IN VIVO STIMULATION OF AXILLARY BUD INITIATION, GROWTH AND DEVELOPMENT OF PLANTAIN (*MUSA* AAB.) USING COCONUT WATER AND INDOLE-3-ACETIC ACID

BY

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DECLARATION

I hereby declare that except for references to the work of other researchers which have been duly cited, this work is a result of my original research and that this thesis has neither in whole nor in part has been presented elsewhere for the award of another degree.

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DEDICATION

To my family who helped me get this far.

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My sincere gratitude, appreciation and thanks to Jehovah and various individuals who were instrumental in the course of my studies and research. There were many others, who contributed to this piece of work, but it is not possible to mention the names of all individuals but I fully recognize and appreciate your valuable contributions. The ones listed below are just a few of the many contributors.

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ABSTRACT

The study was carried out at the University of Ghana Forest and Horticultural Crops Research Centre (FOHCREC), Kade from 2008 to 2012. Two experiments were conducted to investigate the effects of coconut water (CW) alone or in combination with varying concentrations of auxin (IAA) on axillary bud initiation, growth and development of plantain (cv. Asamienu).

In the first experiment, coconut water from fully matured dried fruits was supplemented with three different IAA 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. Three weeks after the injection treatments, variations were seen in the rate of axillary bud formation and growth. The highest number of well-differentiated axillary buds was produced after injecting with a combination of 2ml coconut water and 6ml 10^{-2}M IAA whilst the highest number of fully developed plantlets was produced with a treatment of 8ml coconut water alone. One month after the application of the bud manipulation technique, the highest number of additional well-differentiated axillary buds and fully developed plantlets was produced with 2ml coconut water plus 6ml 10^{-2}M IAA and 4ml coconut water plus 4ml 10^{-2}M IAA respectively.

In the second experiment, the endogenous content of cytokinin (trans- zeatin riboside) and auxin (IAA) in coconut water from fruits at four different fruit maturity stages: liquid endosperm formation stage (6 months after flowering), solid endosperm formation stage (8 months after flowering), semi matured endosperm stage (10 months after flowering), fully matured dried fruit stage (13 months after flowering) and their effects on the proliferation of axillary bud initiation and development were evaluated. In both experiments, distilled

water was injected as the control. Results of the quantitative analysis of the endogenous content of trans- zeatin riboside and IAA showed major changes at the different fruit maturity stages. The results indicated that as the coconut fruit matured the IAA content in the coconut water decreased while the trans- zeatin riboside content inreased with fruit maturity.

Three weeks after coconut water injection 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 treatment. 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 from treatments with coconut water at the liquid endosperm formation stage. Furthermore, treatments with coconut water from fruits at liquid endosperm formation stage produced the highest additional well-differentiated axillary buds one month after the application of the split corm technique whereas the highest additional fully developed plantlets was obtained from treatments with a mixture of coconut water from liquid endosperm formation stage and fully matured dried fruits. In general, treatments with coconut water from fully matured dried fruits produced the largest and the most vigorously growing plantlets.

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CHAPTER 1

INTRODUCTION

General 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 (Stover and Simmonds, 1987). It provides about 70 million people in sub- Saharan Africa with more than 25% of their carbohydrate needs (Swennen, 1990). Plantain is grown across all the humid agro-ecological zones and forms an integral component of most of the complex farming systems (Swennen and Vuylsteke, 1991).

In Ghana, plantain is ranked third after yam and cassava in the food crop sector (FAO, 2006). National production is about 3.6 million metric tonnes per annum (African News Network, 2007) and about 90% of plantains produced is consumed locally (African News Network, 2007). It contributes about 13.1 % of the Agricultural Gross Domestic Product, AGDP (FAO, 2006).

One major challenge in the production of plantain is the lack of large numbers of healthy uniform -sized planting materials (suckers) to plant (Hotsonyame, 1991). Farmers usually plant suckers infected with pests and diseases including nematodes and plantain weevils (Afreh-Nuamah, 1994), banana streak and cucumber mosaic viruses (Osei, 1995). 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 epidemics if steps are not taken to produce large numbers of disease and pest free suckers for farmers (Osei, 1995).

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 (Vuylsteke and Swennen, 1992). 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 (Dallot *et al*., 2001). 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 vigorous as sword suckers especially when corms are splitted into more than four pieces (Robinson and Alberts, 1983; Ahiekpor, 1993). 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. Osei (2006) showed that axillary bud

formation and development of plantain could maximally be promoted when injected with 8ml coconut water from fully matured dried fruits or 4 ml of 10^{-2} M benzyl adenine. A multiplication ratio of up to 1: 15 was obtained when 3-month old split-corm derived suckers were used. However, 50% of the plantlets generated from this technique did not survive due to poor root development. In another study, Nyamekye (1999) observed that 10^{-3} M solution of indole-3-acetic acid (IAA) significantly improved root production of plantain split corms. However, the initial shoot emergence was delayed which could affect time of flowering and hence maturity period. There is therefore the need to develop rapid field multiplication techniques which will produce multiple shoots that can form roots to improve plant survival and growth and will improve root production and at the same time enhance shoot emergence of split corms.

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 (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 (Cronauer and Krikorian, 1984).

Coconut water is a natural source of phytohormones such as auxins and cytokinins (Salisbury and Ross, 1985; 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 may contain varying ratios of cytokinins and auxins concentrations at different fruit maturity stages as has been found for many fruits

(Salisbury and Ross, 1985). 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 (Salisbury and Ross, 1985).

It is therefore possible that by varying the ratios and time of application of coconut water 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 apply *in vitro* micro-propagation principles to develop an efficient rapid field multiplication technique of plantain that will allow for production of many vigorous and uniform growing suckers at a reduced cost.

Specific objectives of this study were to determine:

i. The rate of initiation and development of axillary buds of split corm-derived plantain suckers into plantlets after treatment with different ratios of IAA and coconut water (from fully matured dried fruits).

ii. The rate of initiation and development of axillary buds of split corm-derived plantain suckers into plantlets after treatment with coconut water from fruits at pre-endosperm formation stage, post-endosperm formation stage and fully matured dried fruits.

CHAPTER 2

LITERATURE REVIEW

2.1 Botany of plantain

Plantain is a herbaceous perennial crop with a short underground stem called the corm. The corm consists of short internodes from which the roots, leaves and apical meristem are initiated.

2.1.1 Corm size and structure

Corm size of plantain depends on the cultivar type and age. The diameter ranges from 15-18 cm at maturity for AAB plantain to 20-25 cm for mature Cavendish banana (Price, 1995). Internally, the corm comprises a central cylinder of storage tissue surrounded by a protective cortex. Roots develop from the outer surface of the central cylinder, whereas new suckers develop from buds on the outer surface of the cortex (Speijer and Gold, 1996).

2.1.2 Root system

Roots grow laterally from the corm in the superficial soil layer (Champion and Sioussaram, 1970). Fewer roots grow vertically or deeper, although rooting density and distribution is influenced by the texture and depth of the soil (Irizarry *et al*., 1981). New

roots are produced continuously until the plant flower (Beugnon and Champion, 1966), which may occur 7-9 months after planting.

2.1.3 Leaves and Pseudostem

Leaves arise from the upper part of the corm. The leaf sheaths tightly wrap around each other to form the pseudostem. Growth in girth and height of the pseudostem is related to foliar growth (Stover and Simmonds, 1987). Young leaves emerge from the heart of the pseudostem and are more or less vertical whilst the lamina is rolled in a cylindrical form. This later unfurls and becomes horizontal which will drop as the leaves age (Price, 1995).

2.1.4 Vegetative and reproductive phases

The duration of the vegetative phase may be considerably longer if climatic or soil conditions are unfavorable and may last more than 1-2 years (Anon, 1986). Lavigne (1987) stated that suckers are of great benefit to the mother plant during reproductive phase by providing additional anchorage and a supplementary source of nutrients for the maturing fruits. The fruits are slender, angular pointed and contain an orange-yellow pulp which remains starchy at maturity (Simmonds, 1966).

2.1.5 Inflorescence

The apical meristem is transformed into inflorescence after the vegetative growth phase. The inflorescence consists of groups of male, female and neutral flowers. Female flowers are located at the top of the inflorescence and they form the edible fruits. Each group of edible fruits is called a hand, and individual fruits are called fingers (Stover and Simmonds, 1987; Swennen, 1990). Neutral or intermediate flowers occur in the middle of the inflorescence and do not form fruits. At the end of the inflorescence is a purple bud called the male bud. This consists of bracts covering groups of male flowers. A fully matured inflorescence is called a fruit bunch.

2.2 Major cultivars of plantain

Plantain may be classified into three main groups according to their fruit types: False horn, True horn, and the French plantain. There are several intermediaries within each group (Swennen, 1990; Hemeng *et al.*, 1996b). The False horn plantains have between three and six hands on a bunch. The fingers are big but few. The inflorescence is incomplete as the male bud degenerates at maturity. Bunches weigh between 5 and 15 kg. They have relatively shorter cropping cycle of not more than 12 months. A major local cultivar within this group is called ‘Apantu’.

The French plantain cultivars have between six and ten hands and many but relatively smaller fingers. The inflorescence is complete at maturity and the male bud is large and persistent. Bunches weigh between 30-45 kg. They have a long cropping cycle of 15 to

18 months. 'Apem' is a major local cultivar within the French plantains group (Swennen, 1990; Hemeng *et al*., 1996b).

The true Horn plantains usually have between one and three hands. The fingers are long and big, but are very few. The inflorescence is incomplete as there are no neutral flowers or male bud. A major local cultivar within this group is called 'Asamienu'.

Hemeng *et al*., (1996b) identified 29 cultivars of plantain in Ghana which may be classified into the three main groups according to their fruit types: 14 French plantains, 12 False horn plantains and 3 True horn have been identified. The two major local plantain cultivars grown extensively in Ghana are 'Apem' and 'Apantu' (Akomeah *et al*., 1995)

2.3 Plantain production systems

Plantain is mainly grown in a mixed cropping system with root crops, cereals and vegetables and sometimes as a shade crop for young cocoa plants. It is rarely grown as a sole crop (Afreh-Nuamah and Hemeng, 1995; Schill *et al*., 1997).

