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DEVELOPMENT OF APPROPRIATE *IN VIVO* TECHNIQUE FOR RAPID FIELD MULTIPLICATION OF PLANTAIN (*MUSA* AAB) USING COCONUT (*COCOS NUCIFERA L.*) WATER AND INDOLE-3-ACETIC ACID

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ABSTRACT

Plantain production in Ghana is challenged with inadequate healthy and uniform sized planting materials for cultivation. *In vitro* and rapid field multiplication techniques of plantains have been developed to overcome the problem of obtaining many vigorous and uniform suckers free from pest and diseases. However, *in vitro* techniques require expensive equipment and expertise that are unavailable to most plantain farmers in Ghana. Therefore, an appropriate and inexpensive technique has to be developed *in vivo* to increase the multiplication rate of plantain planting materials. This study investigated the effects of coconut water (CW) alone or in combination with varying concentrations of auxin (indole-3-acetic acid, IAA) on axillary bud initiation, growth and development of plantain (cv. Asamienu). Coconut water from fully matured dried fruits (13 months old) was supplemented with three different indole-3-acetic acid concentrations, 10^{-4} M, 10^{-3} M, and 10^{-2} M to produce five different coconut water : IAA ratios (v:v), viz: 8:0, 6:2, 4:4, 2:6, 0:8. Distilled water was used as control. These treatment solutions were then injected into the base of developing suckers of plantain. Three weeks after injection treatments, the highest number of well differentiated buds was obtained from 2 ml coconut water and 6 ml of 10^{-2} M IAA (2:6 ratio). Eight ml of coconut water alone (8:0 ratio) also produced the highest number of fully differentiated plantlets. One month after the application of the bud manipulation technique, the highest number of additional well-differentiated axillary buds and fully developed plantlets were produced with 2 ml coconut water plus 6 ml 10^{-2} M IAA and 4 ml coconut water plus 4 ml 10^{-2} M IAA, respectively. The study generally showed that treatments with coconut water from fully matured dried fruits alone produced the largest and the most vigorously growing plantlets. On the other hand, coconut water from fully matured dried fruit combined with IAA induced significant proliferation of axillary bud and shoot formation of plantain significantly over the control treatment. These findings demonstrated the potential of using coconut water from fully matured dried fruits alone or in combination with IAA at ratios of (2:6) and (4:4) to improve the multiplication rate of plantain planting materials.

Key words: coconut water, indole-3-acetic acid, plantain, axillary buds, multiplication rate, *in vivo*

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INTRODUCTION

Plantain (*Musa* AAB) is an important source of high-calorie energy in the diet of many people of the entire West African sub-region [1]. It provides about 70 million people in sub- Saharan Africa with more than 25 % of their carbohydrate needs [2].

In Ghana, plantain is ranked third after yam and cassava in the food crop sector [3]. It contributes about 13.1 % of the Agricultural Gross Domestic Product, AGDP [3]. National production is about 3.6 million metric tonnes per about 90 % of plantains produced [4].

One major challenge to the production of plantain is the lack of large numbers of healthy uniform -sized planting materials (suckers) to plant [5]. Farmers usually plant suckers infected with pests and diseases including nematodes and plantain weevils, banana streak and cucumber mosaic viruses [6, 7]. All these diseases and pests are transmitted through infected suckers and adversely affect the growth and yield of plantains. Since there is unrestricted movement of infected plantain suckers in the country, there is a real danger of an epidemic if steps are not taken to produce large numbers of disease and pest free suckers for farmers.

In vitro and rapid field multiplication techniques of plantains have been developed to overcome the problem of obtaining many vigorous and uniform suckers free from pest and diseases [8]. However, suckers obtained from *in vitro* techniques are prone to the banana streak virus disease which is known to be integrated in the plantain genome [9]. The *in vitro* techniques again require expensive equipment and expertise that are unavailable to most plantain farmers in Ghana. These challenges call for the development of appropriate and adoptable field techniques for farmers.

