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Response of Pigeon Pea Landraces [*Cajanus cajan* (L.) Millsp.] to Exogenous Application of Plant Growth Regulators

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Authors' contributions

This work was carried out in collaboration between all authors. Author OUU designed the experiment, performed the statistical analysis, interpreted and formatted the final manuscript. Author EAE wrote the first draft of the manuscript; author EVI wrote the protocol while author MIN did the literature search and collected the data. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Aim: Optimizing plant growth regulators in the manipulation of growth physiology, development and seed yield of pigeon pea landraces is cardinal, especially as it complements other improvement techniques. The research was aimed at evaluating the effect of IAA, NAA and paclobutrazol singly and in combination, but especially paclobutrazol in reducing plant height.
Methods: Thirty (30) seeds each of brown "Fiofio" [*Cajanus cajan* (L.) Millsp.] were soaked in 0, 100, and 150mg/l concentration of IAA, NAA, paclobutrazol, paclobutrazol + IAA and paclobutrazol + NAA, respectively for 48 hours.
Results: Results obtained revealed that treating pigeon pea seeds with paclobutrazol caused reduction in plant height and inter-node length, which did not translate to higher yield. However, plants raised from pigeon pea seeds soaked in 100 and 150 mg/l paclobutrazol + NAA did excellently well in both yield and yield – related traits.
Conclusion: The implication of these findings is that though treating seeds with paclobutrazol caused significant reduction in plant height and increased branch numbers, it needed to interact with NAA to cause holistic improvement, especially in seed yield.

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Keywords: Pigeon pea; "Fiofio" IAA; NAA; paclobutrazol; improvement.

ABBREVIATIONS

IAA - Indole acetic acid; NAA – Naphthalene acetic acid; PAC. – Paclobutrazol; PGRs – Plant growth regulators.

1. INTRODUCTION

Agricultural sustainability and food security are twin cardinal issues in the millennium development goals (MDGs) of the present Nigerian government. It therefore becomes pertinent and timely to develop techniques and programmes that could drive the vision [1]. Crops of importance are the extinct-threatened species of indigenous origin, which reports abound on their adaptability, variability and unique nutritive profiles [2,3,4,5,6,7]. One of such landraces is ''Fiofio'', an indigenous pigeon pea. It is an annual/ perennial, tall, erect and single stemmed legume with glandular pubescent growing to a height greater than 180cm after 6months period [5,8]. It should be highlighted that the annuality or perenniality of pigeon pea depends on the time of maturity and subsequent harvest. The implication is that they are ready for harvest within one growing season and could enter the next growing season and still produce pods.

Pigeon pea serves as food source as well as therapeutic purposes, especially in most developing countries of Africa, Asia and Latin America [9,10,11,12]. The economic and therapeutic values notwithstanding, pigeon pea, especially the landraces are faced with under exploitation in term of cultivation, genetic breeding, research and utilization, which obviously might be linked to the preferences of both consumers and breeders to improved varieties [5].

Interestingly, significant progress has been made towards improving them, especially through mutation breeding [1,3,13,14]. Presently, our research team in the University of Calabar, Nigeria is testing M_2 seeds for their stability while looking at the optimal dose of these mutagens that will give the best result in yield. We have however, observed from our previous reports [1,3,13,14] that the height of this crop might affect seed yield, though indirectly, but particularly because it is usually accompanied with the production of limited number of branches, fewer flowers and pods, which probably might culminate into low seed yield.

Nowadays, phytohormones have been found to play an important role in plants growth and development. Plant growth regulators have enormous applications in agriculture such as delaying or accelerating maturity, stimulating flowering, abscission and organic controlling of weeds etc. [15]. Undoubtedly, hormones accumulation in plant is a key process, which determines seed yield, plant vigor, sex expression and shoots weight [16]. According to [17] seed development is genetically programmed and involves transcriptional and physiological reprogramming, which is modified by sugar and hormones responsive pathway. Plant growth regulators (PGRs) are chemicals, which enhance plant growth, especially when applied in trace quantity [18]. The effectiveness of the PGRs depends on the concentration of the chemicals present and the sensitivity of the organ concerned. Many researcher have documented interesting results on the exogenous applications of PGRs to different crop plants [19,20,21,22,23,24,25,26,27]. Specifically, paclobutrazol blocks the biosynthesis of active gibberellic acid (GA) thus decreasing plant growth and development [28]. Some

researchers indicated that paclobutrazol exert biochemical effect on plant including increase in levels of proline, antioxidants [29], chlorophyll content [30].

