

## Effect of coconut (*Cocos nucifera* L.) water of different fruit maturity stages on axillary bud initiation, growth and development of plantain (*Musa* AAB.)

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### Abstract

The effect of endogenous content of cytokinin (trans- zeatin riboside, T-ZR) and auxin (indole-3-acetic acid, IAA) in coconut water from fruits at four different fruit maturity stages: liquid endosperm, solid endosperm formation stage, semi matured endosperm stage and fully matured dried fruit stage on proliferation of axillary bud initiation and development of local plantain cultivar (cv. Asamienu, *Musa* AAB) was investigated at the University of Ghana Forest and Horticultural Crops Research Centre, Kade. The results indicated that the IAA content decreased while the T-ZR content increased with fruit maturity. Three weeks after treatments, the highest number of well-differentiated axillary buds was obtained for suckers treated with coconut water from fruits at liquid endosperm formation stage whilst the highest number of fully developed plantlets was produced from treatments with coconut water at semi-endosperm formation stage. One month after the application of the split corm technique, the highest number of additional well-differentiated axillary buds and fully developed plantlets were produced from treatments with coconut water at the liquid endosperm formation stage. In general, treatments with coconut water from fully matured dried fruits produced the largest and the most vigorously growing plantlets.

**Key words:** Coconut water, trans- zeatin riboside, indole-3-acetic acid, axillary bud, plantain

### 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 (Fernande, 2011). In Ghana, plantain is ranked third after yam and cassava in the food crop sector (FAO, 2010). National production is about 3.6 million metric tonnes per annum (Dzomeku *et al.*, 2011) and about 90% of plantains produced is consumed locally (Dzomeku *et al.*, 2011). It contributes about 13.1 % of the Agricultural Gross Domestic Product, AGDP (FAO, 2010).

The present low production level is due to several constraints including lack of adequate certified planting material (Thiemele *et al.*, 2015), disease and pest problems, poor soil fertility management and lack of adequate knowledge about agronomic practices. Traditionally, farmers obtain suckers from old farms. Such suckers may be infected with pests and diseases including nematodes (*Helicotylenchus* spp, *Practelenchus* spp and *Rhadopholus* spp), the plantain weevil, *Cosmopolites sordidus* (Were *et al.*, 2015), banana streak and cucumber mosaic viruses (Kouassi, 2010), all of which are transmitted through infected suckers and adversely affect the growth and yield of plantains.

Various methods have been developed to address the lack of adequate certified planting material in large numbers and this include the split-corm, tissue culture (*in vitro*) and decapitation techniques as well as other micro and macro propagation multiplication methods. All these techniques have yielded some good results (Langford *et al.*, 2017).

An appropriate and inexpensive method of 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 vigorous as sword suckers especially when corms are split into more than four pieces. 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. Hence, stimulation of axillary bud formation on plantain corm could possibly increase the multiplication rate of the split corm technique. Osei (2006) showed that axillary bud formation and development of plantain could be promoted when injected with 8 mL coconut water from fully matured dried fruits or 4 mL of 10<sup>-2</sup> M benzyl adenine.

The optimal ratio of cytokinin and auxins is very important during differentiation of plant tissues into roots, shoots and flowers (organogenesis) (Haq and Dahot, 2007). 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 (Jafari, *et al.*, 2010).

Coconut water is a natural source of auxins and cytokinins (Yong *et al.*, 2009). Auxin (indole-3-acetic acid, IAA) and cytokinin

(trans- zeatin riboside) are the most active phytohormones in coconut water (Yong *et al.*, 2009). Coconut water contains varying ratios of cytokinins and auxins concentrations at different fruit maturity stages as has been found for many fruits (Lazim *et al.*, 2015). Generally, the auxin: cytokinin ratio is higher at the early fruit maturity stage while cytokinin: auxin ratio is higher at later stages of fruit maturity (Lazim *et al.*, 2015). It is therefore possible that application of coconut water from fruits at different maturity stages to plantain plant, could stimulated the cells to develop into multiple shoots (suckers) and/or roots. Objective of the study was to evaluate the rate of proliferation of plantain axillary buds formation and growth into suckers after treatment with coconut water from fruits at different maturity stages.