An appropriate and inexpensive rapid field multiplication of plantains is the split corm technique. The technique involves paring and splitting plantain corms into smaller pieces and sprouting them in moist sawdust or soil to permit dormant axillary buds to sprout. However, the multiplication ratio of the conventional split corm technique is about 1:4 when a regular sized plantable sucker is used. Again, suckers generated from the technique do not grow as vigorously as sword suckers especially when corms are cut into more than four pieces [8]. It is, therefore, necessary to develop other approaches to improve the efficiency of the split-corm technique.

Plantain suckers develop from axillary buds pre-formed on the corm. Therefore, stimulation of axillary bud formation on plantain corm could possibly increase the multiplication rate of the split corm technique. Research has shown that axillary bud formation and development of plantain could optimally be promoted when the corms are injected with 8 ml coconut water from fully matured dried fruits or 4 ml of 10^{-2} M benzyl adenine [8]. A multiplication ratio of up to 1: 15 was obtained when 3-month old split-corm derived suckers were used [8].

Differentiation of plant tissues into roots, shoots and flowers is referred to as organogenesis in tissue culture. The optimal ratios of cytokinin and auxins are very

important during organogenesis [10-12]. In *in vitro* micro-propagation of plantain, high auxin: cytokinin ratios induce root formation whereas low auxin: cytokinin ratios promote shoot formation. Intermediate auxin: cytokinin ratios promote callus formation which may differentiate into root and shoot [13].

Coconut (*Cocos nucifera*) water is a natural source of phytohormones such as auxins and cytokinins [14- 17]. Cytokinin (trans- zeatin riboside) is the most active phytohormone in fully matured dried coconut water [14, 15]. It is, therefore, possible that by varying the ratios of coconut water from fully matured dried fruits and IAA in plantain plant, the cells may be stimulated to develop into multiple shoots (suckers) and/or roots.

Objective of the study

The main objective of the study was to evaluate *in vitro* micro-propagation principles to develop a rapid field multiplication technique of plantain that would allow for production of many vigorous and uniform growing suckers at a reduced cost.

A specific objective of this study was to determine the rate of initiation and development of axillary buds of split corm-derived plantain suckers into plantlets after treatment with different ratios of IAA mixed with coconut water (from fully matured dried fruits).

MATERIALS AND METHODS

Plant Material

Maiden suckers of local plantain cultivar Asamienu were used for the experiment. The suckers were obtained from corms of mother plants. The corms were pared and split into mini-sets of about 300 g. The mini-sets were treated with Bendazim (carbendazim fungicide) solution at a concentration of 2 g per litre of water to prevent fungal infection and rotting. The mini-sets were then nursed in sterilized sawdust in nursery boxes to sprout. Watering was done immediately the mini-sets were planted in sawdust and afterwards whenever necessary. Sprouted mini-sets were selected after one month of nursing and planted in a field at a spacing of 1 m × 0.5 m. Then 100 g of NPK (15-15-15) compound fertilizer was applied to each plant six weeks after planting. Watering and weeding were done whenever necessary. Three months after planting, the developing suckers were used in two experiments.

Preparation of the coconut water

The coconut water was boiled for six minutes, cooled and filtered through a filter paper. The filtrate obtained was used in the application of treatments. Distilled water was used for the control treatment.

Treatments

The treatments consisted of a combination of coconut water (CW) from 13 month- old fully matured dried fruit and indole-3-acetic acid (IAA) at ratios of 8: 0, 6: 2, 4: 4, 2:6, 0:8 v/v. Three different concentrations of IAA were used (10^{-4}, 10^{-3} and 10^{-2} M) to produce a total volume of 8 ml each as shown in Table 1.

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Application of the treatments *in vivo*

The treatments were administered by injecting the treatment solutions at the base of the developing suckers at about 5 cm from the soil surface. Plant height, girth, and number of leaves were measured prior to the application of the injection treatments.

The treatments were repeated three times on three consecutive alternate days. Each treatment consisted of six suckers and was replicated three times in a randomized complete block design. After three weeks of application of the different treatments, the plant height, girth, and number of leaves of the suckers were measured.

