AUXINS AND CYTOKININ AS A BIOSTIMULANT FOR CASSAVA ROOT INITIATION AND TUBERIZATION

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Abstract
Plant biostimulant is any substance applied to plants with the aim to enhance nutrition efficiency, abiotic stress tolerance and/or crop quality traits, regardless of its nutrients content. In view of this, a field experiment was conducted to investigate auxins and cytokinins as a biostimulant for cassava root initiation and tuberization at different concentration rates grown under rainfed conditions. The experiment was arranged in a randomized complete block design with three replications. A 3x3x2 factorial arrangement was used, corresponding to three varieties (TME419, Umucass 37 and CR41-10) and three concentrations of two PGRs which were α-naphthalene acetic (NAA) acid as auxins (0, 0.1 and 0.2mM) and 6-benzyladenine (BA) as cytokinins (0, 0.2, and 0.5mM). The Result showed that application of auxins and cytokinins promotes slightly early growth of cassava. The response to the biostimulant depends on the cassava varieties. The biostimulant application caused the greatest growth increase in CR 41-10 than in Umucass 36 and TME419 varieties. Application of auxins enhances growth and root development while cytokinins had no significant effect on root initiation and tuberization.

Keywords: Auxins, cytokinins, biostimulant, root initiation and tuberization

Introduction
Cassava is one of the major crops grown in Nigeria. Cassava roots have a high capacity to store starch, which is the reason they are the organs of the plant with the greatest economic value. Despite its importance to the country, management strategies to ensure higher crop yields have been little studied (Silveira *et al.,* 2012). The use of plant growth regulators or bioregulators in agriculture has been a means of promoting quantitative and qualitative increases in crop production, as when these substances are applied directly to the plants, they promote changes in vital and structural processes, increasing sucrose content, early ripening and crop yields (Martin and Castro, 1999; Caputo *et al.,* 2007). Because of the benefits these substances bring to cultivated plants, combinations of these products have also been studied. These mixtures are called plant stimulants or biostimulants, and are effective when applied in small doses, favoring the growth and development of the plant even under adverse environmental conditions (Casillas *et al.,* 1986). The benefits of biostimulants include: increase in plant growth rate, which is stimulated by cell division, differentiation and elongation. Contradictory results about the application of the biostimulant are reported in the literature. While biostimulant increased growth and productivity of soybean and blackberry (Vieira, 2001; Ferreira *et al.,* 2007a), other authors reported no significant changes in sweet potatoes, soybeans and corn (Dario *et al.,* 2005; Ferreira *et al.,* 2007b; Rós *et al.,* 2015). This variation has been attributed to different application times, doses applied and genotypes. Information about the use of biostimulant in cassava is still scarce. Therefore, the objective of this research was to evaluate the effects of different doses of auxins and cytokinins as biostimulant on the initiation and tuberization of three cassava varieties.

Material and Methods
The experiment was conducted in the experimental site of the National Root Crops Research Institute, Umudike. The soil properties were: pH 5.04; organic matter content 1.17 g kg^{-1}; P, K, and Ca at 14.40, 0.23 and 3.20 cmol kg^{-1} soil respectively; Mg, and effective cation exchange

capacity (ECEC) at 2.00 and 7.92 cmol kg^{-1} soil respectively. The experiment was arranged in a randomized complete block design with three replications. A 3x3x2 factorial arrangement was used, corresponding to three varieties (TME419, Umucass 37 and CR41-10) and three concentrations of two PGRs which were α-naphthalene acetic (NAA) acid as auxins (0, 0.1 and 0.2mM) and 6-benzyladenine (BA) as cytokinins (0, 0.2, and 0.5mM). Planting was carried out in May 2015. Cassava cuttings of approximately 20 cm long, were immersed in a solution of biostimulant diluted in distilled water for 10 minute and immediately planted in the field, five cuttings per plot, in a slanting position. The biostimulant doses were calculated according to use recommendations, along with the control, which was immersed in distilled water only for 10 minute, to make factors equal, totalizing three doses. At 90 days after planting, leaf area, plant height, stem diameter, root volume and leaf number were evaluated. Leaf area was determined by separating the leaves of all plants, scanning and digitizing with the software Digital Area Determinator (DAD). Harvest index is the index of the root weight across the varieties used at their different concentration.

$$Harvest\ index\ = \frac{root\ weight}{root\ weight + biomass}$$

The analysis of variance confirmed the interaction between the factors cassava varieties, biostimulant and doses. Means of the quality factor (varieties) were compared by the F-test ($p < 0.05$) and significances were subjected to Least significance differences.