## Materials and methods

**Analysis of coconut water on local plantain cultivar Asamienu:** Maiden sucker of local plantain cultivar Asamienu was used for the study. The suckers were obtained from mother plants and the corms were pared and split into mini-setts of about 300 g. The mini-setts were treated with Bendazim (carbendazim fungicide) at the rate of 2g per litre of water to prevent fungal infection and rotting. The mini-setts were planted in sterilized sawdust in nursery boxes to sprout. Watering was done immediately the mini-setts were planted in the sawdust and afterwards whenever necessary.

One month after nursing the split corms, uniformly sprouted mini-setts were selected and planted in a field at a spacing of 1m× 0.5m. After six weeks of planting the sprouted mini- setts, 100g NPK (15-15-15 compound fertilizer) was applied to each plant. Watering and weeding were done whenever necessary. Three months after planting the sprouted mini- setts, the developing suckers were injected with coconut water from fruits at different maturity stages: liquid endosperm formation stage (6 months after flowering) - LE, solid endosperm formation stage (8 months after flowering) -SE, semi matured endosperm stage (10 months after flowering) -SME, fully matured dried fruit stage (13 months after flowering) - FMD and combination of LE and FMD.

The coconut water was boiled for six minutes, cooled and filtered through Whatman No. 4 filter paper before using the filtrate for the treatments. A control with distilled water (DW) was included. The treatments were administered at the base of the developing suckers at about 5 cm from the soil surface. The injection was repeated three times on three consecutive alternate days. Each treatment consisted of six plants. The treatments were replicated three times in a randomized complete block design.

The suckers were 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. Six plants from each treatment were selected randomly for growth measurements. After removing the well developed plantlets, the split corm technique was used to produce additional plantlets for each injection treatment. Corms of the treated suckers were planted in sterilized sawdust. The sawdust was steam sterilized at 100 °C for 30 minutes in 200-litre iron drums. After sterilization, the sawdust was left overnight to cool before being used. Watering was done immediately after planting and at 2 days interval afterwards. Six weeks later, the number of additional plantlets produced by the treated corms was counted.

The additional plantlets produced were removed from the corms and potted as described earlier. 20 g NPK (15-15-15 compound fertilizer) was applied to each potted plant. Each treatment was replicated three times.

Data was collected on: percentage increase in plant height and girth, number of fully differentiated axillary buds and fully developed plantlets produced, number of roots, root length and root dry weight of the injected suckers, plant height, plant girth, number of leaves and total leaf area of the plantlets.

**Analysis of cytokinin (trans-zeatin riboside) and auxin (indole-3-acetic acid, IAA) in coconut (*Cocos nucifera L.*) water:** The endogenous content of trans-zeatin riboside and indole-3-acetic acid, IAA was determined by the modifications of those described by Bollmark *et al.* (1988) and He (1993).

**Coconut water pretreatment and T-ZR and IAA isolation:** Coconut water from the fruits at the different maturity stages namely, liquid endosperm formation stage, solid endosperm formation stage, semi matured endosperm stage, fully matured dried fruit stage were boiled for 6 minutes and allowed to cool. The cooled coconut water samples were then filtered through Whatman No. 4 filter paper to remove suspended matters. 100 mL filtrate of the each of the coconut water samples were passed through Chromosep C<sub>18</sub> columns (C<sub>18</sub> Sep-Park Cartridge, Millford, MA) which had been previously washed sequentially with 10 mL methanol-acetic acid (100: 1, v/v), methanol- water- acetic acid (50: 50:1, v/v/v), methanol- water- acetic acid (30: 70:1, v/v/v) and finally with water. The hormone fractions were eluted with 10 mL of 100 % methanol and 10 mL ether. The elutes were then dried under N<sub>2</sub> and then dissolved in 2 mL phosphate buffered saline (PBS) containing 0.1 % Tween 20 and 0.1 % gelatine (pH 7.5) for quantification by ELISA (Enzyme Link Immuno- Sorbent Assay) method.

