Original Research Article

EFFECTS OF AUXIN AND GIBBERELLINS HORMONES ON THE REGENERATION OF PINEAPPLE PROPAGULES FROM CROWN LEAVES (WITH BUDS) IN KILIFI COUNTY, KENYA

ABSTRACT

Kilifi county has a large potential of over 2,000 Ha suitable for pineapple cultivation that has not been fully exploited due to lack of adequate clean planting material. Thus, the aim of the study was to evaluate the effects of auxin and gibberellin hormones on regeneration of pineapple propagules from pineapple crown leaves (with buds). The study was carried out at Pwani University Crop Science farm. A randomized complete block design replicated three times was used. Treatments included auxin and gibberellins hormones where the cut crown leaves (with buds) were dipped and planted at a spacing of 4 cm by 6 cm in plastic containers filled with coir dust. Five plastic containers were used for each hormone treatment. In each container, three plants were tagged for data collection. The plastic containers (the plots) measured 30cm by 36cm by 15cm height. Data collected from the tagged plants included, time to shoot emergence, plant height, leaf length and breadth, number of leaves per plant, final root length and final number of roots. These were used to determine effects of auxin, gibberellins on growth of the propagules up to a height of 15-20 cm. Obtained data was subjected to ANOVA analysis using Genstat Statistical software 14th Edition and means obtained compared using Tukey's honest significant difference test ($p \leq 0.05$). The results indicated auxin treated propagules had 50.7%, 50.6%, 64.2%, 52.6%, 24.4%, 10.4%, 39.0%, 9.9% and 5.2% higher final values of plant weight, shoot weight, dry shoot weight, fresh root weight, plant height, leaf number, root number, dry root weight, root length and average root length, respectively, compared to those treated with gibberellins. The study also showed that auxin treated propagules had their initial roots originating from the base of the seedlings, while gibberellins treated propagules had their initial roots originating from the base of the cut crown leaves.

Key words: Pineapple, crown leaves, propagules, regeneration, auxin, gibberellins

Introduction

Pineapple (*Ananas Comosus* (L.)) is an important tropical fruit grown in Kenya for domestic and export market in processed form and as fresh fruits [1][2][3]. Main growing areas include Thika, Malindi/Magarini and Kisii where the climate is mild with temperatures ranging between 16 and 32°C. The major exporter of processed pineapple products in Kenya is Delmonte Kenya Ltd situated in Thika and grows pineapples year-round. Important varieties grown include Smooth Cayenne, Queen, and newly developed Delmonte varieties such as Del Monte Gold®, Extra Sweet, Honeyglow®, and Pinkglow® [1][3]. The functional benefits of eating pineapples include protection against macular degeneration, antioxidant protection and immune support, potential anti-inflammatory and digestive benefits, manganese and thiamin for energy production and antioxidant defenses [4]. The nutritional benefits include helping reduce malnutrition that is rampant in the area, especially for children under five years and the elderly [5][6]. It is rich in vitamins such as vitamins A, B, C and minerals that include Manganese which prevents osteoporosis. It has no fat and is therefore cholesterol free [4].

In Kilifi county, pineapple cultivation occurs in Malindi and Magarini sub-counties and is a major source of employment and income for majority of the communities in these areas of Kilifi County through sale of fresh fruits, pineapple juice and exports [7]. The variety grown is the smooth cayenne and is highly suitable for fresh market and canning. Other local varieties grown include Marafa Sweet and Mwanazi [3]. The demand for fresh sweet pineapples from Kilifi county is high and the County has never met the increased demand yet there exists a large potential of over 2,000 ha for expansion of pineapple farming. Pineapple farming in Kilifi County is faced by a number of challenges which include inadequate availability of clean planting material, high initial investment costs, drought, high transport cost to markets, pests and diseases. Of these challenges, availability of planting materials is the most limiting factor (. Currently, pineapple planting material is obtained from pineapple suckers, slips and sometimes from pineapple crowns. To get these materials ready for planting takes about 18-24 months to reach maturity and harvest [3]. This is a long period to wait. The current acreage of pineapple growing area in Kilifi county is estimated at 2,000 ha and 20,000ha for the larger Coastal region [7]. Using the required seed rate of 37,000-48,000 suckers per ha, over 80 million suckers are required for planting every growing season. However, the current production of planting material in Kilifi County is much less, between 10,000-15,000 suckers per ha due to drought, disease infestation and poor soils, giving a deficit of 50 million suckers per planting season [7]. This is what limits expansion of pineapple farming in the county and Coastal region as a whole. In order to cover the deficit, there is need to explore other scientific methods of producing and increasing disease-free planting materials within the shortest time possible without compromising on the quality of final product. In Kenya, there are limited studies on production of pineapple planting material from pineapple crown leaves for rapid growth of pineapple plantlets in vivo [9]. Therefore, this study was conceived to evaluate the use of pineapple crown leaves treated with auxin and gibberellin hormones for mass regeneration of planting material for enhancing pineapple production in Kilifi County. The hypothesis under test was that, auxin and gibberellins hormones had no effect on generation and growth of pineapple propagules from crown leaves.