The suckers were then carefully dug out from the soil. The roots were removed and corms washed to expose the developing axillary buds and well- developed plantlets (plants with leaves) for counting. The well- developed plantlets were removed from the mother corms and the mother corms then subjected to the bud manipulation technique to produce additional plantlets for each treatment. The bud manipulation technique involved cutting off the pseudostem close to the corm and then carefully removing leaf sheaths from the corm, layer by layer to expose the lateral buds. Resulting corms after sheath removal were planted in humidified chambers filled with sterilized sawdust and covered with transparent polyethylene sheets to maintain high relative humidity. The sawdust was sterilized by steam heating at 100 ^{0}C for 30 minutes in 200-litre metal drums. It was left to cool off overnight before being used. Watering was done immediately after planting the sheath-free corms and at 2-day intervals. Six weeks later, data was collected on additional plantlets produced.

Measurements were taken on plant height and girth**,** produced number of fully differentiated axillary buds and fully developed plantlets, plant height, plant girth number of leaves and total leaf area of the plantlets, number of roots, root length, root dry weight of the injected suckers. Percentage increase in plant height and girth were also calculated.

Statistical analysis

Data were statistically treated using analysis of variance (ANOVA). The least significant difference (LSD) ($p \leq 0.05$) test was used to separate the means. Statistical analyses were performed using the Genstat 5.0 Release 4.23DE, Discovery Edition 1.

RESULTS AND DISCUSSION

Cytokinin and Auxins have been comprehensively studied for their roles in organogenesis on a wide range of cultivated crops [10- 13]. The stimulatory effect of coconut water alone or in combination with IAA on *in vivo* proliferation and development of axillary buds into suckers of plantain was established in this study. Coconut water alone or in combination with IAA gave varied responses for the *in vivo* proliferation and development of axillary buds due to the varying ratios of cytokinin and auxins present in the treatments.

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The effect of treatment with varying ratios of coconut water and indole-3-acetic acid on growth of 3-month old split corm-derived Asamienu suckers.

Growth in girth was significantly increased from treatment with coconut water alone and in combination with IAA over the control (Figure 1A). The highest percentage increase in girth (85 %) was observed with suckers treated with 4 ml coconut water plus 4 ml IAA (4:4 ratio). Treatments with distilled water (control treatment) resulted in the lowest increase in girth. It was observed that all the treatments with 10^{-2} M IAA produced greater growth in girth than the other treatments with lower concentrations of IAA (10^{-3} or 10^{-4} M).