**Quantitative analysis of T-ZR and IAA by ELISA in coconut water:** ELISA was performed on 96-well microtitration plate. Each well on the plate was coated with 100 µL coating buffer (pH 9.6, 1.5 g L<sup>-1</sup> Na<sub>2</sub>CO<sub>3</sub>, 2.93 g L<sup>-1</sup> NaHCO<sub>3</sub> and 0.02 g L<sup>-1</sup> NaN<sub>3</sub>) and contained 0.25 µg mL<sup>-1</sup> antigens against T-ZR and IAA. The coated plates were incubated for 16 hrs at 4 °C for T-ZR and overnight at 4 °C for IAA and then kept at room temperature for 40 to 50 minutes. After washing with Phosphate Buffered Saline (PBS) and 0.1 % Tween 20 buffer (pH 7.4) four times, each well was filled with 50 µL of either the coconut water extracts or T-ZR and IAA standards (0 - 10 µg mL<sup>-1</sup> dilution range) and 50 µL of 20 µg mL<sup>-1</sup> antibodies against T-ZR and IAA. The plates were incubated for 1 hr at 37 °C and overnight at 4 °C for T-ZR and IAA respectively. 100 µL of 1.25 µg mL<sup>-1</sup> G- horse radish peroxidase substrate was added to each well and incubated for 1 hr at 30 °C. The plates were rinsed five times with Tween 20 buffer containing PBS and 100 µL color –appearing solution containing 1.5 mg mL<sup>-1</sup> o-phenylenediamine and 0.008 % H<sub>2</sub>O<sub>2</sub> was added to each well. The reaction progress was stopped by adding 50 µL 6N H<sub>2</sub>SO<sub>4</sub> per well when the 100 µg mL<sup>-1</sup> standard had a pale color and the 0 µg mL<sup>-1</sup> standard had a deeply thick color in the wells. Color development in each well was detected using an ELISA Reader (Model EL 310, Bio-TEK) at OD A490. Contents of T-ZR and IAA were calculated by the method of Weiler and Ziegler (1981).

**Statistical analysis:** Data collected in each experiment were

analyzed using analysis of variance procedure with Genstat 5.0 Release 4.23DE, Discovery Edition 1 (GENSTAT, 2003). Duncan Multiple Range Test was employed for mean separation. Correlation analysis was used to determine the relationship between the T-ZR and IAA contents in coconut water from fruits at different maturity stages. Graphs were used for the presentation of results.

## Results and discussion

**Changes in endogenous content of T-ZR and IAA in coconut water at different fruit maturity stages:** Endogenous contents of T-ZR and IAA in coconut water at different fruit maturity stages is shown in Fig. 1. The contents of T-ZR and IAA in coconut water varied at the different fruit maturity stages. IAA content decreased from the liquid endosperm formation stage ( $1.51 \mu\text{g mL}^{-1}$ ) to fully matured dried stage ( $0.75 \mu\text{g mL}^{-1}$ ). The T-ZR content on the other hand increased from the liquid endosperm formation stage ( $0.44 \mu\text{g mL}^{-1}$ ) to fully matured dried stage ( $1.01 \mu\text{g mL}^{-1}$ ). The correlation coefficient ( $r$ ) between T-ZR and IAA measured was highly significant ( $P < 0.05$ ). There was a strong negative linear relationship ( $r = -0.93$ ) between T-ZR and IAA. The strong negative correlation between T-ZR and IAA contents indicated that as the coconut fruit matures, the IAA content in the coconut water decreases and *vice versa* for T-ZR. This finding is in agreement with studies by a number of researchers (Ge *et al.*, 2007; Ma *et al.*, 2008, Wu and Hu, 2009). These observations indicated varied effects of coconut water at different fruit maturity stages on the proliferation and development of axillary buds from the treated plantain suckers.