In Ghana, the crop is mainly grown by small-scale farmers who produce an average yield of 6.0 tonnes per hectare (Akomeah *et al*., 1995) on a total land area of about 129,000 hectares (Anon, 1991). A yield of approximately 20 tonnes per hectare was obtained under optimum conditions where pest and diseases were controlled (Anon, 1997). Plantain is a highly priced staple used in the preparation of the national dish, 'fufu' (Afreh-Nuamah and Hemeng, 1995). Per capita consumption of plantain in Ghana is

estimated to be 83 kg per head and is higher than maize and second after cassava (Anon, 1991).

In Ghana, planting of plantain is carried out with sword or maiden suckers with roots and soil attached. Ahiekpor (1996) identified three cropping systems with plantain as the dominant crop, however, maize and the various vegetables are often planted first after land clearing, followed by plantain and cocoyam (Afreh-Nuamah and Hemeng, 1995). The only maintenance activity carried out in plantations involves two to four weedings per year (Schill *et al*., 1997). Plantain is also grown in backyard gardens and tends to produce bunches for many years in this situation due to regular mulching with organic waste (Wilson *et al*., 1986).

2.4 Challenges to plantain production

In many developing countries, where plantain and banana are grown, production has been affected by certain challenges, which have led to diminishing yields and decreasing crop cycles. For example, in some parts of West Africa, plantain is now considered as an annual crop whereas it used to ratoon for 25 years or more (Gold, 1993). The primary production challenges include the following:

- Decreasing soil fertility due to continues cultivation without proper soil management or short fallow periods as a result of increasing population pressure
- Lack of clean planting materials
- Post-harvest losses, and

- Pest and disease complex including black sigatoka, plant parasitic nematodes and banana weevil (Anon, 1992; Akomeah *et al*., 1995; Hemeng *et al*., 1996a; Schill *et al*., 1997).

2.5 Plantain planting material

Lack of healthy uniform-sized planting material has been a major challenge to plantain production in West Africa (Tezenas du Montcel, 1987; Wilson *et al*., 1987). Types of plantain planting materials used for propagation include peepers, sword suckers and maiden suckers (Swennen, 1990). Peepers are small suckers emerging from the soil. The sword suckers are large suckers with sword-like leaves without lamina. Maiden suckers are large suckers with foliage leaves. These planting materials develop from axillary buds but due to apical dominance exerted by the apical meristem prior to flowering, suckering in plantain is very poor. Between 5 and 10 suckers are produced per plant in a year (Swennen *et al*., 1984; Tezenas du Montcel, 1987).

Most farmers in West Africa usually plant suckers obtained from old fields and these suckers may be infected with pest and diseases including nematodes and plantain weevils (Afreh-Nuamah, 1994), banana streak and cucumber mosaic viruses (Osei, 1995). All these diseases are transmitted through infected suckers and adversely affect the growth and yield of plantains.

The problem of obtaining large number of vigorous, uniform and healthy planting materials can be overcome by the use of *in vitro* and rapid field multiplication techniques (Anon., 1955; Vuylsteke and De langhe, 1985; Anon 1995; Adelaja, 1996a).

2.6 *In vitro* rapid sucker multiplication of plantain

In vitro multiplication involves the culture of small plant tissues or cells under aseptic conditions on an artificial media consisting of sugars, plant nutrients and plant growth regulators. A variation of this is the shoot-tip culture which enables large numbers of plants to be obtained from apical meristem. *In vitro* rapid sucker multiplication is highly efficient and allows for a large number turnover of plants in a very short time within a very little space (Cronauer and Krikorian, 1984).

In plantain, *in vitro* micropropagation is made possible by varying the concentrations of cytokinin and auxins in the media (Ortiz and Vuylsteke, 1994; Vuylsteke, 1989). Cytokinin (benzyl adenine, BA) is used in plantain tissue culture techniques to induce shoot formation (Vuylsteke, 1989). Auxins (Alpha-naphthalene acetic acid, NAA, Indole-3-acetic acid, IAA and Indole butylic acid, IBA) are frequently used to induce root initiation in plantains and bananas (Vuylsteke, 1989, Cronauer and Krikorian, 1984). Reports indicate that for regeneration in a well differentiated tissue, cytokinin (BA) and auxin (IAA) are best for plantain while benzyl adenine alone induces mass proliferation (Hirimburegama and Gamage, 1997, Daniells, 1997; Kadota and Niimi, 2003).

The use of in vitro derived plants ensures pest-free planting materials, homogenous fruiting and good quality fruits (Vuylsteke and De langhe, 1985; Martin, 1998). Virus attacks are however spectacular and devastating during the juvenile stage. Variants or off-types may also occur (Martin, 1988; Osei, 1998). The technique also requires special equipment and expertise and therefore highly sophisticated for most farmers in West Africa.

2.7 Split-corm method for rapid field sucker multiplication

The split-corm technique involves paring and splitting the corm into smaller pieces to permit the dormant axillary buds on the corm to sprout (Anon, 1995; Adelaja 1996a). Adelaja (1996a) observed that twenty new plants can be obtained when a corm is split into 50 g pieces. A modification to this technique known as Split Bud Technique (S.B.T), involves removing sprouted corm pieces from the nursery bed and splitting them longitudinally into four sections. This method is capable of generating up to 500 plants from a corm within twenty-four weeks (Adelaja 1996a). In Ghana, however, the method which has been successfully used at the Crop Research Institute (C.R.I) Kumasi, and the University of Ghana Forest and Horticultural Crop Research Centre (FOHCREC) Kade, involves splitting the corm into 8-10 pieces depending on the size of the corm (Anon, 1995). Corms of flowering, pre- flowering, maiden or sword suckers can be used as starting material. Using this method, suckers can be ready within 8-12 weeks after shoot emergence (Anon, 1995). It is a simple method and can easily be adopted by farmers.

Several advantages can be derived from the use of split-corm technique in plantain propagation. Large numbers of planting materials can be produced in a small area with few corms reducing production cost (Ngeze, 1994; Anon, 1995). The production of large numbers of suckers in a nursery facilitates the selection of uniform–sized planting materials which is highly desirable in intensive cultivation (Robinson and Alberts, 1983; Tezenas du Montcel, 1987). Furthermore the process of paring and splitting enables the farmer to eliminate nematodes and discard weevil- infested corms or suckers, thereby minimizing the transfer of these pests from old to new fields. It is easier and more

economical to transport split corm than suckers (Ngeze, 1994; Anon, 1995). The farmer is also sure of the cultivar to be planted (Anon, 1995).

Robinson and Alberts (1983) observed that there was better uniformity in terms of plant height, cycle interval and leaf numbers in split-corm derived material than sword suckers. Kuhne (1980) and Watson (1982) have shown that suckers from split-corms produced larger bunch weight than from sword suckers. However time to bunch emergence was shorter for suckers than split-corms. Watson (1982) again indicated that the size of the split-corms did not influence bunch weight but smaller pieces less than 500 g delayed bunch emergence by 18 days compared to pieces bigger than 1 kg. It has also been observed that shoots of suckers on the average emerged 17 days earlier than split-corms. Larger corm pieces also emerged earlier than smaller ones, and mortality or shooting failures was higher in smaller corm pieces than bigger ones (O' Farrell, 1980; O' Farrell *et al*., 1989; Ahiekpor, 1993).

2.8 Relationship between root and shoot growth

Newly planted suckers first produce adventitious roots and then develop new leaves (Champion and Olivier, 1961). Vigorous growth of plantain suckers is known to result in an early and high yield. However, this can only be achieved if ample nutrient and water can be taken up by a healthy and abundant root system (Swennen and De langhe, 1985; Tezenas du Montcel, 1987). Mohan and Rao (1984; 1985) compared labeled phosphate (^{32}P) uptake in three cultivars and noted that the cultivar that had the most roots had highest uptake while the least was taken by the cultivar with the fewer number of roots. A

study carried out at International Institute of Tropical Agriculture (IITA) Ibadan, on twelve plantain genotypes indicated that there was a significant correlation between above-ground parameters and root growth. Leaf area, plant height and girth were significantly correlated with root dry weight (Anon, 1995).

According to Swennen (1984), sucker initiation and development are closely related to root development. Lassoudiere (1978) observed that there is high and positive correlation between bunch weight and the quality of roots produced.

The effectiveness of anchorage increases with both the overall size and spread of the root system (Mckay and Coutts, 1989). Cultural practices such as application of mulch, in-organic fertilizers and organic manures improve plantain growth, development and subsequent yield (Swennen, 1984; Wilson *et al*., 1986; Tezenas du Montcel, 1987; Swennen, 1990), partly due to their promotory effect on root growth and development. Mulch provides optimum day and night temperatures for root growth (Turner and Lahav, 1983) and inorganic fertilizers are known to promote root ramification (Russel, 1977). Most organic manures are rich sources of auxins which are root promoting hormones. Poultry manure on dry weight basis contains as much as 7.66 mg Indole-3-acetic acid (IAA) per 100 g of manure (Hemence, 1945).

2.9 Plant growth regulators

Plant Growth regulators are also referred to as Phytohormones. Phytohormones are a group of naturally occurring organic compounds that play crucial roles in regulating plant growth in a wide range of developmental processes. They influence physiological

processes at concentrations far below those of nutrients or vitamins (Davies, 1987). Phytohormones are known to act in conjunction or in opposition to each other such that the final condition of growth or development represents the net effect of the hormonal balance (Leopold, 1980). Initially, the term phytohormone was synonymous with auxin. The synthesis of plant hormones may be localized but may also occur in a wide range of tissues or cells within tissues. Phytohormones are usually transported and have their action at a distance but this is not always the case (Davies, 1987; Sponsel, 1987).

The initiation and growth of adventitious roots, lateral roots and root hairs are stimulated by auxins (Sebanek and Jesko, 1992). Sebanek and Jesko (1992) also observed that auxin produced by the shoot and cytokinin produced by the root is in an inverse relationship in the regulation of apical dominance. Application of cytokinin enhances apical dominance of the root whilst auxin weakens the apical dominance of the root.