2.0 Methodology

2.1 Study site

The study was conducted in two seasons, from July to September and from October to December of 2022 at Pwani University Crop Science farm located 60 km from Mombasa lying between latitudes 3°S and 4°S and longitudes 39°E and 40° E, and at an altitude of about 30m above sea level (ASL) in Coastal lowland zone 3 (CL3) [10]. The average annual rainfall ranged between 600-1100 mm. long rains occurred between April and July while the short rains occurred from September to December. Annual mean temperatures ranged between 28°C - 32.0°C [11]. The pre-dominant of soil type in the study area was sandy-loam characterized by poor water holding capacity due to low organic matter content and poor in nutrients [12].

2.2 Material used

These included: pineapple crowns which were obtained from local pineapple markets selected from big mature pineapple fruits with clean crown leaves of uniform size with a bud eye [3]. Plant hormones used were auxin under registered name as “Azatone” or “Rootex” (containing 33% auxin). It was sourced from Nairobi Agrovet Shops. Auxin hormones induce and stimulate cell division and elongation thereby promoting root and shoot growth. Gibberellins hormones are responsible for inducing cell extension, expansion and differentiation thereby promoting root and shoot development. Gibberellins was sourced from Amiran Kenya Ltd, in Nairobi under registered name as “Tivag 40 SL” (soluble liquid, gibberellins at 40g/L).

2.3 Experimental design and treatments

A randomized complete block design with split plot arrangement of treatments, replicated thrice, was used (Figure 1). Two factors, namely auxin hormones and type of media at various levels were used as treatments.

	REP I	REP II	REP II
Auxin A	A	A	A
	A	A	A
	A	A	A
	A	A	A
	A	A	A
Gibberellins G	G	G	G
	G	G	G
	G	G	G
	G	G	G
	G	G	G

Key: Auxin = A; Gibberellins = G
Figure 1: Experimental plot layout in split-plot arrangement

The hormones were at two levels namely, Auxin and gibberellins. Allocation of the treatments in the plots was done randomly (Figure 1). Each plot consisted of a plastic box measuring 30 cm by 36 cm by 15cm height, and filled with the coir media up to 10cm. Spacing between plots was 5 cm and between blocks 15 cm (Figure 1). Spacing of the crown leaves (with buds) between rows was 6 cm and within rows was 4 cm, giving a total of 9 plants per plot. With a total of 30 plots, (15 for auxin and 15 for gibberellins) this gave a grand total of 270 plants for the whole experiment in season I.

2.4 Preparation of crown leaf cuttings, treatment and planting in propagation trays

One or two healthy pineapple crown leaves of more than 5 cm in length, were carefully and individually cut off from the stem of selected pineapple crowns, and checked for healthy eye buds. Crown leaf buds are shared by two leaves at leaf-overlap; hence either a single leaf or two leaves were hived off to obtain an intact bud (Plate 1). The hived off leaves were dipped in 70% alcohol for sterilization, and in a solution of Ridomil fungicide for treatment against fungal pathogens. The leaves were then dipped in auxin hormone powder (Azatone) or in gibberellins solution depending on the respective treatment (Plate 1).

Plate 1: Treatment of individual crown leaves (with a bud) with auxin hormone.

At initial stages, the treated crown leaves were raised in large metallic propagation trays containing coir media to facilitate selection of healthy "germinating" pineapple propagules from crown leaf buds (Plate 2).

Plate 2: Propagation tray, with coir media and planted crown leaf cuttings.