**Effects of coconut water at different fruit maturity stages on percentage increase in height and girth of 3-month old split corm-derived Asamienu suckers:** Fig. 2 shows the percentage increase in growth in height and girth of the 3-month old split corm-derived Asamienu suckers 3 weeks after injection with coconut water of different fruit maturity stages. Coconut water from fruits of the various maturity stages significantly decreased percentage growth in height compared with suckers treated with distilled water. The suppressive effect of coconut water on plant growth in height increased as the fruit matured. Suckers treated with distilled water had the highest percentage increase in plant height (36.5%) and the lowest percentage increase in plant girth

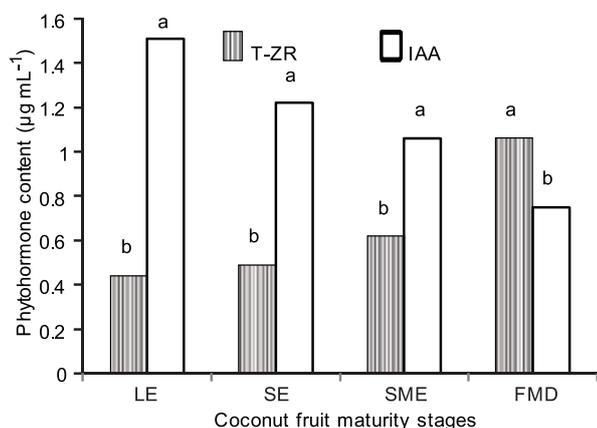


Fig. 1. Changes in endogenous contents of T-ZR and IAA in coconut water at different fruit maturity stages. Mean values with the same letters are not significantly different at ( $P = 0.05$ ) as per DMRT.

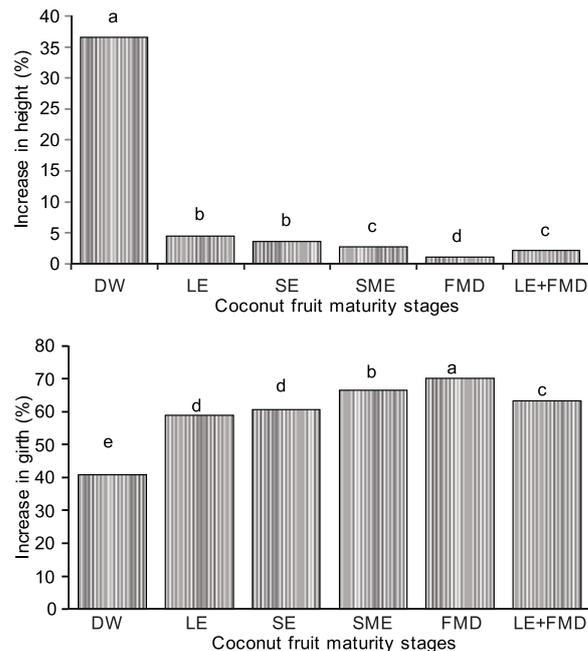


Fig. 2. Effect of coconut water from fruits at different maturity stages on percent increase in height and girth of 3-month old split corm derived Asamienu suckers three weeks after injection. Mean values with the same letters are not significantly different at ( $P = 0.05$ ) as per DMRT.

(40.8%). On the other hand, the increasing effect of coconut water on plant growth in girth increased as the fruit matured. The results also indicated that, coconut water of the fully matured dried stage recorded the lowest percentage growth (1.1%) in height (Fig. 2) and also the highest increasing effect on the percentage growth in girth of 70.1% (Fig. 3). Coconut water from fruits at the various maturity stages recorded significantly increased percentage growth in girth compared with the distilled water treatment.