It does suggest that PGRs have the capacity to stimulate and /or inhibit physiological processes, which directly or indirectly might affect crop yield. Our present research x-rays the effect of IAA, NAA and Paclobutrazol on the yield of pigeon pea. Of a particular interest is the effect of paclobutrazol as reports point to its height reducing ability [31,32,33] while taking into cognizance the possibility of synergism in interaction of these PGRs, which could lead to better morphological and yield performances.

2. MATERIALS AND METHODS

2.1 Seed Collection and Plant Growth Regulator Treatment

Seeds of brown "Fiofio" (*Cajanus cajan* (L) Millsp) were obtained from Dr. Udensi O. Ugorji seed collections in the Department of Genetics and Biotechnology, University of Calabar, Nigeria. Thirty (30) seeds each were soaked in 0, 100, and 150mg/l concentration of paclobutrazol, IAA, NAA, paclobutrazol+IAA and paclobutrazol+NAA, respectively for 48 hours.

2.2 Planting and Cultural Practices

A plot size of 12x12 meters was manually cleared in the University of Calabar Experimental Farm. Five beds were made with a spacing of 2 meters between them. Three seeds were sown in a hole of 4cm deep according to the method of the [34] as modified by [6] using 5 x 3 factorial layout on a randomized complete block design (RCBD) with 6 replications. Spacing of 20 x75 cm was maintained. Weeding was done as the need arose. There was no fertilizer application.

2.3 Data Collection and Analysis

Data on percentage seed germination, days to seedling emergence and survival percentage (phenological traits) were collected while morphological and yield traits were recorded at 3 and 6 months, respectively. They were subjected to analysis of variance (ANOVA), using PASW version 18.0 Microsoft statistical software. The means were separated using least significant different (LSD) test.

3. RESULTS AND DISCUSSIONS

3.1 Results

3.1.1 Phenological parameters

Result on seedling emergence revealed that there was slight increase on the time it took seedlings to emerge as the concentration of PGRs increased, though this trend was not consistent. Rate of seed germination was also affected by increase in PGRs concentration, especially for seeds soaked in 150mg/l of paclobutrazol (Figs. 1- 3). There were significant effects (P=0.05) of plant growth regulators both singly and in combination on days to seedling emergence and percentage seed germination while percentage seed survival

showed no significant different (P > 0.05) (Fig. 1). Plants raised from seeds soaked in 150mg/l paclobutrazol and paclobutrazol + NAA (100mg/l) took a longer time to emerge. Days to flowering and maturity were significantly affected (P = 0.05) by PGRs treatment singly and in combination. Generally, there was significant reduction in these parameters, especially when compared with their respective controls (Figs. 4 & 5).

3.1.2 Morphological traits

Three months after planting, plant raised from seeds soaked in 100 and 150mg/l paclobutrazol grew shorter (116.4cm and 101.20 cm) when compared with other treatment groups. They produced more branches (8; 10) with shorter inter-node length and quite low number of leaves. Plants raised from seeds treated with 150mg/l IAA produced the highest number of leaves (250.0), the tallest plants (155.80cm) while NAA soaked seeds produced plants with the longest inter-node (5.56cm) (Table 1). Comparatively, plants raised from PAC. + NAA treated seeds produced plants with broader leaves (20.04cm) at 150mg/l. In 6 months, 150mg/l IAA soaked seeds gave rise to the tallest plants (299.75cm), number of leaves (369.25) and the longest inter-node (6.70cm). Paclobutrazol (100 & 150 mg/l) soaked seeds produced plants whose height were short, (185.25 cm and 185.0 cm) though the combination of paclobutrazol +NAA soaked seeds produced plant with the highest number of branches, plants raised from seeds soaked in 150mg/l paclobutrazol produced comparatively high number of branches (~20.0) with reduced inter-node length. Additionally, combining the PGRs did not have much effect on plant height, number of leaves and inter-node length but however, caused significant (P =0.05) increase in the number of branches plant^{-1} when the pigeon pea seeds were soaked in 150mg/l PAC. + NAA (Table 2). These effects were concentration dependent.