2.9.1 Major groups of plant growth regulators

There are 5 major plant hormones: auxin, cytokinins, gibberellins, ethylene and abscisic acids. Auxin is produced by apical tissues and developing leaves, the function is for cell division and elongation. Auxin is also the cause of apical dominance, phototropism and gravitropism. Cytokinins are another group of plant hormones that can stimulate cell division. Cytokinins are derivatives of the purine adenine. Function of cytokinins includes stimulation of cell division; the major source is the roots and apical meritstem. Cytokinins are opposite to auxin in that they move upward and they promote growth of lateral buds. They prevent leaf senescence and are essential for plant cell

culture. Gibberellins include over 30 structurally related compounds. The function of Gibberellins includes promoting stem elongation, breaking dormancy of seeds, buds and stimulation of flowering in mature plants. Abscisic acids are the major plant hormone response to stress. Their main functions include bud dormancy, seed maturation and dormancy, abscission of leaves and fruits (opposite to auxin) and closing of stomata. Ethylene is the only gas form of plant hormone; it is produced by the fruit. Major function of ethylene includes promoting fruit ripening and stimulating senescence and abscission in leaves and fruits.

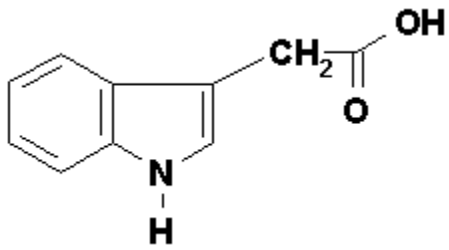
In respect to this study auxins and cytokinins will be described into details. Coconut water is a natural source of plant homones especially cytokinins and auxins.

2.9.2 Auxin

Indole-3-acetic acid (IAA) is the main naturally occurring auxin in plants. It is a weak acid (pKa = 4.75) that is synthesized in the meristematic regions located at the shoot apex and subsequently transported to the root tip in plants (Blakeslee *et al*., 2005). For many years, tryptophan was assumed to be the precursor of IAA and this was later confirmed using experiments involving seedlings of *Phaseolus vulgaris* subjected to stable isotope labeling studies (Bialek *et al*., 1992). IAA occurs not only in the free form, but is also conjugated to various amino acids, peptides, or carbohydrates. These IAA conjugates are biologically inactive and appear to be the IAA storage forms in seeds and are probably involved in hormonal homeostasis (Jakubowska and Kowalczyk, 2005). Auxin is implicated in many regulatory processes in plants especially those relating to plant

growth and development (Berleth *et al*., 2004; Dharmasiri *et al*., 2005). Auxin functions in the relay of environmental signals such as light and gravity, the regulation of branching processes in shoots and roots, and as discovered more recently, the patterned differentiation of cells in meristems and immature organs (Berleth *et al*., 2004). Undoubtedly, it is a versatile spatial-temporal signal. Auxin transport generates auxin concentration maxima and gradients within tissues that are instrumental in the diverse regulation of various plant developmental processes, including embryogenesis, organogenesis, vascular tissue formation and tropisms.

2.9.2.1 Nature of Auxins



Indole-3-acetic acid

Auxins were the first plant hormones discovered. The term auxin is derived from the Greek word *auxein* which means to grow. Compounds are generally considered auxins if they can be characterized by their ability to induce cell elongation in stems and are substances which share similar activity to indole-3-acetic acid, IAA (the first auxin to be isolated from plants) in physiological activity (Davies, 1995; Arteca, 1996). IAA is chemically similar to amino acid tryptophan which is generally accepted to be the

molecule from which IAA is derived. Auxins usually affect other processes in addition to cell elongation of stem cells but this characteristic is considered critical of all auxins and thus "helps" define the hormone (Mauseth, 1991; Raven, 1992; Salisbury and Ross, 1992; Arteca, 1996). The enzymes responsible for the biosynthesis of IAA are most active in young tissues such as shoot apical meristems and growing leaves and fruits. The same tissues are the locations where the highest concentrations of IAA are found.

2.9.2.2 Functions of Auxins

The following are some of the responses that auxin is known to cause (Davies, 1995; Mauseth, 1991; Raven, 1992; Salisbury and Ross, 1992).

- Stimulates root initiation on stem cuttings and lateral root development in tissue culture
- Stimulates cell elongation
- Stimulates cell division in the cambium and, in combination with cytokinins in tissue culture
- Stimulates differentiation of phloem and xylem
- Mediates the tropistic response of bending in response to gravity and light
- The auxin supply from the apical bud suppresses growth of lateral buds
- Delays leaf senescence
- Can inhibit or promote (via ethylene stimulation) leaf and fruit abscission
- Can induce fruit setting and growth in some plants

- Involved in assimilate movement toward auxin possibly by an effect on phloem transport
- Delays fruit ripening
- Stimulates growth of flower parts
- Promotes (via ethylene production) femaleness in dioecious flowers, and
- Stimulates the production of ethylene at high concentrations

2.9.3 Cytokinins

Cytokinins were discovered in the 1950s (Miller, *et al*., 1955; Werner *et al*., 2001; Amasino, 2005). Natural cytokinins are *N6*-substituted adenine derivatives with various substituted groups, and the physicochemical behaviour of cytokinins is a function of side chain(s), sugar, phosphate and degree of purine ring and/or side chain modification (Amasino, 2005).

The auxin-cytokinin hypothesis predicted that cytokinins, together with auxins, play an essential role in plant morphogenesis by controlling the formation of roots and shoots and moderating their relative growth (Werner *et al*., 2001). Cytokinins are a class of phytohormones that exert various roles in the different aspects of plant growth and development, e.g., cell division, formation and activity of shoot meristems, induction of photosynthesis gene expression, leaf senescence, nutrient mobilization, seed germination, root growth and stress response (Mok and Mok, 1994; Tantikanjana *et al*., 2001; Werner *et al*., 2001; Haberer and Kieber, 2002; Amasino, 2005). Evidently, cytokinin-deficient plants generally develop stunted shoots with smaller apical meristems. The plastochrone

of these cytokinin-deficient plants is prolonged, and leaf cell production is only 3- 4% of wild type plants (with normal cytokinin metabolism), indicating an absolute role of cytokinins in leaf growth. Cytokinins are required during leaf formation, both to drive the cell division cycle at normal rates and to obtain the required number of divisions in order to produce a normal leaf size (Werner *et al*., 2001).

In addition, cytokinins are also involved in promoting the transition from undifferentiated stem cells to differentiated tissues (Frank and Schmulling, 1999). Unlike the growth-promoting role of cytokinins in the shoot apical meristem, cytokinins have a negative regulatory function in root growth whereby it suppresses cell division in plant roots (Werner *et al*., 2001).

Kinetin was the first cytokinin discovered and so named because of the compounds ability to promote cytokinesis (cell division) (Miller *et al.*, 1955). It was a degradation product of herring sperm DNA and was found to be able to promote cell division in plants (Miller *et al*., 1955, Miller *et al*., 1956). Kinetin was previously assumed to be an unnatural and synthetic compound, until Barciszewski *et al.*, (1996), detected it in freshly extracted cellular DNA from human cells and in plant cell extracts.

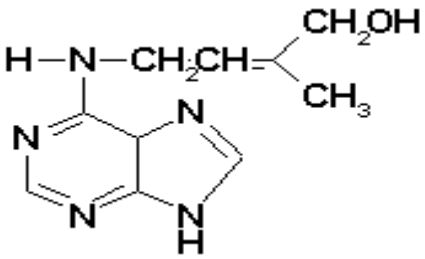
Recently, Ge *et al.*, (2005), identified kinetin and kinetin riboside from coconut water. Being one of the cytokinins, kinetin has the effects on the plant developmental processes that could be influenced by cytokinins, such as leaf expansion and seed germination.

Zeatin was the first naturally-occurring cytokinin identified from a plant source (*Zea mays*) by Letham, (1963). Letham, (1974) identified *trans*-zeatin in coconut water and a year later, van Stadens and Drewes (1975) verified the presence of both *trans*-zeatin and

trans-zeatin riboside in coconut water. *Trans*-zeatin riboside is the most abundant type of cytokinin found in coconut water (Yong *et al*., 2009) and is normally used to induce plantlet regeneration from callus in plant tissue culture.

Based on experimental data, *trans*-zeatin plays a key role in the G2-M transition of tobacco cells. It was found to override the blockade of mitosis caused by lovastatin which inhibits cytokinin biosynthesis and controls cellular entry in mitosis (Laureys *et al*., 1998)

2.9.3.1 Nature of Cytokinins



Zeatin

Cytokinins are compounds with a structure resembling adenine which promote cell division and have other similar functions as kinetin. Cytokinin biosynthesis happens through the biochemical modification of adenine (Salisbury and Ross, 1992; McGaw, 1995).

The most common form of naturally occurring cytokinin in plants today is called zeatin which was isolated from corn (*Zea mays*). Cytokinins have been found in almost all higher plants as well as mosses, fungi, bacteria, and also in tRNA of many prokaryotes and eukaryotes.

Today there are more than 200 natural and synthetic cytokinins combined. Cytokinin concentrations are highest in meristematic regions and areas of continuous growth potential such as roots, young leaves, developing fruits, and seeds (Mauseth, 1991; Salisbury and Ross, 1992; Arteca, 1996).

2.9.3.2 Functions of Cytokinin

A list of some of the known physiological effects caused by cytokinins are listed below. The response will vary depending on the type of cytokinin and plant species (Mauseth, 1991, Raven *et al*., 1992, Salisbury and Ross, 1992, Davies, 1995, Arteca 1996,).

- Stimulates the growth of lateral buds (release of apical dominance)
- Stimulates morphogenesis (shoot initiation/bud formation) in tissue culture.
- Stimulates cell division.
- Stimulates leaf expansion resulting from cell enlargement.
- May enhance stomatal opening in some species.
- Promotes the conversion of etioplasts into chloroplasts via stimulation of chlorophyll synthesis.

2.9.4 Coconut fruit

Coconut (*Cocos nucifera* L.) is an important fruit tree in tropical climates and the fruit can be made into a variety of foods and beverages. The edible part of the coconut fruit (coconut meat and coconut water) is the endosperm tissue. Endosperm tissues undergo

one of three main modes of development, which are the nuclear, cellular and helobial modes (Lopes and Larkins, 1993) and the development of coconut endosperm belongs to the nuclear mode.

Initially, the endosperm is a liquid containing free nuclei generated by a process, in which the primary endosperm nucleus undergoes several cycles of division without cytokinesis (the process in which the cytoplasm of a single eukaryotic cell is divided to form two daughter cells). Cytokinesis then occurs, progressing from the periphery towards the center, thus forming the cellular endosperm layer. At first, the cellular endosperm is translucent and jelly-like, but it later hardens at maturity to become white flesh (coconut meat).