**Effects of coconut water from different maturity stages on fully differentiated axillary buds, plantlets and root production of 3-month old split corm-derived Asamienu suckers:** Fig. 3 shows the mean number of fully differentiated axillary buds, plantlets and root production, produced by injecting 3-months old split corm derived Asamienu suckers with coconut water from fruits at different maturity stages. The number of well-differentiated axillary buds decreased in treatments with coconut water at liquid endosperm to solid endosperm formation stages and increased again at the semi-endosperm formation stage. The highest number of well-differentiated axillary buds (9.5) was obtained from suckers treated with coconut water from fruits at liquid endosperm formation stage. However, the number of plantlets that developed from the well-differentiated axillary buds with the various injection treatments was highest (5.5) at the semi-endosperm formation stage and the lowest number (0.3) was produced from treatment with distilled water.

Generally, mean root number, mean root diameter and mean root dry weight of the treated suckers increased from liquid endosperm formation stage to fully matured dried stage. Suckers treated with coconut water from liquid endosperm formation stage produced the highest root number (41.1), root diameter (7.9 cm) and root dry weight (14.2 g).

Enhanced root production and proliferation of fully differentiated axillary buds at the liquid endosperm formation stage over the control can be attributed to higher content of auxin (IAA) in

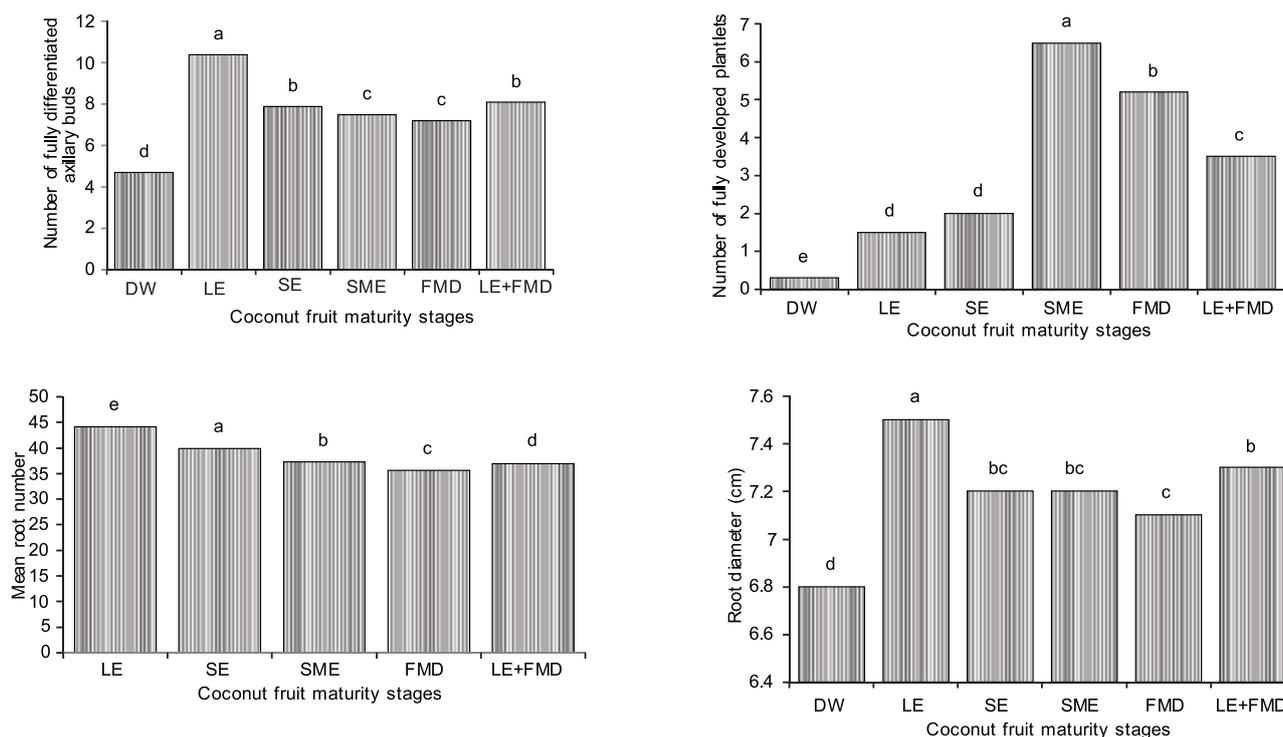


Fig. 3. Effect of coconut water from fruits at different maturity on number of fully differentiated axillary buds, plantlets and root production of 3-month old split corm derived Asamienu suckers three weeks after injection. Mean values with the same letters are not significantly different at ( $P=0.05$ ) as per DMRT.