3.1.3 Yield traits

It was observed that from our current result plants raised from seeds treated with 150mg/l IAA produced the highest number of flowers plant^{-1} (134.50) and the longest pod (6.43) comparing the single treatments while paclobutrazol and NAA soaked seeds gave rise to plants with the least number of flowers plant^{-1}. Our results also revealed that plants raised from seeds soaked in 150mg/l paclobutrazol + NAA performed better. They produced the highest number of flowers (~144), pods per plant (318.0), seeds per pod (7.00), longest pod (7.27cm), and highest seed yield per treatment (1.919kg) while at 100 mg/l paclobutrazol + NAA, the seed yield was 1.321kg per treatment (Table 3). Comparatively, plants from seeds soaked in 150mg/l IAA produced 135 flowers while those from combination of paclobutrazol + IAA at 100mg/l produced 136 flowers. For number of pods per plant, paclobutrazol + IAA treated seeds produced 244 pods and seed yield of 1.236 kg per treatment at the concentration of 150 mg/l. These effects were concentration specific.

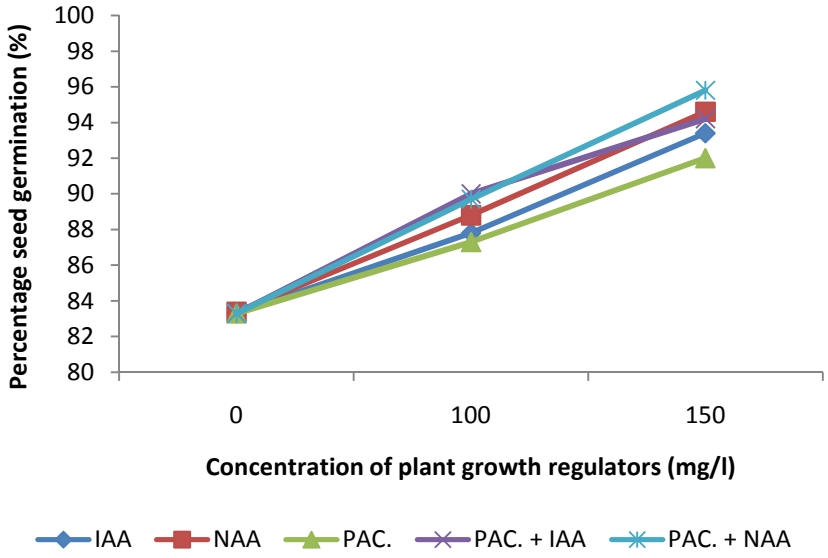

Fig. 1. Effect of plant growth regulator on percentage seed germination (%) of pigeon pea