Results and Discussion

The results of the growth and yield parameters of the different level of the plant growth regulators on three cassava genotypes used, are presented in Figure 1 to 5 and Table 1. Figures 1 - 5 showed the effect of Auxins and cytokinins concentrations on the growth parameters of different cassava varieties at 3 months after planting, Table 1 showed the effect of auxins and cytokinins concentrations on the yield parameters of different cassava varieties at 3 months after planting.

The Effect of NAA and BA Concentration on growth parameters

In this experiment, the findings demonstrated the effects of auxins and cytokinins on sprouting and shoots formation where the use of NAA and BA had slightly significant ($p \leq 0.05$) effect on sprouting and shoot formation except for stem diameter (Table 4.1). It was observed that the produced number of leaf/node, plant height, leaf area and sprouting increased with increase in concentration for the auxins but decreased with cytokinins. A significantly ($p \leq 0.05$) highest number of leaves/nodes (18.00±1.76), leaf area (164.33±23.71) and plant height (27.08±4.18) were observed when 0.02mM of the auxins was used on CR41-10 but showed no significant effect on the stem diameter and the sprouting when compared to the control (Figure 1 – 5). Cytokinins showed slightly mean increase at the lower concentration (0.02mM) compared to the control on the growth parameters where higher concentration of cytokinins above 0.02mM did not enhance plant height, leaf area, sprouting and number of leaves. Generally, this study indicates that increasing concentration of NAA for the cassava varieties enhanced the shoot proliferation but not sprouting rate. The concentration at 0.02mM of the auxins showed best results compared to the lower concentration which is 0.01mM (Figure 1, 2, 3, 4 and 5). Increased number of leaves and leaf area are desirable because the cassava plant will have a greater photosynthetic active area, with greater contributions to productivity gains, as well as increasing the soil cover and possibly reducing the interference of weeds. Similar result finding was also reported by (Silva *et al.*, 2012).

Effects of auxin and cytokinin on root initiation and tuberization of cassava

In this experiment, different concentrations of auxins and cytokinins were tested to compare their effectiveness on root initiation and tuberization of cassava. All the characters evaluated were found to be influenced by the different concentrations of the auxins (NAA) except the

cytokinins (BA). The total weight, biomass, root weight, root number and the harvest index were significantly ($P \leq 0.05$) affected by the NAA but the BA showed no significant ($P \geq 0.05$) effect on root initiation and tuberization of cassava when compared to the control (Table 2). This revealed that application of both the 0.01 and 0.02mM of the auxins produced significantly more total weight, biomass, root weight and number of root while there is no significant different across the harvest index. The cytokinins has no significant effect on the potential yield of the cassava compared to the control. A more vigorous root development revealed by the application of auxins can promote the absorption, mainly of water, at the beginning of the crop establishment, which is essential for the survival of most plants in the field and to lower the number of failures in the plant stand. Reghin *et al.*, (2000) found significant effect of increasing doses of biostimulant on the number and length of roots of peruvian carrot (*Arracacia xanthorriza* Bancroft) up to the limit of 7.0 mL L-1, indicating that it enhances growth and root development. Peressin & Carvalho (2002) reported that, from 7 to 90 days after planting the cassava cuttings, the phase of root system formation starts, especially the fibrous roots, with some of these turning into storage roots later. Thus, the increments of dry mass become important for producing higher yields of roots.