coconut water at the liquid endosperm formation stage (Ge *et al.*, 2007; Ma *et al.*, 2008). IAA is known to promote root development (Woodward and Bartel, 2005). However, since T-ZR which promotes formation of axillary buds is produced in the roots (Hopkins and Huner, 2004), increased root production will in turn increase the content of endogenous cytokinin produced. It can be deduced from this study that the increased content of endogenous T-ZR due to the increased root production enhanced proliferation of axillary buds. This study confirmed the observations made by Shimizu-Sato and Mori (2001) that cytokinin derived from roots promotes axillary buds outgrowth and auxin derived from a shoot apex regulates cytokinin transport in plants.

The total number of fully developed plantlets produced per plant increased as the coconut fruit matures from liquid endosperm stage to semi-matured endosperm stage and then declined at the fully matured dried fruits stage. This observation indicates that the optimum ratio of IAA and T-ZR to enhance maximum number of axillary buds growth into plantlets or shoots is at the semi-matured endosperm stage. The decrease in number of fully

developed plantlets at the fully matured dried fruits stage is an indication of increased endogenous content of basipetally flow of IAA due to increased leaf production. Shimizu-Sato *et al.* (2009) suggested that apically derived-auxin transported basipetally inhibits outgrowth of axillary buds.

**Effects of coconut water from different maturity stages on the growth of plantlets produced three weeks after the injection treatment and one month after the application of the split corm technique:** Fig. 5 shows the mean plant height, girth, leaf number and total leaf area of plantlets generated from the corms of Asamienu suckers treated with coconut water from fruits at different maturity stages. The results indicated that, plant height, girth, leaf number and total leaf area of plantlets obtained from suckers treated with coconut water from fully matured dried fruits was significantly higher than that derived from suckers treated with distilled water and liquid endosperm formation stage. Plantlets generated from suckers treated with coconut water of all the different stages were bigger than that obtained from the distilled water treatment.

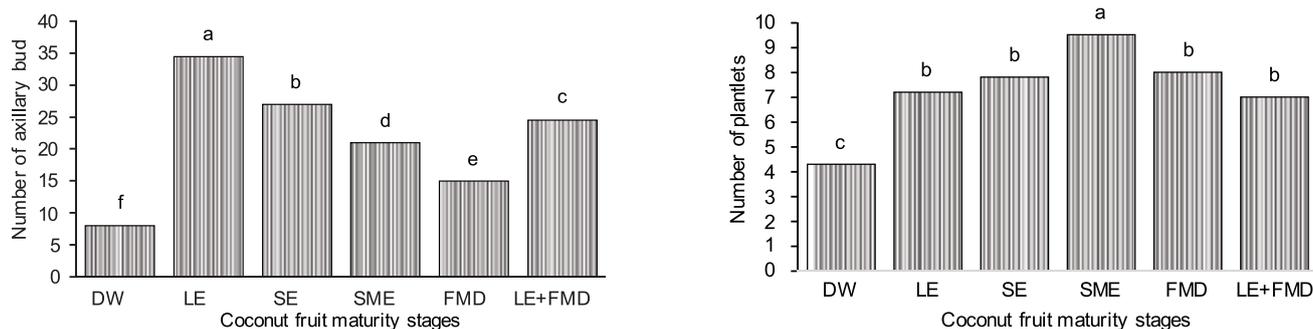


Fig. 4. Mean number of additional axillary buds and plantlets produced three weeks after injection with coconut water from fruits at different maturity stages and one month after application of the split corm technique. Mean values with the same letters are not significantly different at ( $P=0.05$ ) as per DMRT.