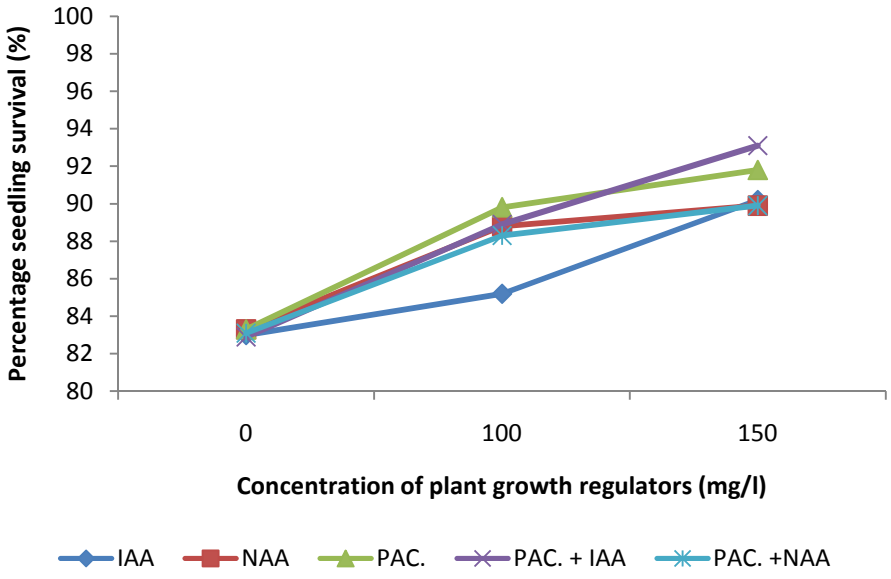

Fig. 2. Effect of plant growth regulator on percentage seedling survival (%) of pigeon pea

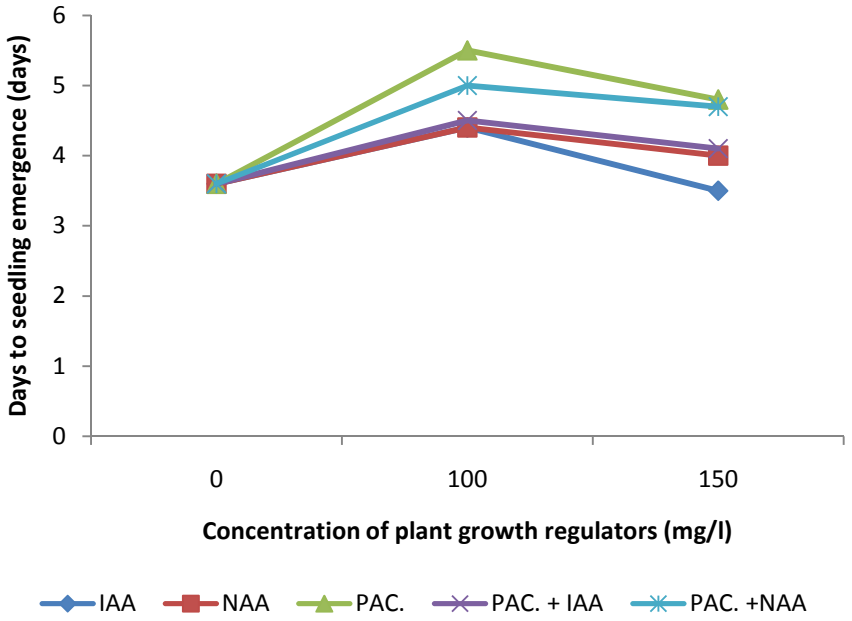

Fig. 3. Effect of plant growth regulator on days to seedling emergence of pigeon pea