2.9.4.1 Coconut water

Unlike the endosperms of other plants (e.g., wheat and corn), the cellularization process in a coconut fruit does not fill up the entire embryo sac cavity, but instead leaves the cavity solution-filled. This solution is commonly known as coconut water and it is of cytoplasmic origin (Janick and Paull, 2008).

Nutrients from coconut water are obtained from the seed apoplasm (surrounding cell wall) and are transported symplasmically through plasmodemata, which are the connections between cytoplasms of adjacent cells into the endosperm (Patrick and Offler, 2001).

Coconut water should not be confused with coconut milk, although some studies have used the two terms interchangeably (Kobayashi *et al*., 1997; Sandhya and Rajamohan, 2008). The aqueous part of the coconut endosperm is termed coconut water, whereas coconut milk, also known as "santan" in Malaysia and Indonesia, and "gata" in the Philippines refers to the liquid products obtained by grating the solid endosperm, with or without addition of water (APCC, 1994).

Coconut water has been extensively studied since its introduction to the scientific community in the 1940s. It is widely used in the plant tissue culture industry (van Overbeek, *et al*., 1941; Ang and Yong, 2005; Arditti, 2008). The extensive use of coconut water as a growth-promoting component in tissue culture medium formulation can be traced back to more than half a century ago, when van Overbeek *et al.* (1941) first introduced coconut water as a new component of the nutrient medium for callus cultures in 1941.

Coconut water appears to have growth regulatory properties, e.g., cytokinin-type activity (George and Sherrington, 1984). One of the most significant and useful components in coconut water are cytokinins, which are a class of phytohormones (Kende and Zeevaart, 1997). The first cytokinin, *N*6-furfuryladenine (kinetin) was isolated from an autoclaved sample of herring sperm DNA in 1955 (Miller, *et al*., 1955; Miller, *et al*., 1956). In 1963, Letham isolated *trans*-zeatin, the first naturally-occurring cytokinin, from a plant source (unripe corn seeds) (Letham, 1963).

Coconut water is also reported to contain indole-3-acetic acid (IAA), the primary auxin in plants (Ma *et al*., 2008; Wu and Hu, 2009). Other components found in coconut water include sugars, sugar alcohols, lipids, amino acids, nitrogenous compounds, organic acids

and enzymes (Tulecke, *et al*., 1961; Santoso, *et al*., 1996; Arditti, 2008; USDA, 2009), and they play different functional roles in plant due to their distinct chemical properties. The chemical components in coconut water which contribute to its bioactivity are essential to the plant industry, biotechnology and biomedical fields. Undoubtedly, cytokinins are currently the most important components in coconut water (Yong *et al.*, 2009.*).*

The chemical composition of coconut water is affected by several factors. Jackson *et al.* (2004) showed that coconut water of different stages of maturity contains different concentrations of compounds, and that the chemical contents also varied.

Coconut water (coconut liquid endosperm), with its many applications, is one of the world's most versatile natural product. It is traditionally used as a growth supplement in plant tissue culture/micropropagation. The wide applications of coconut water can be justified by its unique chemical composition of sugars, vitamins, minerals, amino acids and phytohormones (Yong *et al*., 2009.). One advantage of coconut water is that it results in considerable plant cell proliferation without increasing the number of undesirable mutations (Arditti, 2008).

This study attempts to evaluate the role of auxin and cytokinin present in coconut water on axillary bud formation and development in plantain.

2.9.4.2 Composition of auxins and cytokinins in coconut water

Coconut water at different maturity stages in the fruit contains auxin and various cytokinins as shown in the Table 1 below.

Table 1. Naturally-occurring phytohormones unequivocally identified in coconut water.

Phytohormone	**Coconut Type**	
	Young green	**Mature***
Auxin	**(**nM)	(μg mL^{-1})
Indole-3-acetic acid	150.60	0.25 ± 0.03
		0.75 ± 0.04
		1.46 ± 0.13
		0.71 ± 0.12
		0.78 ± 0.10
Cytokinins		
*N*6-isopentenyladenine	0.26	
Dihydrozeatin	0.14	
trans-zeatin	0.09	
kinetin	0.31	
ortho-topolin	3.29	
dihydrozeatin *O*-glucoside	46.60	
trans-zeatin *O*-glucoside	48.70	
trans-zeatin riboside	76.20	
kinetin riboside	0.33	

* Five coconut water samples were analysed.

Source information: Ge *et al*., 2007; Ma *et al*., 2008**;** Wu and Hu, 2009

2.9.5 Importance of cytokinin and auxins during organogenesis

Auxin and cytokinin play fairly important roles in many aspects of plant growth and development. The interaction between auxin and cytokinin is particularly important to control a few developmental processes such as the formation of meristems that are essential to establish the whole plant body. For example the shoot meristems give rise to the above ground parts whereas the root meristems produce the below ground parts.

The optimal ratios of cytokinins and auxins are very important during organogenesis (Sangwan and Harada, 1975; 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 (Sangwan and Harada, 1975, Cronauer and Krikorian, 1984, Jain *et al*., 1988).

2.9.6 Auxin and cytokinin interaction in control of axillary bud formation and growth.

In many plant species, the intact main shoot apex grows predominantly and axillary bud outgrowth is inhibited. This phenomenon is called apical dominance and has been analysed for over 70 years (Shimizu-Sato *et al*., 2009). Decapitation of shoot apex releases the axillary buds from their dormancy and they begin to grow out. Auxin derived from an intact shoot apex suppresses axillary buds outgrowth whereas cytokinin induced by decapitation of the shoot apex stimulates axillary buds outgrowth (Shimizu-Sato and Mori, 2001).

The axillary buds derived from the primary shoot apical meristem and in a developmental process generally involve two phases: during the first phase, the axillary meristem is formed from groups of meristematic cells which originate directly from detached parts of the primary shoot apical meristem of the main shoot. The axillary meristem produces axillary buds located on the axil of the leaf primodia. In the second phase, after the axillary buds are fully developed and have reached certain size, depending on the plant species, growth ceases and the axillary buds become dormant (Shimizu-Sato *et al*., 2009). Shimizu-Sato *et al*., 2009 indicated that it was the basipetal auxin flow in the stem which suppresses the axillary buds outgrowth. Thimann and Skoog (1934) reported that decapitation of *Vicia spp.* plants induced axillary bud outgrowth whereas the application of indole-3-acetic acid (IAA) to the stump prevented axillary buds outgrowth.

It was also observed that axillary bud growth cannot be prevented by the direct application of the auxin to the axillary buds after decapitation (Thimann and Skoog, 1934). Further, radiolabelled auxin applied to the stump was not transported into the axillary buds (Hall and Hillman, 1975). These findings indicated that auxin did not directly prevent axillary buds outgrowth (Shimizu-Sato *et al.,* 2009). Stump applied cation of 2 4- dichlorophenoxyacetic acid, which cannot be transported basipetally in plants also did not prevent the outgrowth of axillary buds (Brown *et al.*, 1979). The auxin transport inhibitor 2, 3, 5- triiodobenzoic acid (TIBA) in lanolin applied to stem of an intact plant however reduced or abolished apical dominance (Snyder, 1949). These observations indicate that basipetal auxin flow derived from a shoot apex inhibits axillary buds outgrowth.

Auxin produced mainly in the young leaves and shoot apex (Ljung *et al.,* 2001) is transported basipetally down the stem in a polar manner by active transport in the vascular parenchyma (Lomax *et al.,* 1995). Although the molecular mechanisms of active auxin transport are becoming clear, it remains unknown how auxin flow derived from shoot apex induces axillary buds to enter dormancy (Shimizu-Sato *et al.,* 2009).

Cytokinin promotes axillary buds outgrowth. The effects of cytokinin in apical dominance are antagonistic to those of auxin. Direct application of cytokinin to axillary buds promotes axillary buds outgrowth even in intact plants (Wickson and Thimann, 1958). To date, cytokinin is the only chemical known to release axillary buds from dormancy. After decapitation in chickpea, cytokinin levels in the axillary buds increased seven-fold within six hours and 25- fold within 24 hours and axillary buds outgrowth was correlated with the cytokinin level in the axillary buds (Turnbull *et al.,* 1997).

Roots are the main cytokinin- producing organ in plants and cytokinin biosynthesis in the root system is transported into the shoot via the xylem (Hopkins and Huner 2004). Cytokinin levels in bean xylem exudates increase within 16 hours after decapitation and gradually returned to basal levels (Bangerth, 1994). Application of 1- naphthylacetic acid to the stump prevents the increase in cytokinin the bean xylem exudates (Li *et al*., 1995). These observations led to the suggestions that cytokinin derived from roots promotes axillary buds outgrowth after decapitation and auxin derived from a shoot apex regulates cytokinin transport in plants (Letham, 1994).

2.9.7 Effect of cytokinin, auxin and coconut water on callus induction and shoot regeneration

Synthetic cytokinins, auxins and coconut water had been used in several experiments *in vivo* to improve callus induction and plant regeneration in many plants. Pelissier *et al*., (1990) observed callogegenic capacities of hypocotyls *Helianthus annuus* tTCL with respect to NAA, BA, and coconut water. Mamun *et al*., (2004) reported that 2,4-D and 10% coconut water produced maximum amount of regenerative callus from leaf sheath in sugarcane. Similar results were observed by Masteller and Holden (1970) in sorghum. Alagumanian *et al*., 2004 also reported that 10% coconut water with various concentrations of BAP was effective for callus regeneration in *Solanum*.

Amdadul *et. al*., (2012) reported that the highest percentage of shoot regeneration (90%) and maximum number of shoots (10) per explants were observed when meristematic stem cuttings explants were cultured on MS medium containing 4.0 mg/l BAP plus 2.0 mg/l IAA plus13% (v/v) coconut water. Gbadamosi and Sulaiman (2012) indicated that *Irvingia gabonensis* demonstrated varied growth patterns on MS basal media supplemented with varied concentrations of four growth hormones and coconut water. They observed that ¼ MS plus 0.05 mg/l NAA plus 20.0% coconut water gave the highest viability (60%) and the best enhanced root formation (1.67 roots) while medium with ¼ MS plus 0.05 mg/l BAP plus 0.05 mg/l KIN plus 0.05 mg/l IBA plus 10.0% coconut water gave the highest shoot formation (2.17 roots) and leaves (6.00).

CHAPTER 3

MATERIALS AND METHODS

3.1 Production of plantain planting materials using the split corm technique

Maiden suckers 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 (Plate 1).