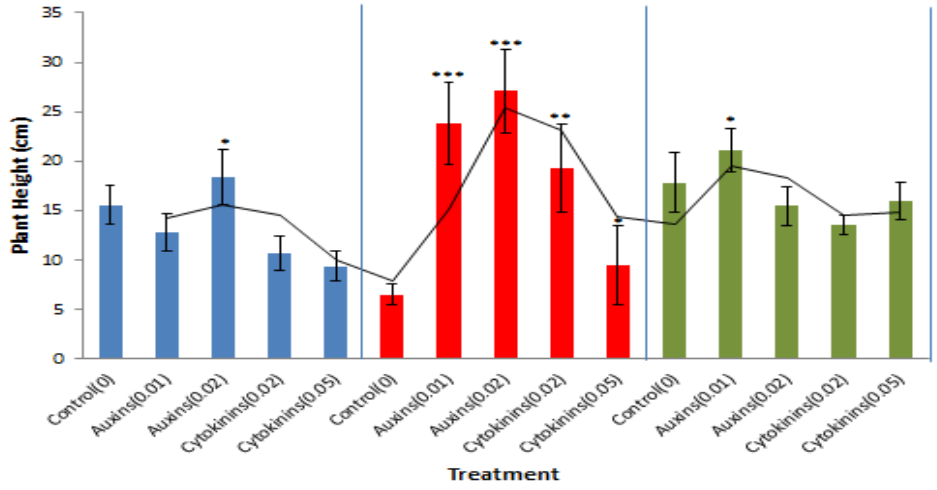

Figure 1: Effect of Auxins and Cytokinins on plant height of cassava varieties

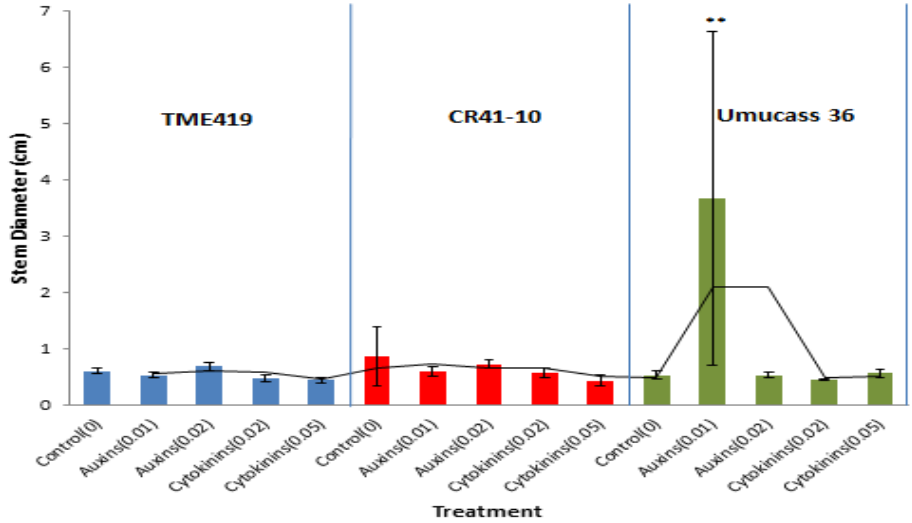

Figure 2: Effect of Auxins and Cytokinins on Stem diameter of cassava varieties

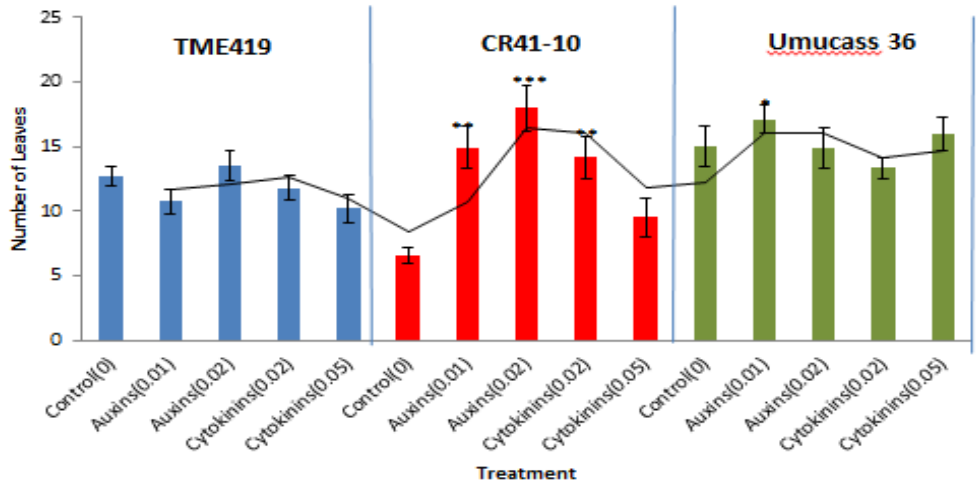

Figure 3: Effect of Auxins and Cytokinins on number of leaves of cassava varieties