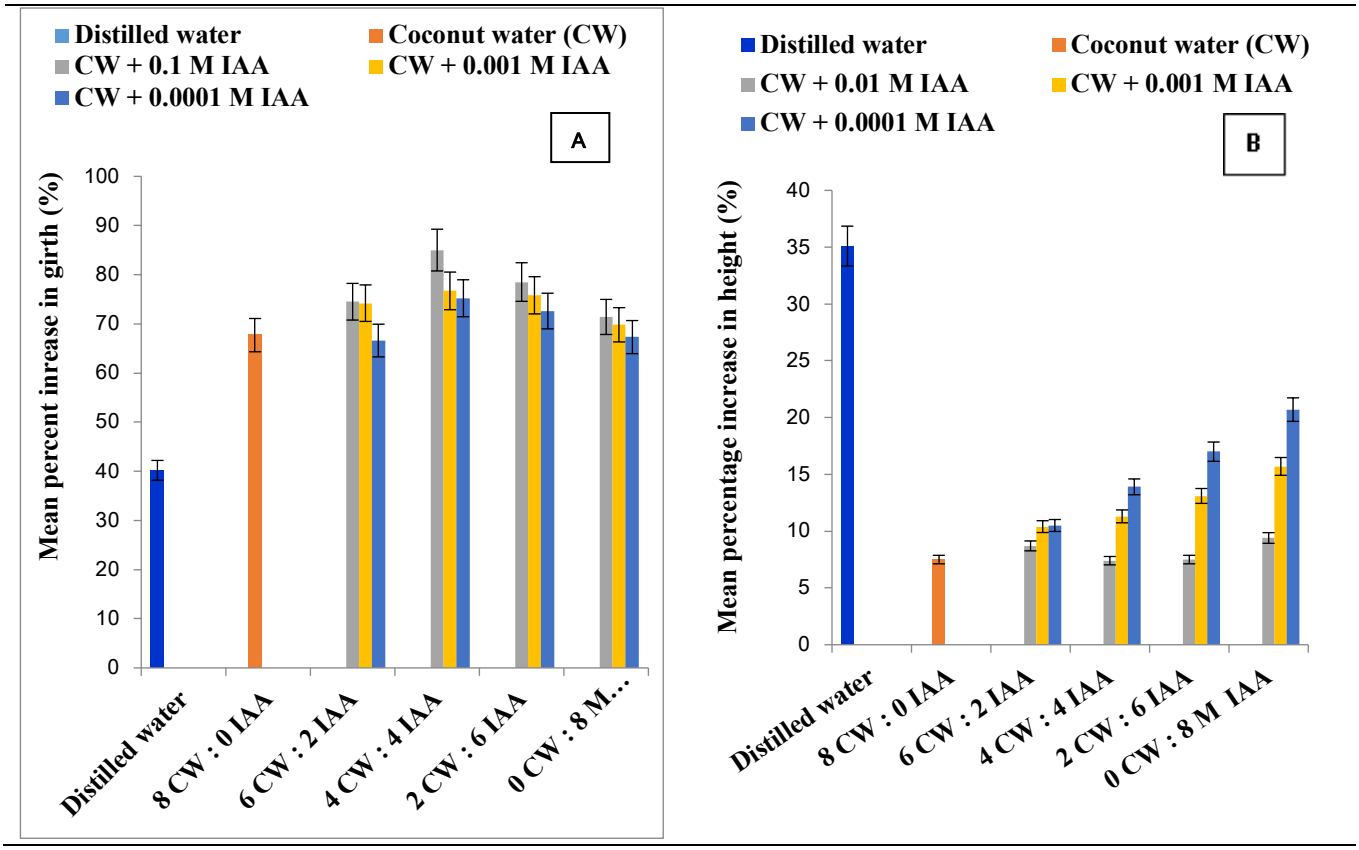

Figure 1: Percentage increase in girth (A) and height (B) of three months old split corm-derived Asamienu suckers three weeks after injection with varying ratios of coconut water CW: indole-3-acetic acid (IAA), viz: (8: 0, 6: 2, 4: 4, 2:6, 0:8 v/v) at three different concentrations of IAA at 0.01M, 0.001M and 0.0001M. Bars shows standard deviation with n=6

All the treatments with coconut water alone or in combination with IAA significantly reduced growth in height (Figure 1B) compared to the control treatment. Suckers treated with 4 ml coconut water plus 4 ml 10^{-2} M IAA increased height by only 7.4 % whilst those treated with distilled water increased height by 35.1% after the injection treatment. It was also observed that all the treatments with 10^{-2} M IAA reduced growth in height more than with the lower concentrations of IAA (10^{-3} or 10^{-4} M IAA).

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The decrease growth in height and significant increase in girth within the 3-week period after the injection with IAA or coconut water resulted from decreased upward growth in the apical meristem and increased lateral cellular activity [18]. The increased lateral cellular activity enhanced development of axillary buds on the corm and also promoted the release of dormant axillary buds from dormancy and developed into plantlets. Similar results were obtained in other studies when three months old split corm-derived plantain suckers were treated with benzyl adenine or coconut water from fully matured dried fruits [8].

The effect of treatment with varying ratios of coconut water and IAA on growth of plantlets derived from three- month old split corm Asamienu suckers

Figure 2 (A, B, C, D) show the mean plant height, girth, leaf number and total leaf area of plantlets generated from the corms of Asamienu suckers treated with different ratios of coconut water and IAA, respectively. Plant height, girth, leaf number and total leaf area of plantlets obtained from treatment with coconut water alone was significantly higher than that derived from the other treatments. Coconut water alone produced bigger plantlets than that of the other treatments.