The mini-setts were treated with Bendazim (carbendazim fungicide) at the rate of 2g per litre of water to prevent fungal infection and rottening. The mini-setts were then 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. Six weeks after 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 used in two experiments:

Plate 1. 300g split corms of plantain used for the experiments

Plate 2. Three-month old split corm-derived suckers ready for the injection treatments

3.2 Experiment 1

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

3.2.1 Methodology

Three-month old developing split corm-derived suckers (Plate 2) of the local plantain cultivar (Asamienu) were treated with different ratios of IAA and coconut water. The coconut water was obtained from fully matured dried fruits. The IAA was bought from MES Equipment Limited, Accra.

Coconut water from fully matured dried fruits was supplemented with three different IAA concentrations, 10^{-4} M, 10^{-3} M, and 10^{-2} M to produce thirteen different treatments of coconut water : IAA ratios (v:v), viz: 8:0, 6:2, 4:4, 2:6, 0:8 as shown in Tables 2, 3 and 4 below.

Table 2. Concentration of IAA in varying ratios of coconut water from fully matured dried fruits and 10^{-4} M IAA to be used for the injection treatments.

Volume of Coconut Water (ml)	8	6	4	2	0
Volume of 10^{-4} M IAA (ml)	0	2	4	6	8
IAA Concentration in the total 8mls aliquot solution (M)	0	2.5×10^{-5}	5.0×10^{-5}	7.5×10^{-5}	10^{-4}

Table 3. Concentration of IAA in varying ratios of coconut water from fully matured dried fruits and 10^{-3} M IAA to be used for the injection treatments.

Volume of Coconut Water (ml)	8	6	4	2	0
Volume of 10^{-3} M IAA (ml)	0	2	4	6	8
IAA Concentration in the total 8mls aliquot solution (M)	0	2.5×10^{-4}	5.0×10^{-4}	7.5×10^{-4}	10^{-3}

Table 4. Concentration of IAA in varying ratios of coconut water from fully matured dried fruits and 10^{-2} M IAA to be used for the injection treatments.

Volume of Coconut Water (ml)	8	6	4	2	0
Volume of 10^{-2} M IAA (ml)	0	2	4	6	8
IAA Concentration in the total 8mls aliquot solution (M)	0	2.5×10^{-3}	5.0×10^{-3}	7.5×10^{-3}	10^{-2}

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 was included.

The treatments were administered by injection (Plate 3) at the base of the developing suckers at about 5cm from the soil surface. Plant height, girth, and number of leaves were measured prior to the application of the injection treatments.

Plate 3. Injection of three-month old developing split corm-derived plantain sucker in the nursery.

The injection was repeated three times on three consecutive alternate days. Each treatment consisted of five plants. The treatments were replicated three times in a randomized complete block design. Three weeks after the injections, plant height, girth, and number of leaves 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. After removing the well developed plantlets, the bud manipulation technique with sheath removal (Plate 4) was used to produce additional plantlets for each injection treatment.

Plate 4. Bud manipulation technique to generate additional plantlets three weeks after removal of the initial developing plantlets.

Corms of the treated suckers were planted in humidified chambers filled with sterilized sawdust and covered with transparent polyethylene sheets to maintain high relative humidity.

The sawdust was steam sterilized at 100^0C for 30 minutes in 200-litre iron drums. After sterilization, the sawdust was left overnight to cool before being used.

Plate 5. Humidified chambers filled with sterilized sawdust and covered with transparent polyethylene sheets to maintain high relative humidity for sprouting of the treated split corms.

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 also removed from the corms and potted

as described earlier. 20g NPK (15-15-15 compound fertilizer) was applied to each potted plant. Two months after potting, six plants from each treatment were selected randomly for growth measurements. Each treatment was replicated three times.

Data were collected on the following: 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, total leaf area of the plantlets produced.

3.3 Experiment 2

The objective of this experiment was to determine the rate of initiation and development of axillary buds of split corm derived plantain suckers after treatment with coconut water from fruits at pre-endosperm formation stage, post-endosperm formation stage and fully matured dried fruits.

3.3.1 Methodology

Three-month old developing split corm derived suckers of the two local plantain cultivars were treated with coconut water from fruits at four different fruit maturity stages:

- Liquid endosperm formation stage (LE) (6 months after flowering)
- Solid endosperm formation stage (8 months after flowering)
- Semi matured endosperm stage (10 months after flowering)
- Fully matured dried fruit stage (FM) (13 months after flowering)
- Combination of LE and FM
- Control (Distilled water)

Six plants were injected with 8 ml boiled and filtered coconut water on three consecutive alternate days. The treatments were replicated three times in a randomized complete block design. Growth measurements were taken as described in experiment 1. Three weeks after the application of the injection treatments, the procedures outlined in experiment 1 were used to determine the total number of well differentiated axillary buds as well as well developed plantlets produced per plant after sprouting treated corms in sterilised moist sawdust.

Plate 6. Coconut endosperm development at four different fruit maturity stages:

I = Liquid endosperm formation stage (6 months after flowering)

II = Solid endosperm formation stage (8 months after flowering)

III = Semi matured endosperm stage (10 months after flowering)

IV = Fully matured dried fruit stage. (13 months after flowering)

The treatments were replicated three times in a randomized complete block design. Each treatment consisted of five plants.

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, total leaf area of the plantlets produced.

3.3.2 Analysis of Cytokinin (trans-zeatin riboside) and auxin (indole-3-acetic acid, IAA) in coconut (*Cocos nucifera L.)* water.

Cytokinin (trans-zeatin riboside) and auxin (indole-3-acetic acid, IAA) which represent major phytohormones in coconut water (Ge *et al*., 2007, Ma *et al*., 2008,Wu and Hu, 2009**)** were isolated and quantified. The endogenous contents of trans-zeatin riboside and indole-3-acetic acid, IAA were determined by the modifications of those described by Bollmark *et al*., (1988) and He (1993).

3.3.3 Coconut water pretreatment and cytokinin (trans-zeatin riboside) and auxin (indole-3-acetic acid, 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_{18} columns (C_{18} 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_2 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.

3.3.4 Quantitative analysis of cytokinin (trans-zeatin riboside) and auxin (indole-3-acetic acid, 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^{-1} Na2CO3, 2.93 g l^{-1} NaHCO3 and 0.02 g l^{-1} NaN3) and contained 0.25 µg ml^{-1} antigens against trans-zeatin riboside and IAA.

The coated plates were incubated for 16 hrs at 4^0C for trans-zeatin riboside and overnight at 4^0C 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 trans- zeatin riboside and IAA standards (0 - 10 µg ml^{-1} dilution range) and 50 µl of 20 µg ml^{-1} antibodies against trans- zeatin riboside and IAA.

The plates were incubated for 1 hr at 37^0C and overnight at 4^0C for trans-zeatin riboside and IAA respectively. 100 µl of 1.25 µg ml^{-1} G- horse radish peroxidase substrate was added to each well and incubated for 1 hr at 30^0C.

The plates were rinsed five times with Tween 20 buffer containing PBS and 100 µl color –appearing solution containing 1.5 mg ml^{-1} o-phenylenediamine and 0.008 % H_2O_2 was added to each well. The reaction progress was stopped by adding 50 µl 6N H2SO4 per well when the 100 µg ml^{-1} standard had a pale color and the 0 µg ml^{-1} 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 trans-zeatin riboside and IAA were calculated by the method of Weiler and Ziegler (1981).

3.4 Statistical analysis and presentation of results

Data collected in each experiment were analysed using analysis of variance (ANOVA) procedure. The least significant difference (LSD) at P= 0.05 was employed for means separation. Correlation analysis was used to determine the relationship between the cytokinin (trans-zeatin riboside) and auxin (indole-3-acetic acid, IAA) contents in coconut water from fruits at different maturity stages. Graphs were used for the presentation of results.

CHAPTER 4

RESULTS

4.1 Experiment 1

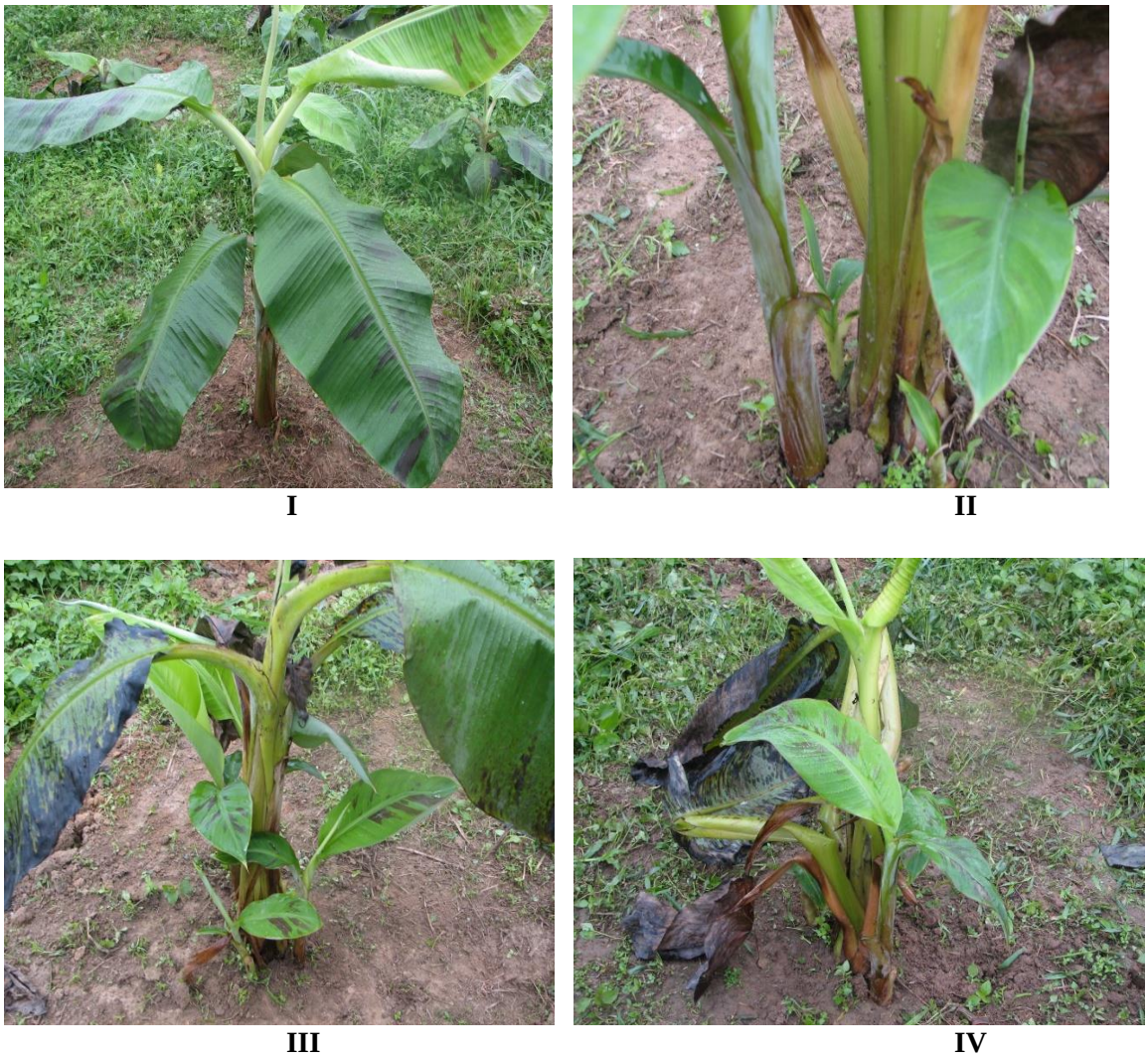
4.1.1 Effects of varying ratios of coconut water and indole-3-acetic acid on percentage increase in plant height and girth of 3-month old split corm-derived Asamienu suckers.