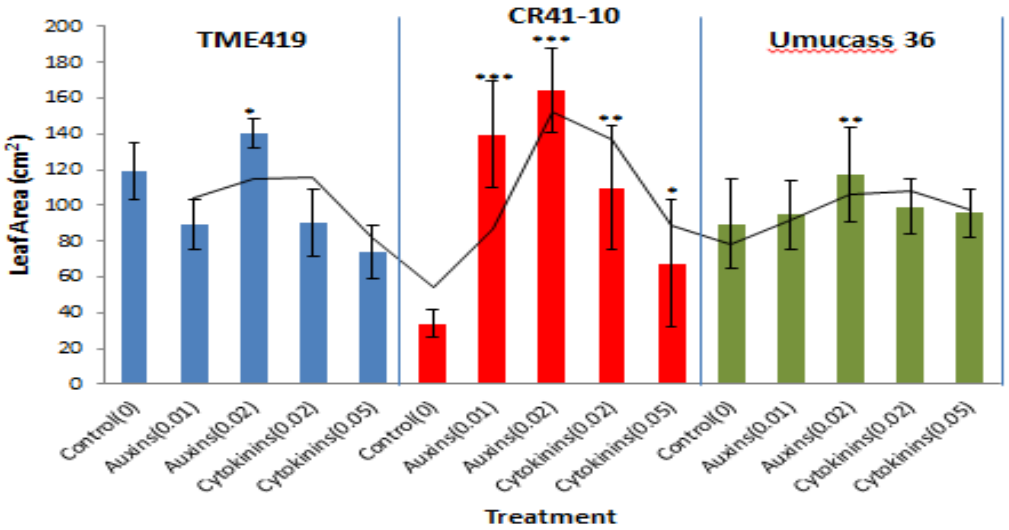

Figure 4: Effect of Auxins and Cytokinins on leaf area of cassava varieties

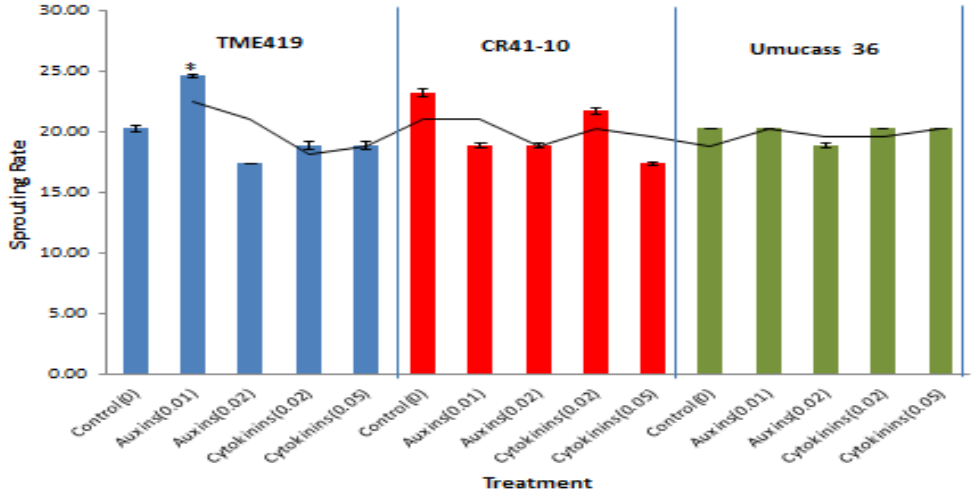

Figure 5: Effect of Auxins and Cytokinins on sprouting rate of cassava varieties

Table 1: Effect of Auxins and cytokinins concentrations on the yield parameters of different cassava genotypes at 3month after planting