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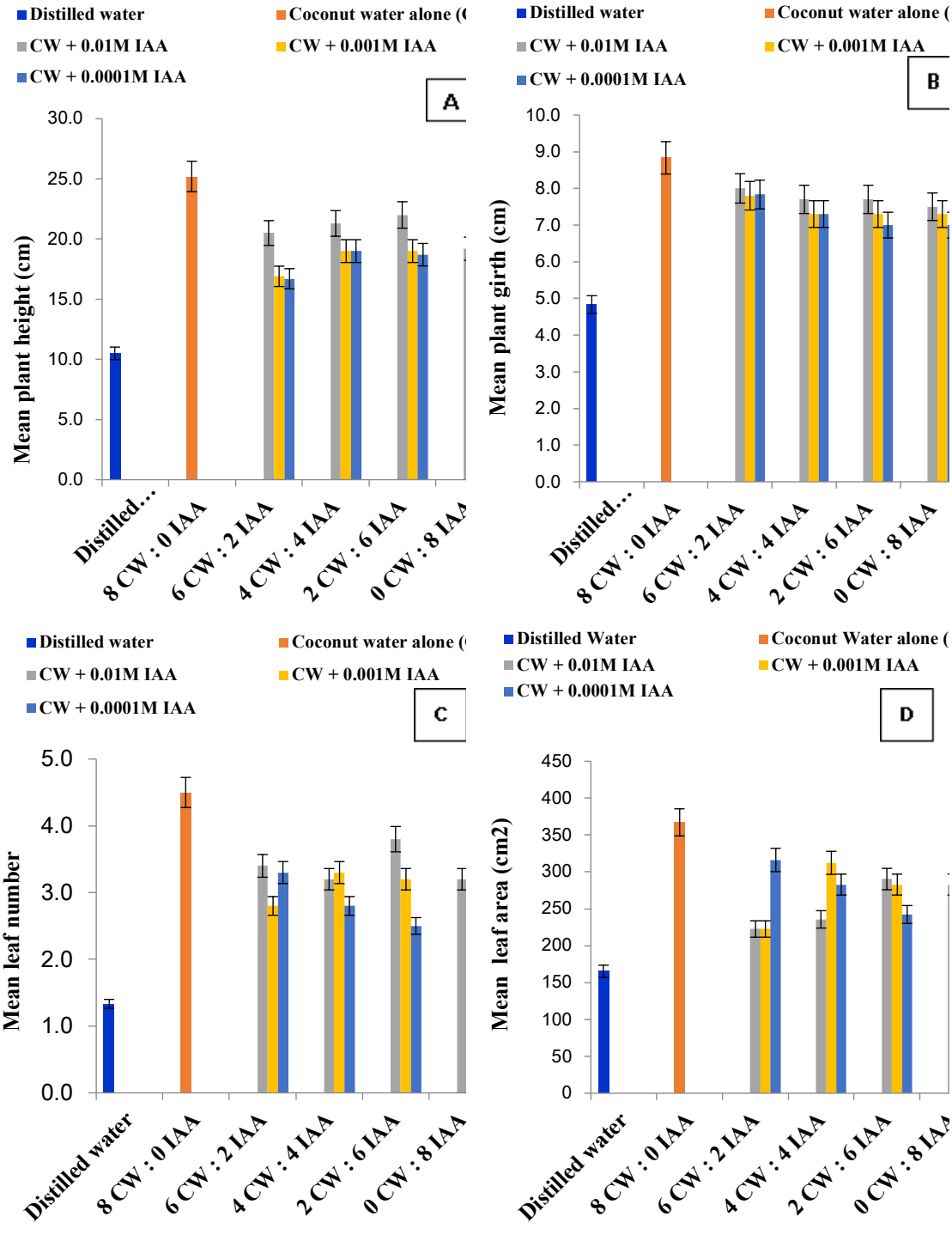

Figure 2: Mean plant height (A), girth (B), number of leaves (C) and leaf area (D) of plantlets derived from three months old split corm Asamienu suckers treated with varying ratios of coconut water CW: indole-3-acetic acid (IAA), viz: (8: 0, 6: 2, 4: 4, 2:6, 0:8 v/v) at three different concentrations of IAA, 0.01M, 0.001M and 0.0001M. Bars shows standard deviation with n=6

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This observation affirms that coconut water from fully matured fruits contains higher amount of cytokinin, especially trans-zeatin riboside which promotes shoot growth [8]. It had been reported from a study that direct application of cytokinin to axillary buds promotes axillary buds' outgrowth even in intact plants [19].

