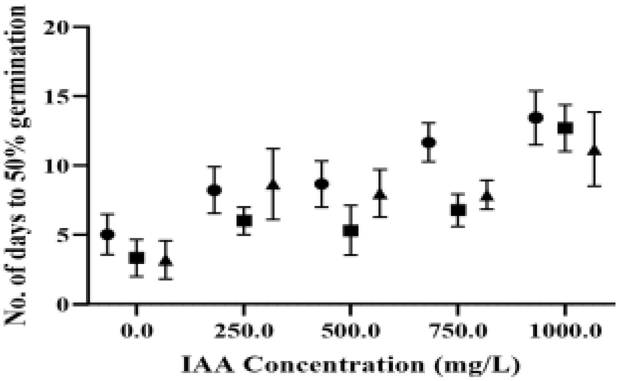
2.1.9 Statistical Analysis

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 ± 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 (*Vignaunguiculata*) 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 treatmentsand 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). PGRshave 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).



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TVU-7
TVU-9
TVU-1945

Figure 1 Number of days to 50% germination of the Indole-3-acetic acid (IAA)treated seeds of cowpea (*Vignaunguiculata*) 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 (*Vignaunguiculata*) accession TVu-7, TVu-9 and TVu-1945 germinated from the various concentrations of

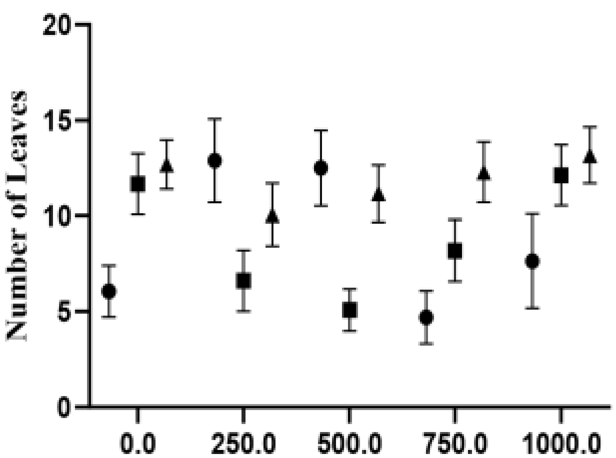
Treatment (mg/L) **TVU-1945** **TVU-7** **TVU-9**
Chl. a (µg/ml) Chl. b (µg/ml) Total Chl. (µg/ml) Chl. a (µg/ml) Chl. b (µg/ml) Total Chl. (µg/ml) Chl. a (µg/ml) Chl. b (µg/ml) Total Chl. (µg/ml)

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[cf] 15 ± 0.62[bc] 30 ± 0.88[cg] 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 ± 0.2[bc] 15 ± 0.76[bcf] 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 ± 0.48[ab] 25 ± 0.54[ace] 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 werevariations 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 500mg/L IAA of the TVU-1945accession plant, recorded the least response to total chlorophyll content compared to others treatment. When exposed to (500mg/l) IAA concentration.The coefficient of variations of Chl*a*, Chl*b*, and total Chl*a*+*b* at 1000mg/L of TVU-7 accession**,** were19, 33and 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 1000mg/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.

TVU-7 ●
■
▲
TVU-9
TVU-1945



IAA Concentration (mg/L)

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

TVU-7 ●
TVU-9 ■
TVU-1945 ▲

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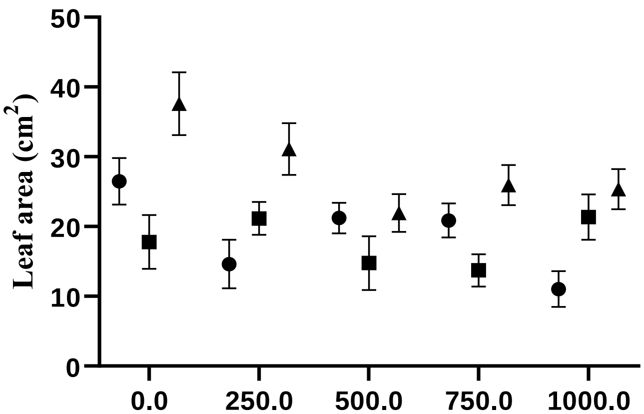
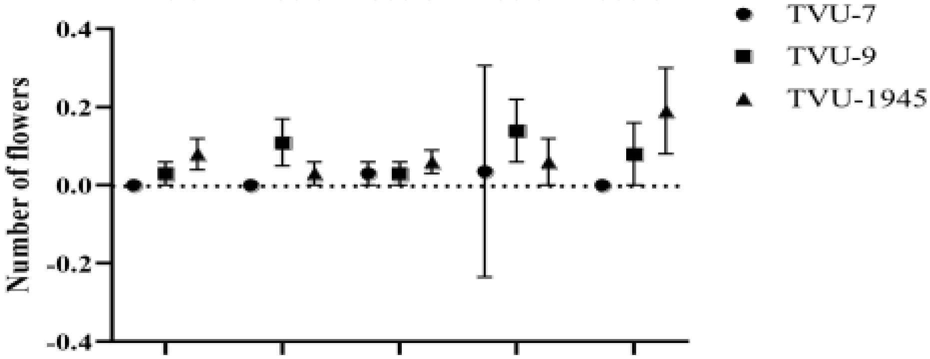