Before planting into propagation trays, the coir media was watered to saturation and left for 5 days to allow for free drainage and settling of the media. After planting in the propagation trays (measuring 90 cm by 60 cm) and irrigating uniformly to field capacity using watering-Can, the propagation trays were transferred into propagation structures roofed with fiber glass for maintaining adequate lighting, constant temperature and high relative humidity which is necessary for sprouting of the leaf buds (Plate 3). Regular watering was maintained every two days to ensure adequate moisture in the media. During the study period, no pests or diseases were noted.

Plate 3: One-month old germinated pineapple propagule seedlings

2.5 Selection and transplanting germinated propagules into plastic containers

About one month after planting, healthy well sprouted and rooted propagules that had attained 3 cm in height were selected for transplanting into plastic containers filled with coir (Plates 3, 4 and 5).

Plate 4: Rooted propagule at 3 cm in height ready for transplanting

Plate 5: Transplanted and three tagged propagules per plot for data collection

2.6 Data collection

In each plot (plastic container) three plants were randomly selected and tagged (Plate 5). It is from these tagged plants where data was collected every 7 days after transplanting until the propagules attained 15-20 cm in height. Data collected included, plant height, leaf length and width, number of leaves, stem diameter, final root length and final number of roots per propagule. This experiment was done in two seasons, namely, January to April 2023 and from May to August, 2023.

2.7 Determination of parameters

Plant height was determined using a meter rule, from the base of the propagule to the tip of the plant. The number of days to shoot emergence was determined from the day of planting until a new shoot emerged above media surface. Leaf length was determined using a ruler from its attachment to the stem to the tip of the leaf blade, while the leaf width, was determined using a ruler at its widest part across the midrib. The number of new leaves were determined by observing and counting the new leaves as they emerged above the media surface. Final root length was determined by uprooting and measuring the root length using a ruler. The final number of roots per propagule was determined by physically counting the number of roots per plant. Stem diameter was determined by placing a string around the stem and obtaining the length on a ruler and dividing the obtained circumference by value of pie (3.14).

2.8 Data analysis

The collected data for each variable was subjected to analysis of variance (ANOVA) using Genstat statistical software 14th edition and the means obtained subjected to Tukey's Honestly significant difference test for comparison at 5% level of significant difference.

3.0 Results

3.1 Effects of hormones on growth parameters of pineapple propagules

The results indicate that application of plant hormones on crown leaves (with buds) resulted in significant differences ($P \leq 0.05$) in propagule growth parameters. In general, auxin hormones (Azaton) resulted in higher values of plant parameters than gibberellins hormones (Figure 2 and Table 1). However, they attained peak stages of growth at the same time (Figure 2). One notable difference due to hormones treatment was on the place of origin of initial roots during the sprouting stage (Plate 6). In auxin treated crown leaves, initial roots originated from the base of the young germinating seedlings, while in gibberellins treated crown leaves, initial roots originated from the base of the cut part of the crown leaves (Plate 6).

Plate 6: Site of origin of initial roots in hormone treated pineapple crown leaf

For auxins treated leaves Plate 6 (a), roots originated from base of the sprouting seedlings, while for gibberellins treated leaves Plate 6 (b), roots originated from the base of cut leaves. The results also indicated that auxin treated propagules had 10.5%, 15.4%, 29.0%, 15.8% and 7.1% more leaves per plant, taller plants, larger stem diameters, leaf lengths and leaf widths respectively than gibberellins treated propagules (Tables 1 and 2).