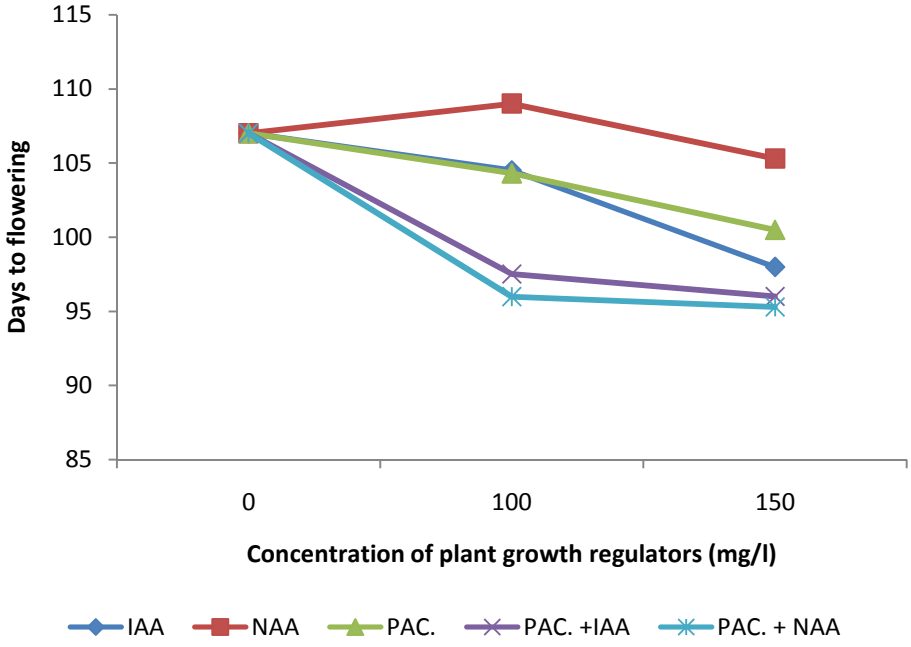

Fig. 4. Effect of plant growth regulator on days to flowering of pigeon pea

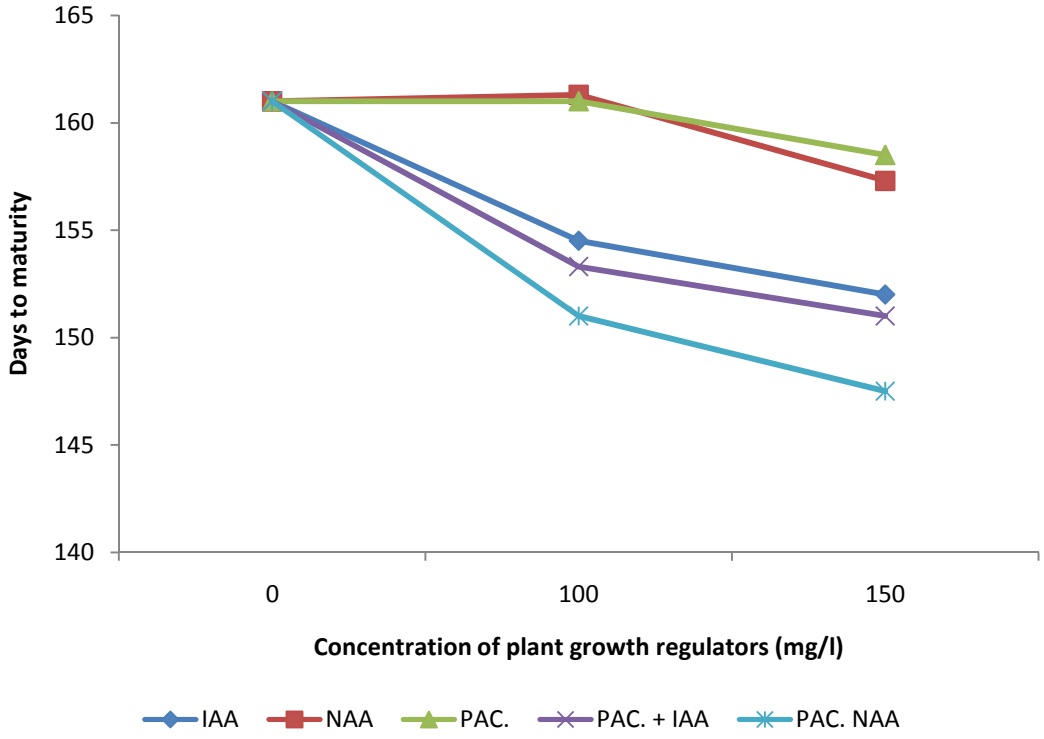

Fig. 5. Effect of plant growth regulator on days to maturity of pigeon pea

Table 1. Effect of plant growth regulators on morphological traits in pigeon pea after three months