Proliferation of axillary buds and plantlets after treatment with varying ratios of (coconut water and IAA) and the bud manipulation technique

Figure 3 (A and C), shows the number of fully differentiated axillary buds and plantlets produced by injecting 3-month old split corm derived Asamienu suckers with varying ratios of coconut water and IAA, respectively. The number of well-differentiated axillary buds increased as the IAA concentration and volume increased up to 6 ml 10^{-2} M IAA concentration and then declined as the volume was increased. The highest number of well-differentiated axillary buds was produced with 2 ml coconut water plus 6 ml 10^{-2} M IAA. By the third week after the injection treatments, some of the well-differentiated axillary buds had already developed into plantlets. All the treatments with coconut water alone or in combination with IAA produced a higher number of fully developed plantlets than the control treatment. The highest number of fully developed plantlets (4.8) was produced with 8 ml coconut water treatment alone.

Figures 3 (B and D) shows the number of fully differentiated axillary buds and plantlets developed from corms of 3-month old split corm-derived Asamienu suckers one month after the application of the bud manipulation technique respectively. The number of well-differentiated axillary buds increased as the IAA concentration and volume increased up to 6 ml 10^{-2} M AA and then declined as the volume increased further. The highest number of well-differentiated axillary buds (22.5) was produced with 2 ml coconut water plus 6 ml 10^{-2} M IAA. Coconut water alone or in combination with IAA produced a higher number of well-differentiated axillary buds than the control treatment. Coconut water in combination with 10^{-2} M IAA produced a higher number of plantlets at the varying ratios than with the other concentrations (10^{-3} M and 10^{-4} M). The highest number of fully developed plantlets (4.0) was obtained with 4 ml coconut water plus 4 ml 10^{-2} M IAA (4:4 ratio). Coconut water alone or in combination with IAA produced higher number of plantlets than the control treatment.

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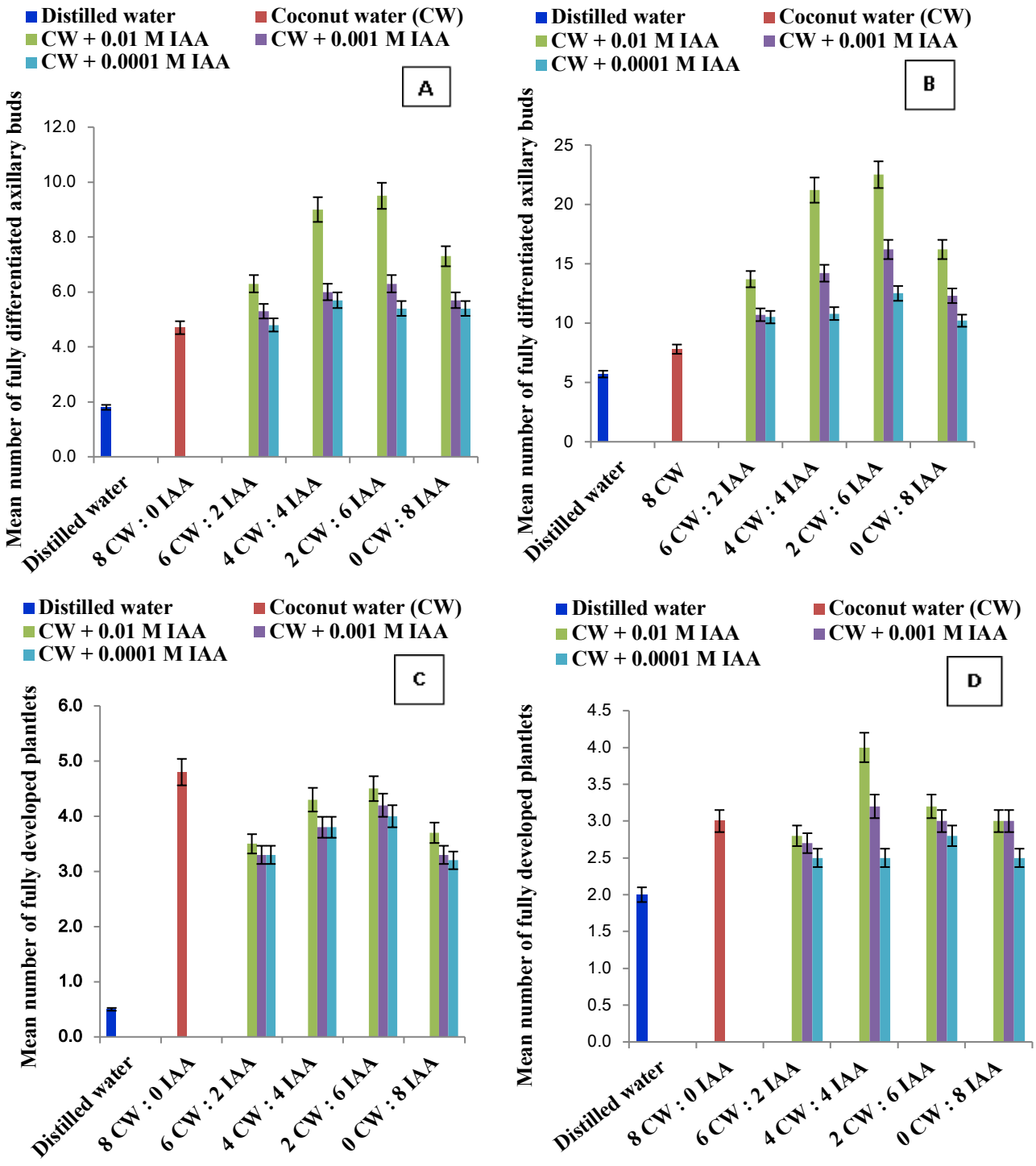