Figures 1 and 2 show the mean growth in height and girth of 3-month old split corm-derived Asamienu suckers 3 weeks after the injection treatments. The significant differences (P= 0.05) in growth in height and girth of the suckers were due to the injection treatments.

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

The other treatments with coconut water alone or in combination with IAA significantly reduced (P=0.05) growth in height (Figure 2) compared to the control treatment (distilled water). Suckers treated with 4ml coconut water plus 4ml 10^{-2}M IAA increased in height by only 7.4% whilst those treated with distilled water increased in height by 35.1% after

the injections. It was also observed that all the treatments with 10^{-2}M IAA reduced growth in height more than the other treatments with 10^{-3} or 10^{-4}M IAA.



I **II**

III **IV**

Plate 7. Effects of varying ratios of coconut water and indole-3-acetic acid on the growth of 3-month old split corm-derived Asamienu suckers three weeks after the injection treatment. I=Distilled water, II= Coconut water alone, III= CW: IAA (50:50 v/v), IV= CW: IAA (25:75v/v)

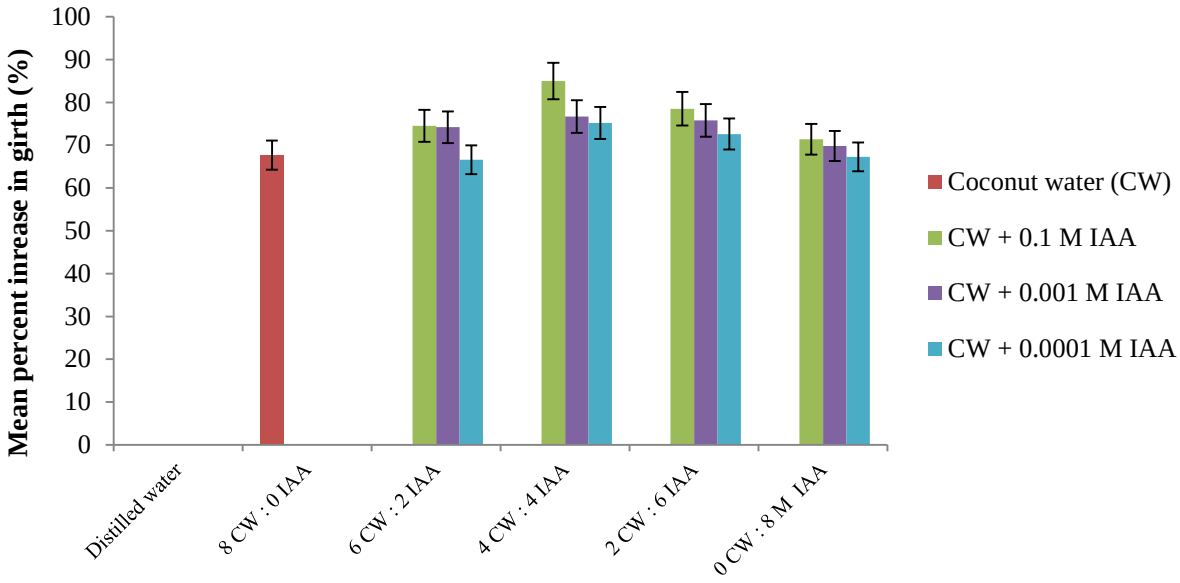


Figure 1. Percentage increase in plant girth of 3-month old split corm-derived Asamienu suckers three weeks after injection with varying ratios of coconut water (CW) and indole-3-acetic acid (IAA). Bars show standard deviation with n= 6.

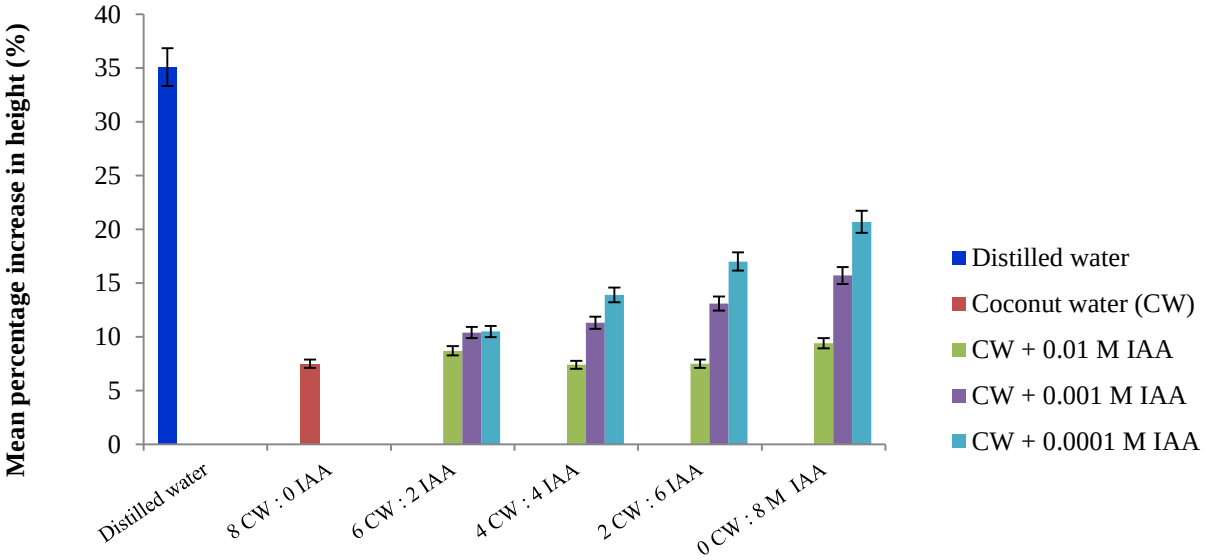


Figure 2. Percentage increase in plant height of 3-month old split corm-derived Asamienu suckers three weeks after the injection with varying ratios of Coconut water (CW) and indole-3-acetic acid (IAA). Bars show standard deviation with n= 6.

4.1.2 Effects of varying ratios of coconut water and indole-3-acetic acid on root production of 3-month old split corm-derived Asamienu suckers

Figures 3, 4 and 5 show the mean root number, root diameter and root dry weight of 3-month old split corm-derived Asamienu suckers respectively three weeks after the injection treatments respectively. Generally, mean root number, root diameter and root dry weight increased with increasing concentration and volume of the IAA. The differences in root number and root diameter among the treatments were significantly different (P= 0.05) from each other. However, root dry weight production was significantly different among the treatments. The treatment that produced the highest root dry weight (15.4 g) resulted from applying 8ml 10^{-2}M IAA.

I **II**

Plate 8. Effects of varying ratios of coconut water and indole-3-acetic acid on root production of 3-month old split corm-derived Asamienu suckers three weeks after the injection treatment. I=Distilled water, II= 10^{-2}M IAA

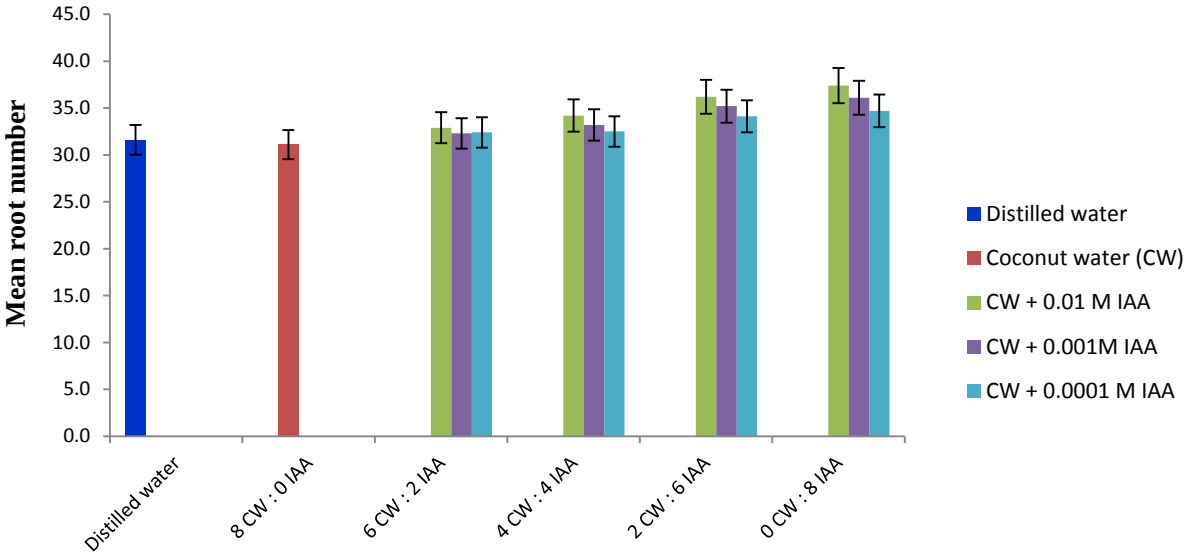


Figure 3. Mean root number of 3-month old split corm derived Asamienu suckers three weeks after treatment with varying ratios of coconut water (CW) and indole-3-acetic acid (IAA). Bars show standard deviation with n= 6.