Genotype	PGRs	Conc (mM)	Total weight (g)	Biomass (g)	Root weight (g)	Number of root	Harvest Index
TME 419 (white root)	Control	0	250.00±35.12^{ab}	205.60±36.22^{ab}	44.40±11.63^{ab}	4.00±0.58^{bc}	0.18±0.06^{a}
	Auxins	0.01	213.33±40.96^{ab}	190.70±26.76^{ab}	22.63±15.17^{ab}	4.67±1.20^{abc}	0.09±0.05^{a}
		0.02	513.33±218.81^{a}	432.37±175.12^{a}	80.97±43.71^{a}	5.33±1.45^{abc}	0.14±0.02^{a}
	Cytokinins	0.02	160.00±46.19^{ab}	137.87±59.58^{ab}	22.13±14.52^{ab}	3.67±0.33^{bc}	0.24±0.20^{a}
		0.05	176.67±121.97^{ab}	164.97±114.82^{ab}	12.23±7.10^{ab}	3.33±1.86^{bc}	0.07±0.04^{a}
CR 41-10 (Latin America)	Control	0	40.00±15.28^{b}	40.00±15.28^{b}	1.47±0.27^{b}	1.00±0.00^{c}	0.02±0.02^{a}
	Auxins	0.01	480.00±182.48^{a}	415.97±145.60^{a}	64.03±38.57^{ab}	6.33±2.33^{ab}	0.10±0.04^{a}
		0.02	470.00±115.90^{a}	425.60±101.73^{a}	44.40±14.77^{ab}	9.67±1.20^{a}	0.09±0.02^{a}
	Cytokinins	0.02	366.67±218.35^{ab}	337.50±199.43^{b}	29.13±19.13^{ab}	6.00±3.61^{abc}	0.08±0.01^{a}
		0.05	30.00±20.00^{b}	28.60±19.60^{ab}	1.40±0.40^{b}	1.33±0.33^{bc}	0.08±0.02^{a}
Umucass 36 (Yellow root)	Control	0	293.33±223.41^{ab}	255.63±186.85^{ab}	39.23±35.79^{ab}	5.00±2.00^{abc}	0.08±0.04^{a}
	Auxins	0.01	320.00±45.09^{ab}	270.27±44.49^{ab}	49.73±6.29^{ab}	5.00±1.00^{abc}	0.16±0.03^{a}
		0.02	256.67±114.65^{ab}	228.13±106.40^{ab}	28.53±11.65^{ab}	6.33±1.33^{ab}	0.11±0.05^{a}
	Cytokinins	0.02	133.33±28.48^{ab}	113.50±36.26^{ab}	19.83±8.18^{ab}	3.00±0.00^{bc}	0.18±0.08^{a}
		0.05	303.33±32.83^{ab}	266.83±26.40^{ab}	36.50±7.24^{ab}	3.67±0.33^{bc}	0.12±0.01^{a}

Means in a column with the same superscript are not significantly different according to Turkey's test ($P \leq 0.05$)

Conclusion

The application of auxins and cytokinins promotes slightly early growth of cassava at 3 month after planting. The response to the biostimulant depends on the cassava varieties. The biostimulant application caused the greatest growth increase in CR 41-10 than in Umucass 36 and TME419 varieties. Application of auxins enhances growth and root development while cytokinins had no significant effect on root initiation and tuberization. Generally, this indicated that auxins promotes cassava growth, initiation and tuberization while cytokinins slightly promote cassava growth but has no effect on the root initiation and tuberization at 3 months after planting. From the findings of this study, the following recommendations are made for future research on effect of PGRs on the tuberization and shooting of cassava plants. The treatment combination is a good suggestion for better improvement for tuberization and shooting formation. Extension of time of evaluation should be encouraged to see the effect of these PGRs at older age. Spraying should also be included in the method treatment applications with a wider range of concentrations.

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