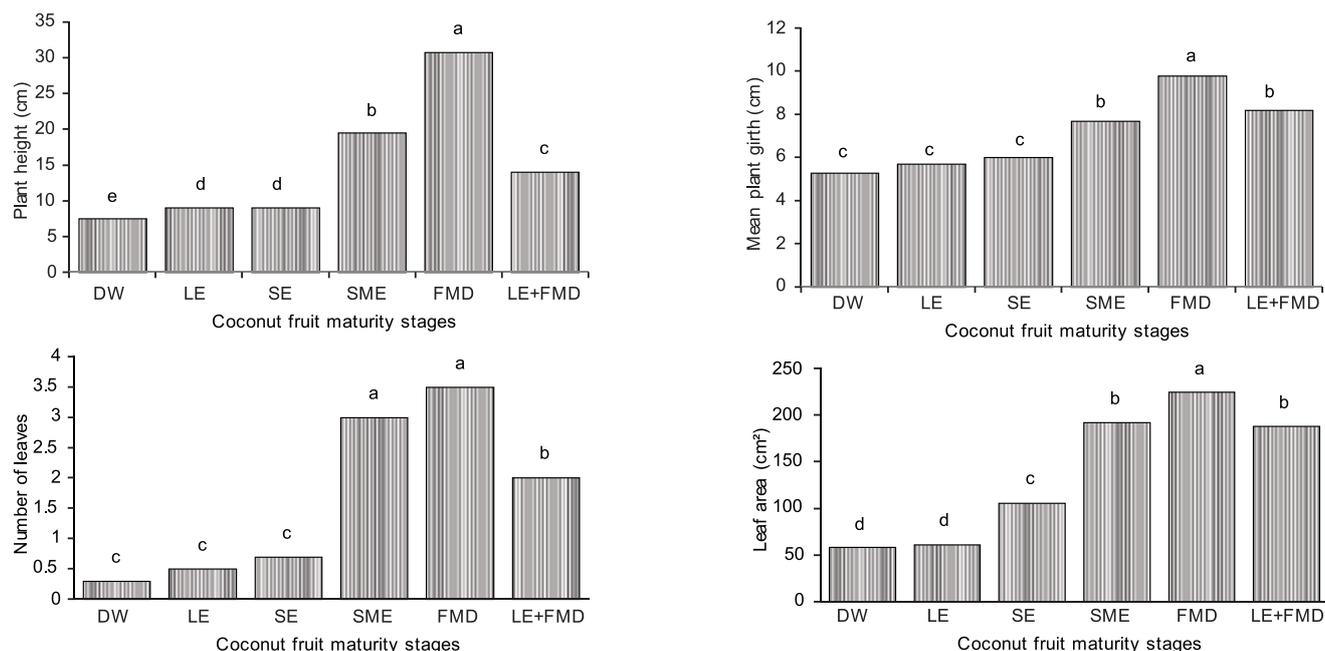


Fig. 5. Effect of coconut from fruits at different maturity stages on growth of plantlets derived from 3-months old split corm Asamienu suckers. Mean values with the same letters are not significantly different at ( $P=0.05$ ) as per DMRT.

The results of these studies indicate that proliferation of axillary buds and their development into suckers in plantain can be induced *in vivo* by injecting 3-months old split corm-derived suckers with coconut water.

Treatment with coconut water from fruits at liquid endosperm formation stage containing higher levels of IAA and lower level trans-zeatin riboside enhanced proliferation of fully differentiated axillary buds. Formation of axillary buds was also promoted 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 (as in coconut water from fully matured dried fruits) enhanced growth of pre-formed axillary buds on the corm into suckers.

The studies show that, rapid field multiplication of plantain suckers could be achieved with this newly developed technique with coconut water.

## Acknowledgements

We thankfully acknowledge financial support from the University of Ghana Research Grant and the staff of the Forest and Horticultural Crops Research Centre-Kade for their field and technical assistance.

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Received: October, 2017; Revised: November, 2017; Accepted: December, 2017