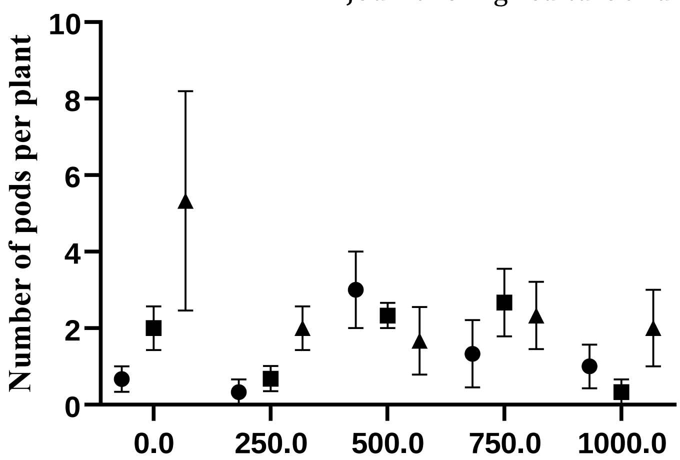
IAA Concentration (mg/L)

Figure 3Leaf area (cm^2) of the cowpea plant (*Vignaunguiculata*) accession TVu-7, TVu-9 and TVu-1945 germinated from the various concentrations of Indole-3-acetic acid (IAA) treatedseed and control.



0.0 250.0 500.0 750.0 1000.0

IAA Concentration (mg/L) Figure 4 Number of flowers of the cowpea plant (*Vignaunguiculata*) accession TVu-7, TVu-9 and TVu-1945 germinated from the various concentrations of Indole-3-acetic acid (IAA) treatedseed and control.



TVU-7
TVU-9
TVU-1945
IAA Concentration (mg/L)

Figure 5 Number of pods of the cowpea plant (*Vignaunguiculata*) accession TVu-7, TVu-9 and TVu-1945 germinated from the various concentrations of Indole-3-acetic acid (IAA) treatedseed 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 cowpeaplant germinatedfrom the IAA treated seeds showed significant difference (Figure 2-5).For example, the number of leaves produced in the cowpea plant accession TVu-7 germinatedfrom 250 and 500mg/L IAA treated seeds recorded higher numbers compared to theirtreatment control (Figure 2). Though results from plant accession TVu-9 recorded low numbers of leaves in the IAA treatment 250, 500 and 750mg/L compared to the control treatment (Figure 2).A different study by ElSaeid*et al.* (2010)

reported that a higher concentration of 1000mg/L IAA increases the number of leaves in cowpea.This is also similar to the case of plant accessionTVu-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 TVu1945 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(Mshelmbula*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 Lakshmipathi*et al*. (2017) demonstrated that IAA at 100ppm which was a moderately used concentration significantly increased the leaf area of cashew plant.
Here in this study, the result in Figure 4showed that there was no significant difference detected innumber 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 ofGibberellic acid (GA3) upto 150 ppm 90 days after planting.The number of pods produced also showed nosignificant difference detected among the different cowpea accessions at the concentrations of 500, 750 and 1000 mg/LIAA treatments (Figure 5). This also quite agrees with the report of Mshelmbula*et al*. (2019).However, another study reported that 25 mg/L and 50 mg/L treatments showed a significant increase in thenumber of pods produced but higher concentrations of IAA treatments showed no effect of a significant increasein the number of pods(El-Saeid*et al.,*2010).

4 Conclusion

The effect of IAA a type of plant growth regulatorshave now been evaluated on seed germination, seedling development and leaf chlorophyll content of the different cowpea accession TVu-7, TVu-9 and TVu1945. 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 1000mg/L further extend the number of days to attend 50 % seed germination in the different cowpea accessions.
Although, an increased concentration of 1000mg/L significantly increased leaf chlorophyll content of the different cowpea accessions.

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