Plant growth regulators	Concentration (mg/l)	Morphological traits				
		Plant height plant^{-1} (cm)	Number of leaves plant^{-1}	Leaf area (cm^2)	Number of branches plant^{-1}	Internode length plant^{-1} (cm)
Indole acetic acid (IAA)	0	139.20^{b}±5.40	217.00^{d}±1.15	16.07^{c}±1.03	5.80^{c}±0.37	4.46^{d}±0.42
	100	130.80^{c}±4.73	236.60^{b}±1.90	15.75^{c}±0.67	7.20^{c}±0.76	4.16^{e}±0.29
	150	155.80^{a}±5.90	250.00^{a}±1.70	16.63^{b}±0.72	8.40^{b}±0.67	5.43^{b}±0.38
Naphthalene acetic acid (NAA)	0	139.20^{b}±5.40	217.00^{d}±1.15	16.07^{c}±1.03	5.80^{c}±0.37	4.46^{d}±0.42
	100	128.00^{c}±6.65	86.20^{h}±1.70	13.62^{d}±0.45	5.80^{c}±0.27	3.70^{f}±0.21
	150	125.60^{c}±4.17	226.00^{c}±2.05	15.05^{c}±0.65	7.60^{c}±0.51	5.56^{a}±0.17
Paclobutrazol (PAC.)	0	139.20^{b}±5.40	217.00^{d}±1.15	16.07^{c}±1.03	5.80^{c}±0.37	4.46^{d}±0.42
	100	116.40^{d}±6.61	171.20^{f}±1.30	7.25^{e}±0.40	8.00^{b}±0.39	3.17^{g}±0.29
	150	101.20^{e}±7.39	180.80^{e}±1.17	5.55^{f}±0.21	10.00^{a}±1.43	3.57^{g}±0.18
PAC. + IAA	0	139.20^{b}±5.40	217.00^{d}±1.15	16.07^{c}±1.03	5.80^{c}±0.37	4.46^{d}±0.42
	100	121.80^{d}±3.43	176.20^{f}±1.15	14.95^{d}±0.88	7.40^{c}±0.39	3.63^{g}±0.48
	150	128.60^{c}±6.17	138.00^{g}±1.20	15.41^{c}±0.79	8.20^{b}±0.58	5.01^{c}±0.34
PAC. + NAA	0	139.20^{b}±5.40	217.00^{d}±1.15	16.07^{c}±1.03	5.80^{c}±0.37	4.46^{d}±0.42
	100	122.40^{d}±3.25	225.00^{b}±1.60	15.96^{c}±0.85	8.80^{b}±0.86	4.43^{d}±0.46
	150	122.60^{d}±4.83	224.20^{b}±1.80	20.04^{a}±0.65	7.80^{b}±0.60	4.65^{d}±0.44

**Means with the same superscript indicate no significant difference (P > 0.05) along the vertical array*

Table 2. Effect of plant growth regulators on morphological traits in pigeon pea after six months

Plant growth regulators	Concentration (mg/l)	Morphological traits				
		Plant height plant^{-1} (cm)	Number of leaves plant^{-1}	Leaf area (cm^2)	Number of branches plant^{-1}	Internode length plant^{-1} (cm)
Indole acetic acid (IAA)	0	236.00^{c}±1.45	329.00^{b}±1.46	21.55^{a}±0.89	17.50^{d}±0.63	5.40^{b}±0.50
	100	246.25^{b}±2.20	348.50^{b}±0.94	20.26^{a}±0.49	17.00^{d}±0.70	5.21^{c}±0.31
	150	299.75^{a}±0.85	369.25^{a}±1.75	24.29^{a}±1.18	17.50^{d}±0.63	6.70^{a}±0.28
Naphthalene acetic acid (NAA)	0	236.00^{c}±1.45	329.00^{b}±1.46	21.55^{a}±0.89	17.50^{d}±0.63	5.40^{b}±0.50
	100	197.00^{g}±1.80	215.25^{d}±1.60	19.67^{a}±0.88	16.75^{d}±0.47	4.77^{c}±0.27
	150	228.25^{e}±1.60	313.25^{c}±1.30	21.00^{a}±1.30	17.50^{d}±0.63	4.62^{d}±0.35
Paclobutrazol (PAC.)	0	236.00^{c}±1.45	329.00^{b}±1.46	21.55^{a}±0.89	17.50^{d}±0.63	5.40^{b}±0.50
	100	185.25^{h}±2.70	335.00^{b}±1.90	23.82^{a}±1.61	15.50^{e}±0.26	3.35^{e}±0.13
	150	228.25^{e}±3.45	287.25^{c}±2.01	17.34^{a}±1.21	20.25^{b}±0.25	3.07^{f}±0.08
PAC. + IAA	0	236.00^{c}±1.45	329.00^{b}±1.46	21.55^{a}±0.89	17.50^{d}±0.63	5.40^{b}±0.50
	100	211.75^{f}±0.85	309.50^{c}±1.03	19.29^{a}±1.22	19.00^{c}±0.90	4.05^{d}±0.23
	150	212.75^{f}±1.10	327.75^{b}±1.30	30.38^{a}±1.37	17.50^{d}±0.83	3.57^{e}±0.30
PAC. + NAA	0	236.00^{c}±1.45	329.00^{b}±1.46	21.55^{a}±0.89	17.50^{d}±0.63	5.40^{b}±0.50
	100	231.75^{d}±2.25	347.00^{b}±1.46	16.82^{a}±0.44	20.50^{b}±0.28	4.52^{d}±0.48
	150	211.75^{f}±1.10	339.00^{b}±1.45	28.36^{a}±0.89	21.75^{a}±0.75	5.00^{c}±0.53