Figure 3: **Mean number of fully differentiated axillary buds (A) and plantlets (C) derived from three months old split corm Asamienu suckers treated with varying ratios of coconut water CW: indole-3-acetic acid (IAA), viz: (8: 0, 6: 2, 4: 4, 2:6, 0:8 v/v) at three different concentrations of IAA, 0.01M, 0.001M and 0.0001M. Mean number of fully differentiated axillary buds (B) and plantlets (D) developed from corms of 3-month old split corm-derived Asamienu suckers one month after the application of the bud manipulation technique. Bars show standard deviation with n= 6**

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These results clearly show that proliferation of fully differentiated axillary buds can be induced in plantain by injecting 3-month old split corm-derived well-developed and actively growing suckers with 2 ml or 4 ml coconut water from fully matured dried fruits combined with 6 ml or 4 ml 10^{-2} M IAA, respectively three weeks after the injection treatment. It has been reported that when meristematic stem cuttings explants were cultured on MS medium containing 4.0 mg/l BAP plus 2.0 mg/l IAA plus 13 % (v/v) coconut water, the highest percentage of shoot regeneration (90 %) and maximum number of shoots (10) per explant were obtained [20]. Two ml or 4 ml coconut water from fully matured dried fruits combined with 6 ml or 4 ml 10^{-2} M IAA, respectively also showed a carryover effect on the treated corms for production of fully differentiated axillary buds after the application of the bud manipulation technique and sprouting the corms in moist sawdust for one month. The synergistic effect of coconut water and IAA was found to be optimum for proliferation of axillary buds' development at these combinations (2 ml or 4 ml coconut water from fully matured dried fruits combined with 6 ml or 4 ml of 10^{-2} M IAA, respectively).

The number of fully differentiated axillary buds per plant declined in the treatments with coconut water alone as a result of increased endogenous content of basipetally flow IAA due to increased leaf production. This finding agrees with the observation that apically derived-auxin transported basipetally inhibits outgrowth of axillary buds [21].

Production of plantlets three weeks after injection treatment and one month after the application of the bud manipulation technique

The total number of plantlets produced from corms of 3-month old split corm-derived Asamienu suckers three weeks after the injection treatment and one month after the application of the bud manipulation technique is represented in Figure 4. Coconut water alone or in combination with all the various IAA concentrations produced a significantly higher number of plantlets than the distilled water treatment. The number of plantlets produced from all the treatments was more than double that for the distilled water. The total number of plantlets produced with coconut water alone was similar to that from 2 ml coconut water plus 6 ml of 10^{-2} M IAA. The highest number of plantlets (8.3) produced was obtained with 4 ml coconut water plus 4 ml of 10^{-2} M IAA and was significantly higher than that obtained from distilled water (2.5).