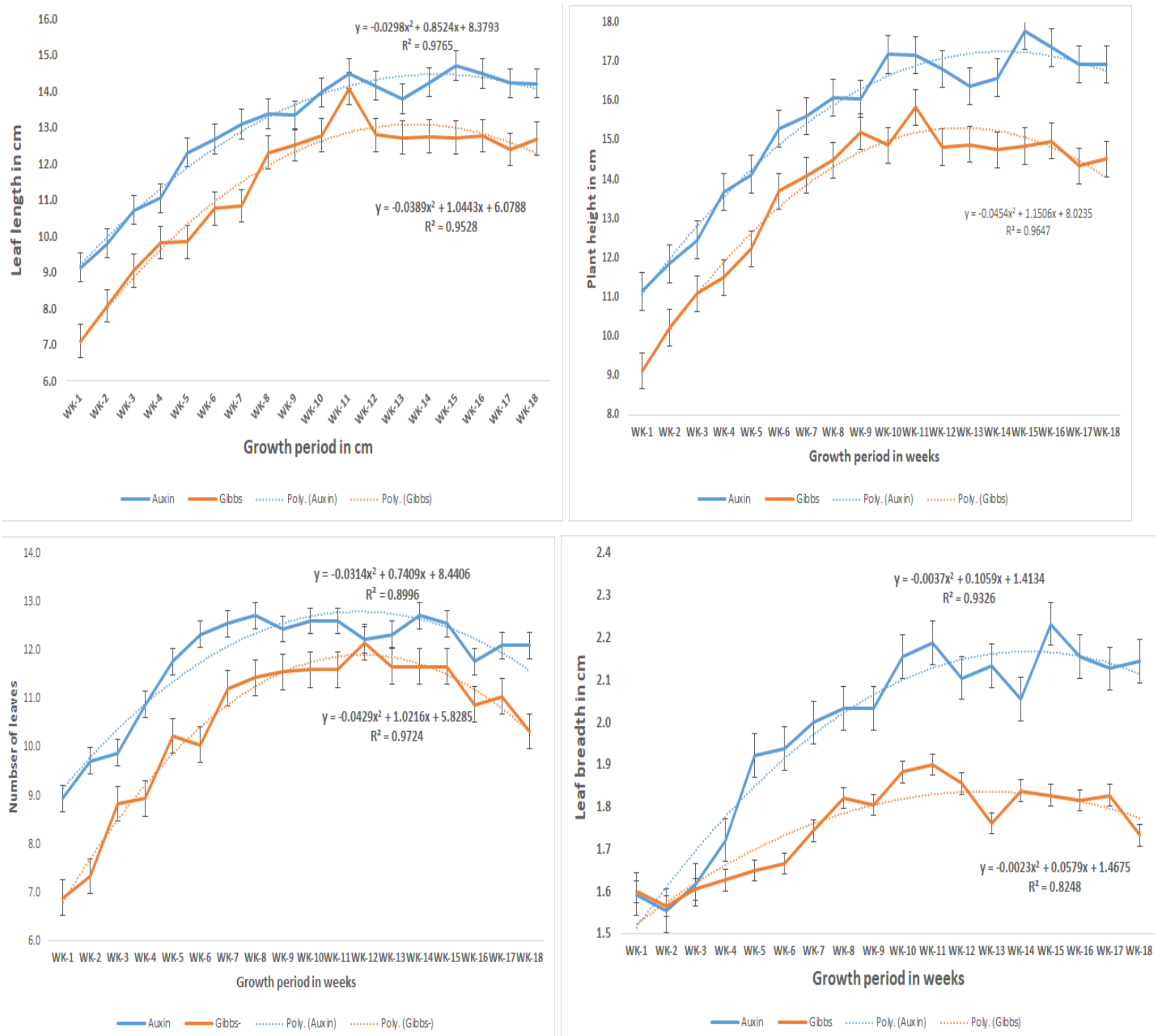

Figure 2: Period at which most growth parameters attained peak growth due to auxin and gibberellins hormones

Auxin hormones maintained significantly higher values for most tested parameters compared to gibberellins, for similar growth period. The growth pattern of most propagule parameters followed a parabolic (polynomial) trend (Figure 2).

Table 1: Effects of hormones on growth parameters of pineapple propagule at 10th week after transplanting

	Propagule parameters				
Hormones	**Leaf number**	**Height**	**Stem diameter**	**Leaf length**	**Leaf breadth**
Auxin	11.65a	17.29a	6.66a	14.64a	2.26a
Gibberellins	10.43b	14.63b	6.06b	12.33b	2.10b
Mean	11.0	16.0	6.4	13.5	2.2
($P < 0.05$)	<.0001	0.000	0.000	0.000	0.012
Sed	0.150	0.250	0.08	0.21	0.03
CV%	1.4	1.6	1.3	1.6	1.4

Means at the same column that do not share a letter are significantly different

3.2 Effects of hormones on final growth parameters of pineapple propagules

Tables 1 and 2 shows that application of auxin hormones on crown leaf (with buds) resulted in significant differences ($P \leq 0.05$) on final propagule growth parameters compared to those treated with gibberellins hormones. Auxin treated propagules occasioned significantly final plants height, longer roots, higher number of leaves and roots per plant than gibberellins treated propagules (Table 2). Thus, auxin treated propagules had 50.7%, 50.6%, 64.2%, 52.6%, 24.4%, 10.4%, 39.0%, 9.9% and 5.2% higher final values of plant weight, shoot weight, dry shoot weight, fresh root weight, plant height, leaf number, root number, dry root weight, root length and average root length, respectively, compared to those treated with gibberellins.