**Means with the same superscript indicate no significant difference (P > 0.05) along the vertical array*

Table 3. Effect of plant growth regulators on yield traits in pigeon pea after six months

Plant growth regulators	Concentration (mg/l)	Yield traits				
		Number of flowers plant^{-1}	Number of pod plant^{-1}	Number of seed pod^{-1}	Pod length plant^{-1} (cm)	Seed yield plant^{-1} (kg)
Indole acetic acid (IAA)	0	118.25^{e}±0.85	130.50^{l}±2.17	4.25^{c}±0.61	5.70^{d}±0.45	0.5193^{h}±0.75
	100	125.50^{d}±3.90	138.25^{h}±1.10	4.25^{c}±0.61	6.06^{c}±0.25	0.5515^{h}±2.50
	150	134.50^{b}±1.90	143.00^{g}±2.29	5.75^{b}±0.47	6.43^{b}±0.43	0.7403^{g}±2.55
Naphthalene acetic acid (NAA)	0	118.25^{e}±0.85	130.50^{i}±2.17	4.25^{c}±0.61	5.70^{d}±0.45	0.5193^{h}±0.75
	100	124.00^{d}±2.12	139.00^{h}±1.75	4.00^{c}±0.38	5.33^{d}±0.22	0.5428^{h}±1.88
	150	116.75^{e}±1.60	144.25^{g}±1.90	4.50^{c}±0.63	6.15^{c}±0.24	0.7050^{g}±1.87
Paclobutrazol (PAC.)	0	118.25^{e}±0.85	130.50^{f}±2.17	4.25^{c}±0.61	5.70^{d}±0.45	0.5193^{h}±0.75
	100	117.25^{e}±1.10	153.00^{f}±2.15	6.00^{a}±0.38	5.32^{d}±0.20	0.9298^{e}±2.05
	150	116.75^{e}±1.79	163.25^{e}±3.79	5.25+±0.61	4.79^{e}±0.36	0.8480^{f}±1.07
PAC. + IAA	0	118.25^{e}±0.85	130.50^{f}±2.17	4.25^{c}±0.61	5.70^{d}±0.45	0.5193^{h}±0.75
	100	135.75^{b}±1.40	211.50^{c}±1.75	5.50^{b}±0.63	5.88^{c}±0.12	0.9980^{d}±2.53
	150	130.75^{c}±1.31	243.75^{b}±2.80	5.25^{b}±0.62	5.64^{d}±0.23	1.2355^{c}±2.09
PAC. + NAA	0	118.25^{e}±0.85	130.50^{l}±2.17	4.25^{c}±0.61	5.70^{d}±0.45	0.5193^{h}±0.75
	100	132.75^{c}±1.10	192.40^{d}±1.03	5.00^{b}±0.70	6.01^{d}±0.38	1.3208^{b}±2.65
	150	144.24^{a}±2.05	318.00^{a}±1.07	6.50^{a}±0.28	7.27^{a}±0.28	1.9185^{a}±2.09

**Means with the same superscript indicate no significant difference (P > 0.05) along the vertical array*

3.2 Discussion

Due to the resurgence of interest in exploring, exploiting and optimizing extinct-threatened pulses, especially pigeon pea ("Fiofio") by our research team in the University of Calabar, Nigeria, concerted efforts have been made in its cultivation and domestication to ascertain its adaptive and productivity capacity, especially in the humid tropics of Cross River State, Nigeria [5, 6]. Interestingly, also, it has undergone several mutation breeding processes using gamma ray irradiation, amiprophos methyl and colchicine and very good promising results have been obtained [1, 3,13, 14, 35]. We discovered that seed yield seems to be lowered owing to the height attained by the plant at anthesis and subsequent maturity. This has led to the treatment of the seeds with plant growth regulators, especially paclobutrazol, a growth retardant to evaluate their effect on the growth and development of the crop.