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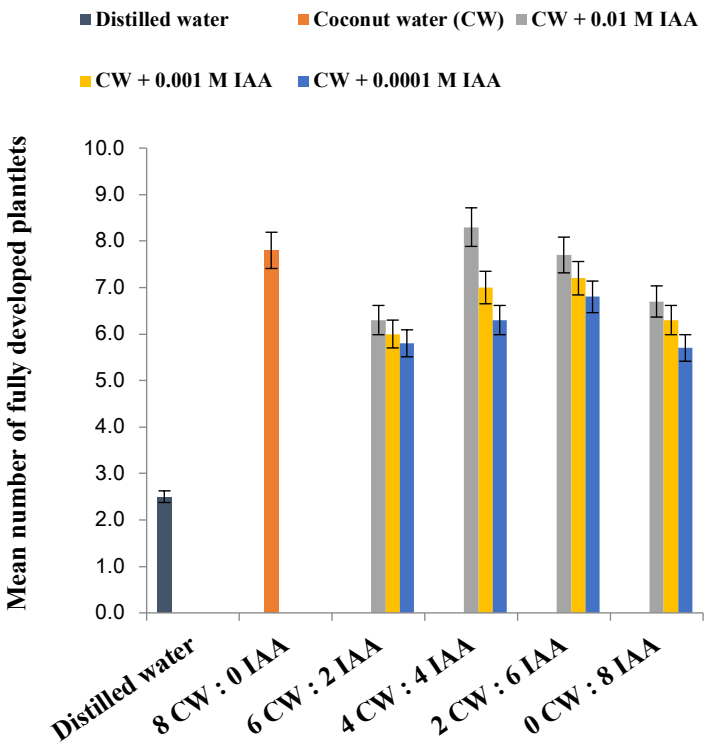

Figure 4: Total number of fully developed plantlets per plant obtained from corms of 3-month old split corm –derived Asamienu suckers three weeks after the injection treatment and one month after the application of the bud manipulation technique. Bars show standard deviation with n=6

A study showed that 2.2 mg L^{-1} N^6-benzylaminopurine with 0.2 mg L^{-1} IAA was most effective for shoot multiplication in several banana cultivars [22]. Also, it was shown that the maximum number of plantlets was obtained when 8.0 µM IAA and 10.0 µM of benzyl aminopurine were used in the micro propagation of banana [22]. It is reported that auxins and cytokinin play a major role in the pattern of growth and development in *Musa* Spp [23].

CONCLUSION

The results from this study demonstrated the potential use of coconut water from fully matured fruits alone or in combination with IAA to improve the multiplication rate of plantain planting materials. The results indicated that proliferation of axillary buds and their development into suckers in plantain could be induced *in vivo* by injecting 3-month old split corm-derived suckers with coconut water alone or in combination with IAA. Formation of axillary buds was probably enhanced as a result of increased endogenous trans-zeatin riboside content produced in the roots. On the other hand, treatments with higher levels of exogenous trans-zeatin riboside (represented by coconut water from fully matured dried fruits) enhanced growth of axillary buds into suckers.

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The study shows that rapid field multiplication of plantain suckers can be achieved using this newly developed technique, employing coconut water alone or in combination with IAA. The technique involves mixing 2 ml or 4 ml of coconut water from fully matured dried fruit with 6 ml or 4 ml of 10^{-2} M IAA, respectively and injecting the mixture into the base of developing suckers to induce proliferation of axillary buds and shoot formation of plantain. Significant growth of plantain axillary buds over the control treatment resulted in production of many viable and uniform size plantain suckers.

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Table 1: Varying ratios of coconut water from fully matured dried fruits with IAA at three different concentrations (10^{-4}, 10^{-3} and 10^{-2} M) used for the injection treatments

Volume of Coconut Water (ml)	Volumes of IAA (ml)		
	10^{-4} M	10^{-3} M	10^{-2} M
8	0	0	0
6	2	2	2
4	4	4	4
2	6	6	6
0	8	8	8

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