Table 1: Effects of hormones on final growth parameters of pineapple propagule

Media types	Plant weight	Shoot weight	Dry shoot weight	Root weight	Plant Height	Leaf number	Root number	Dry root weight	Root length	Ave-Root length
Auxin	78.9a	71.4a	12.0a	7.6a	26.2a	15.4a	30.8a	4.8a	27.2a	15.4a
Gibberellins	38.9b	35.3b	4.3b	3.6b	19.8b	13.8b	18.8b	0.95b	24.5a	14.6a
Mean	58.9	53.35	8.15	5.6	23	14.6	24.8	2.9	25.85	15
P-Value	0.000	0.000	0.000	0.000	0.000	0.006	0.000	0.000	0.070	0.392
Sed	11.7	10.7	1.45	1.17	1.25	0.73	2.48	0.24	1.54	1.48
% CV	19.9	20.1	17.8	20.9	5.4	5.0	10.0	8.3	6.0	9.9

Means in the same column that do not share a letter are significantly different

4.0 Discussion

4.1 Effects of hormones on pineapple propagule growth parameters

Auxin and gibberellins had significant different effects on propagule growth parameters, namely plant height, leaf length and width, number of leaves, stem diameter, final root length and final number of roots per propagule. Similar observations were by [13] and [14] who reported that, in pineapple species, auxin and gibberellins increase cell division, elongation and expansion activities. In this study, auxin hormones in particular resulted in higher number of leaves, taller plants, larger stem diameters, leaf lengths and leaf widths than gibberellins hormones. This could partly be attributed to the functional role and mode of action of each type of hormone on plant cells during growth processes. Auxins are generally known to promote cell elongation, while gibberellins are known to promote lateral cell expansion amongst other functions. This explains why auxin treated propagules had longer leaves, taller plants and wider stem diameter than those treated with gibberellins hormones. These results agree with similar observations by [15], who reported that auxin primarily induced cell division and elongation, stimulated shoot elongation and root initiation and also vascular differentiation in carrots.

4.2 Effects of hormones on final growth parameters of pineapple propagules

Application of auxin hormones on crown leaf resulted in propagules that were taller and heavier in weight with longer roots, higher number of leaves and roots per plant than those treated with gibberellins. This would suggest that auxin hormones had a functional role of enhancing growth through increased cell division of most meristematic tissues and therefore faster accumulation of biomass by the pineapple propagules than gibberellins hormones. Most literature cites the major role of gibberellins as being more of cell differentiation and therefore organ development than biomass accumulation. This observation appears to agree with those reported by [16], who observed that among the functions of gibberellins include delaying senescence in fruits, involved in leaf expansion, break bud and seed dormancy, promote bolting in cabbages and beet, facilitate elongation of fruits such as apples and enhancing their shape, all of which point to developmental functions than growth.

5.1 Conclusions

This study has shown that it is possible to mass generate pineapple seedlings from crown leaves with eye-buds. The study has shown that auxin and gibberellins hormones can hasten regeneration of pineapple seedlings from crown leaves with eye-buds. Although both hormones influenced the growth parameters in a similar way, auxin hormones had profound effects on the different parameters compared to gibberellins. Auxin values for the different parameters were way above those of gibberellins.

5.2 Recommendations

The results suggest that the mode of action of the two hormones in initiating production of initial roots, is different. This needs to be studied further, to ascertain why gibberellins initiates rooting from the cut leaf base surface and why auxins initiate the roots from the base of developing seedlings.

There is need to study and compare how long it takes for the pineapple propagules generated from crown leaf buds to reach flowering and produce a mature fruit, their shelf life and quality with planting material sourced from different parts of the plant such as suckers, slips, crowns, among others.

Recommendations for farmers are that, there is a potential to increase local production of clean planting material through mass generation using pineapple crown leaves with eye-buds using auxin hormones.

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