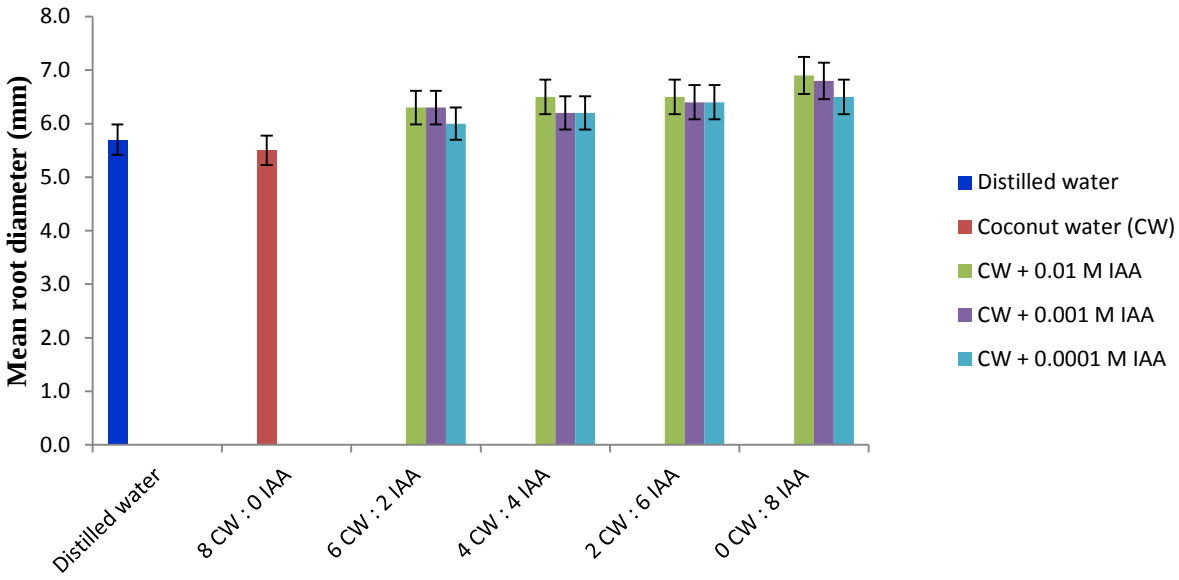


Figure 4 . Mean root diameter of 3- month old split corm derived Asamienu suckers three weeks after treatment with varying ratios of coconut water (CW) and indole-3-acetic acid (IAA). Bars show standard deviation with n= 6.

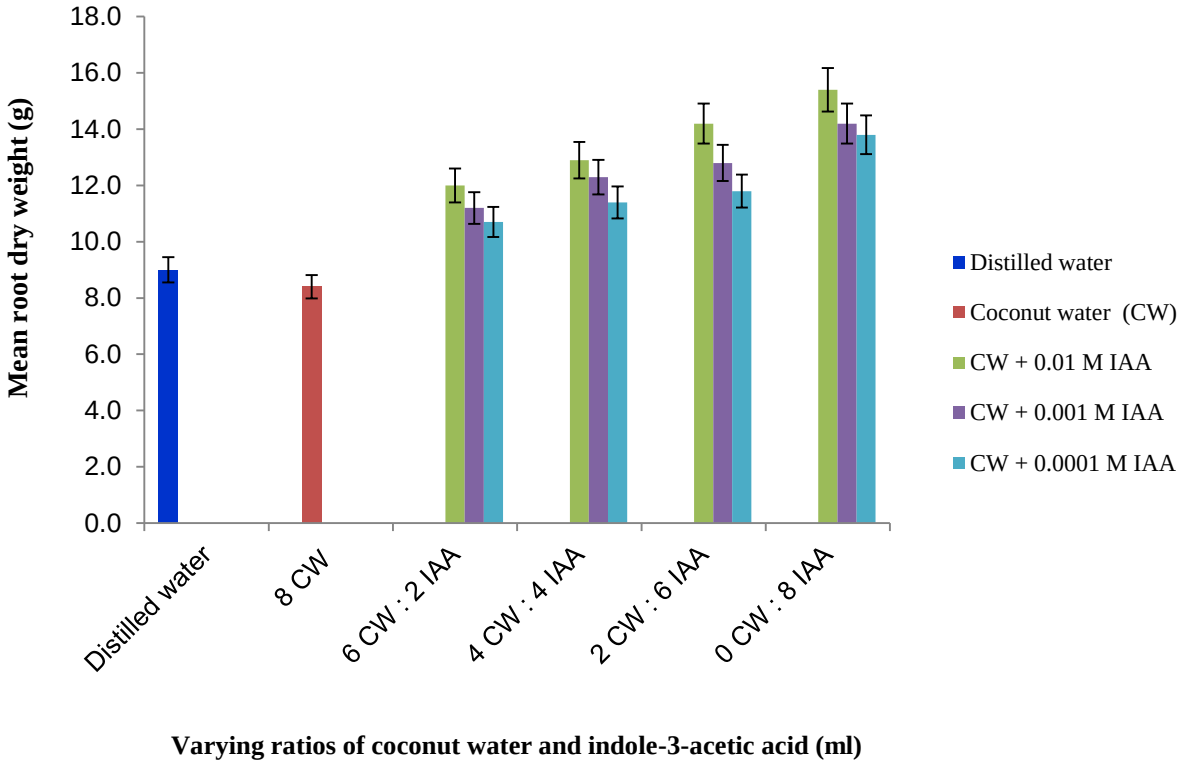


Figure 5. Mean root dry weight of 3-month old split corm derived Asamienu suckers three weeks after treatment with varying ratios of coconut water (CW) and indole-3-acetic acid (IAA). Bars show standard deviation with n= 6.

4.1.3 Effects of varying ratios of coconut water and indole-3-acetic acid on fully differentiated axillary buds and plantlets of 3-month old split corm-derived Asamienu suckers.

Figure 6 shows the number of fully differentiated axillary buds produced by injecting 3-months old split corm derived Asamienu suckers with varying ratios of coconut water and IAA.

The number of well-differentiated axillary buds increased as the IAA concentration and volume increased up to 6ml 10^{-2}M IAA concentration and then declined as the volume was increased.

The highest number of well-differentiated axillary buds was produced with 2ml coconut water plus 6ml 10^{-2}M IAA. By the third week after the injection treatments, some of the well-differentiated axillary buds had already developed into plantlets.

Figure 7 shows the number of plantlets that developed from the well-differentiated axillary buds with the various injection treatments. 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 8ml coconut water alone treatment.

I

II

III

IV

Plate 9. Effects of varying ratios of indole-3-acetic acid and coconut water on fully differentiated axillary buds and plantlets of 3-month old split corm-derived Asamienu suckers three weeks after the injection treatment. I=Distilled water, II= Coconut water alone, III= CW: 10^{-2}M IAA (50:50 v/v), IV= CW: 10^{-2}M IAA (25:75v/v)

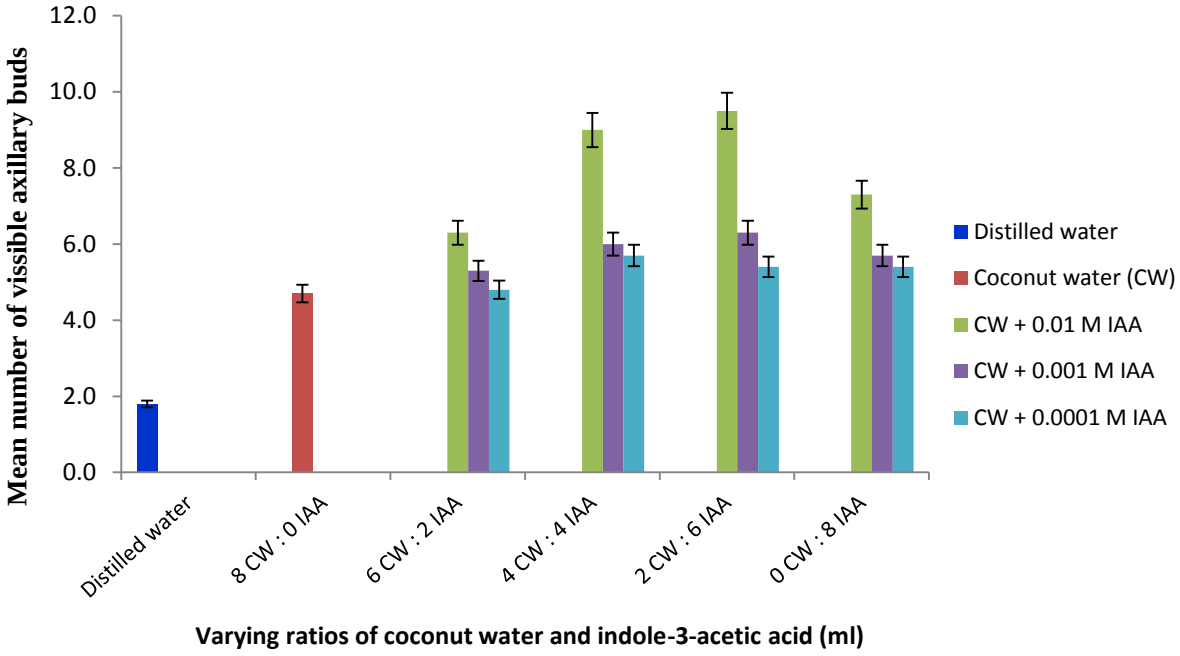


Figure 6. Mean number of fully defferentiated axillary buds of 3-month old split corm-derived Asamienu suckers three weeks after the injection with varying ratios of coconut water (CW) and indole-3-acetic acid (IAA). Bars show standard deviation with n= 6.