According to [17], seed development is genetically programmed, which involves transcriptional and physiological reprogramming that is mediated by sugar and hormone-responsive pathways. Auxins and paclobutrazol have been reported to affect the biosynthesis of gibberellins (GA_3) as well as deactivates their function in several plant, including pea, tobacco, etc.[36, 37, 38,]. It is noteworthy to mention here that gibberellins are hormones responsible for cell elongation. The implication is that their mechanism is to frustrate its synthesis and if already synthesized deactivates the cellular programme thus impeding cell elongation [28,]. [22, 39] independently reported that 50ppm NAA caused significant increase in plant height. On the contrary, plant raised from seeds soaked in 100mg/l and 150mg/l IAA grew taller than other treatment groups, including the control. We observed that there was a synergistic interaction between paclobutrazol, IAA and NAAs in combination. It seems to suggest that the addition of paclobutrazol to either IAA or NAA affected the plant height boosting effects of the single treatments. The inhibition of plant growth and decrease of internode elongation by treating seeds/ plants with paclobutrazol corroborates the reports of [32, 33]. Leaf area at 3 months showed significant differences across treatment groups though it does not agree with positions of [18, 40] concerning the effect of NAA as a leaf area enlarger. Surprisingly, at 6 months, there was no significant differences observed across the treatment groups.

The reported effects of single application of IAA and NAA on root nodulation process [26] in cowpea might be the underlying factor for the increase yield as documented by [41, 42, 43] in mung bean and [44] in soybean. However, our present result showed that combined treatment of pigeon pea seeds with paclobutrazol + NAA did better in all yield traits, resulting to yield enhancement. It is probable that while single treatment of pigeon pea seeds with paclobutrazol increased number of branches, which was evident on the plants raised from seeds soaked in 150mg/l paclobutrazol + NAA with comparable number of leaves per plant, NAA on the other hand, might have enhanced the processes underlying root nodulation thus resulting to holistic improvement in yield traits, including seed yield.

Though IAA treatment did not cause any significant effect on the leaf area as reported by [18, 40], one would have expected that since soaking the seeds in especially 150mg/l IAA caused a significant increase in the number of leaves; photosynthetic activities would have increased as the activation of translocation machinery. This obviously would lead to increase productivity [45, 46]. However, the reverse was the case.

The role of IAA and NAA in plant cell division is to enhance germination and growth of seedlings. It thus would have been logical to observe a positive significant effect of soaking of the seeds in the PGRs on percentage germination and seedling emergence. It was

however, surprising to notice that seed soaking in PGRs rather caused a slight delay in seedling emergence. It should be understood that though IAA and NAA are auxins, their specificity of action seems to be plant-specific. This is premised on the fact that soaking should have increased tissue hydration, enhanced redistribution of nutrient reserves, respiratory activities, leading to increased seedling growth, early flowering and subsequent yield improvement [19, 20]. However, they did not fulfil these functions singly but rather in combination with paclobutrazol. This might suggest that these plant growth regulators might not function optimally singly but would need another one to contribute integrally to the improvement or enhancement cascade.

4. CONCLUSION

Explicitly, plant raised from treating pigeon pea with 100 and 150 mg/l paclobutrazol + NAA did excellently well in both yield and yield – related traits. The implication of this finding is that plant breeders, especially for local pigeon pea ("Fiofio") could try combination of improvement methods, including soaking seeds in plant growth regulators. Though treating seeds with paclobutrazol caused significant reduction in plant height and increased branch numbers, it needed to interact with NAA to cause holistic improvement, especially seed yield.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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