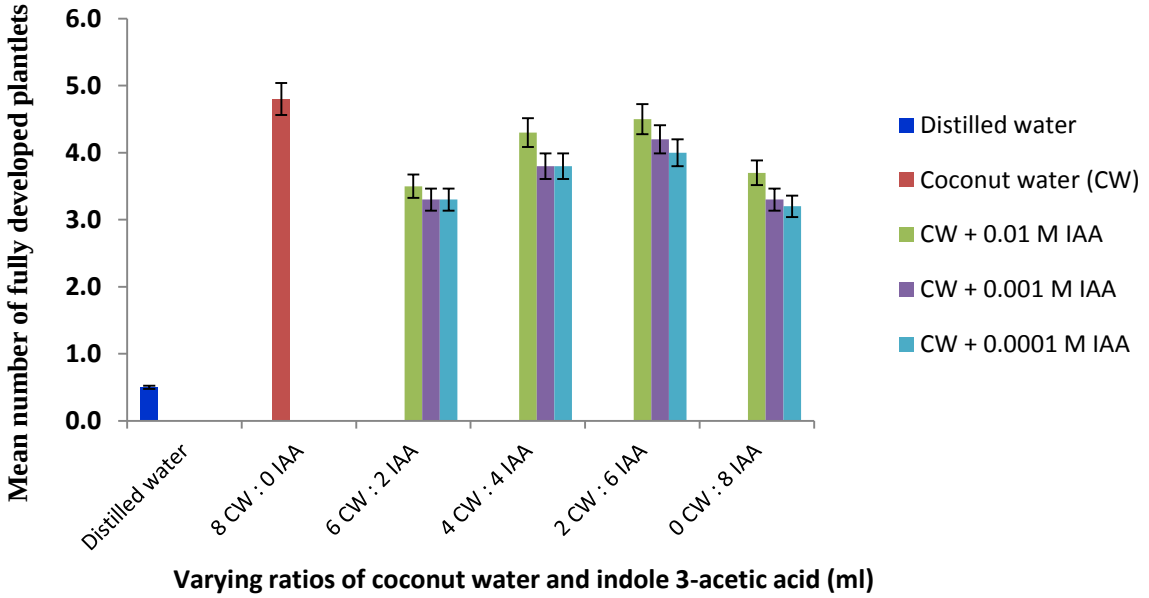


Figure 7. Mean number of fully developed plantlets of 3-month old split corm-derived Asamienu suckers three weeks after the injection with varying ratios of coconut water (CW) and indole-3-acetic acid (IAA) concentrations . Bars show standard deviation with n= 6.

4.1.4 Effects of varying ratios of indole-3-acetic acid (IAA) and coconut water (CW) on plant height girth, number of leaves and total leaf area of plantlets derived from 3-month old split corm-derived Asamienu suckers.

Figures 8, 9, 10 and 11 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 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.

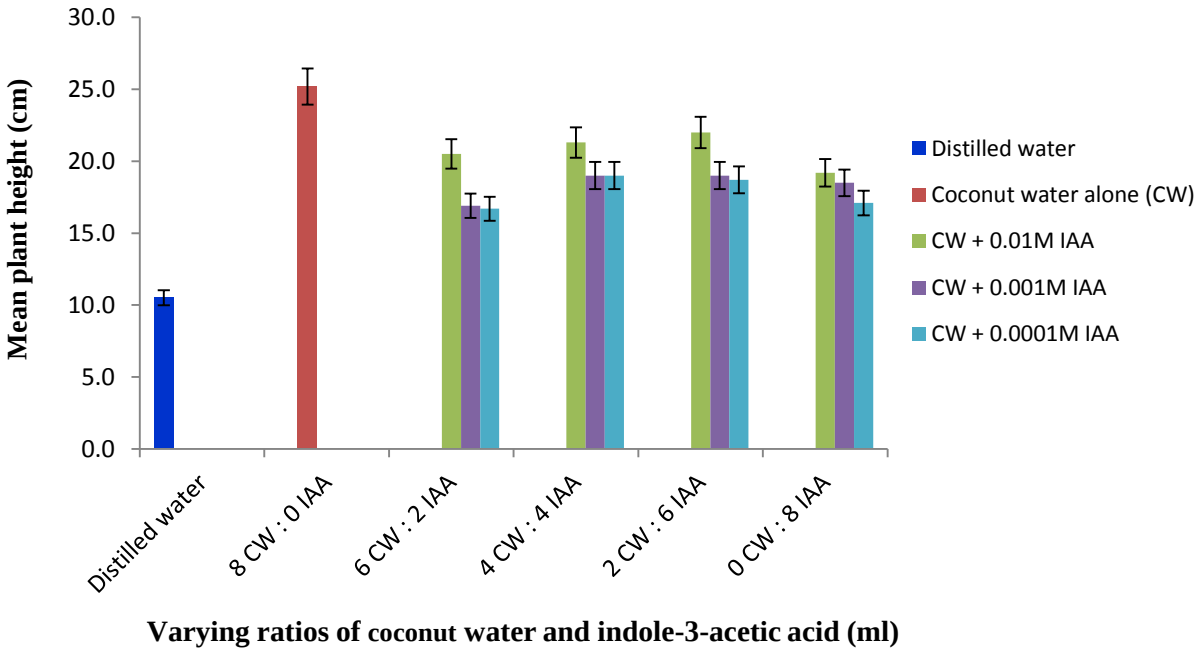


Figure 8. Mean plant height of plantlets derived from 3-month old split corm-derived Asamienu suckersms treated with varying ratios of coconut water (CW) and indole-3-acetic acid (IAA). Bars show standard deviation with n= 6.

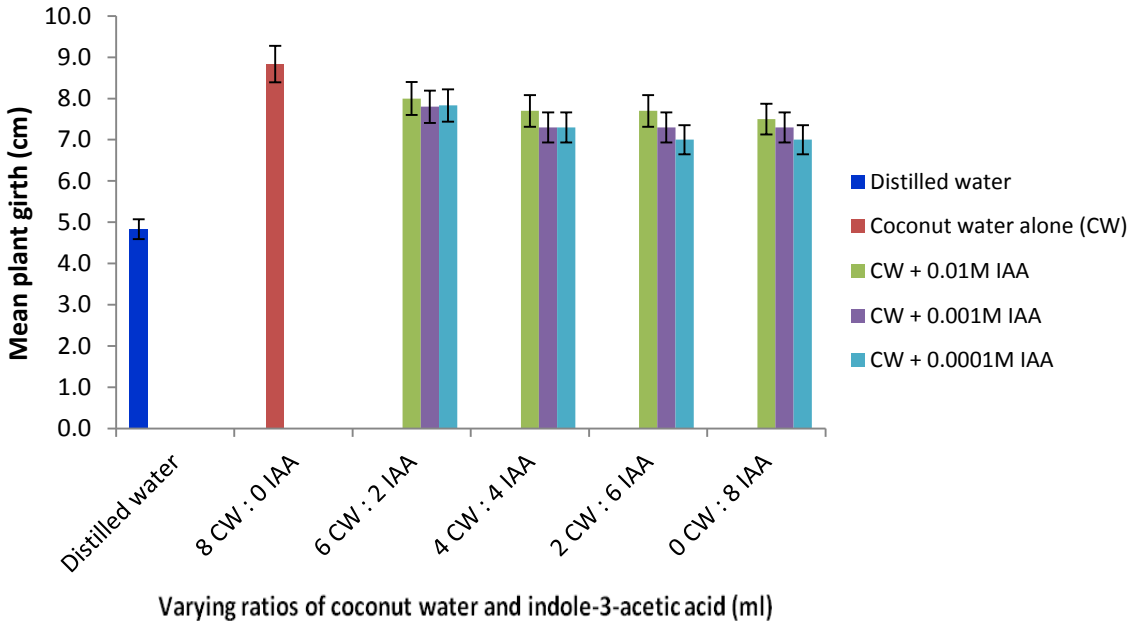


Figure 9. Mean plant girth of plantlets derived from 3-month old split corm-derived Asamienu suckersorms treated with varying ratios of coconut water (CW) and indole-3-acetic acid (IAA). Bars show standard deviation with n= 6.

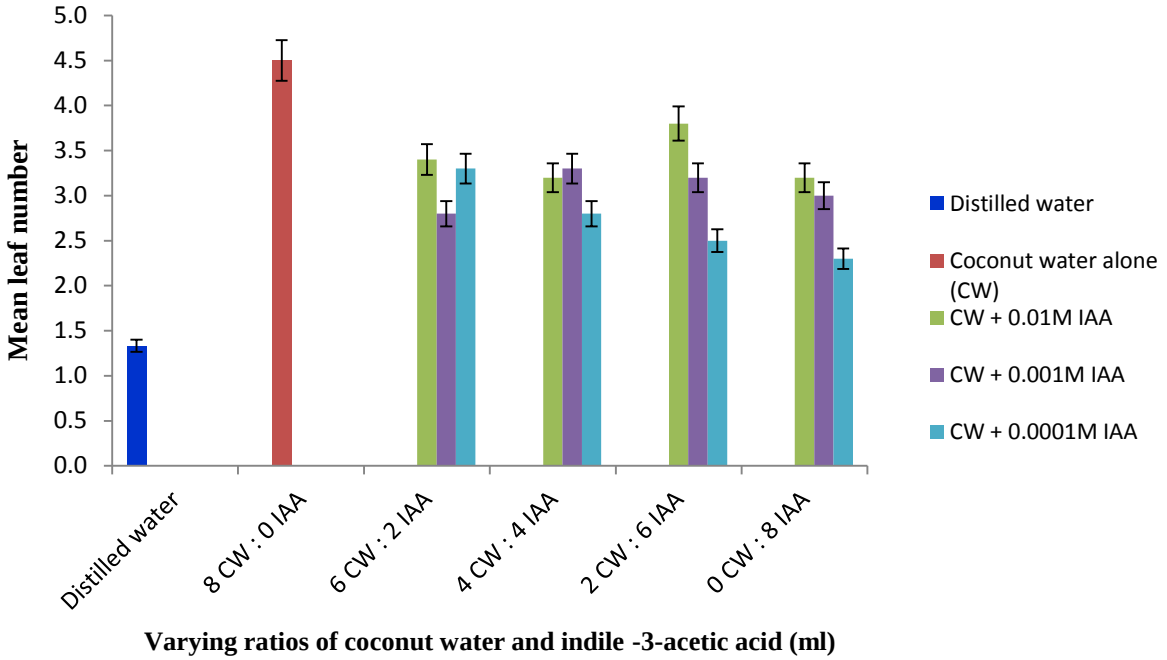


Figure 10. Mean number of leaves of plantlets derived from 3-month old split corm-derived Asamienu suckers treated with varying ratios of coconut water (CW) and indole-3-acetic acid (IAA). Bars show standard deviation with n= 6.

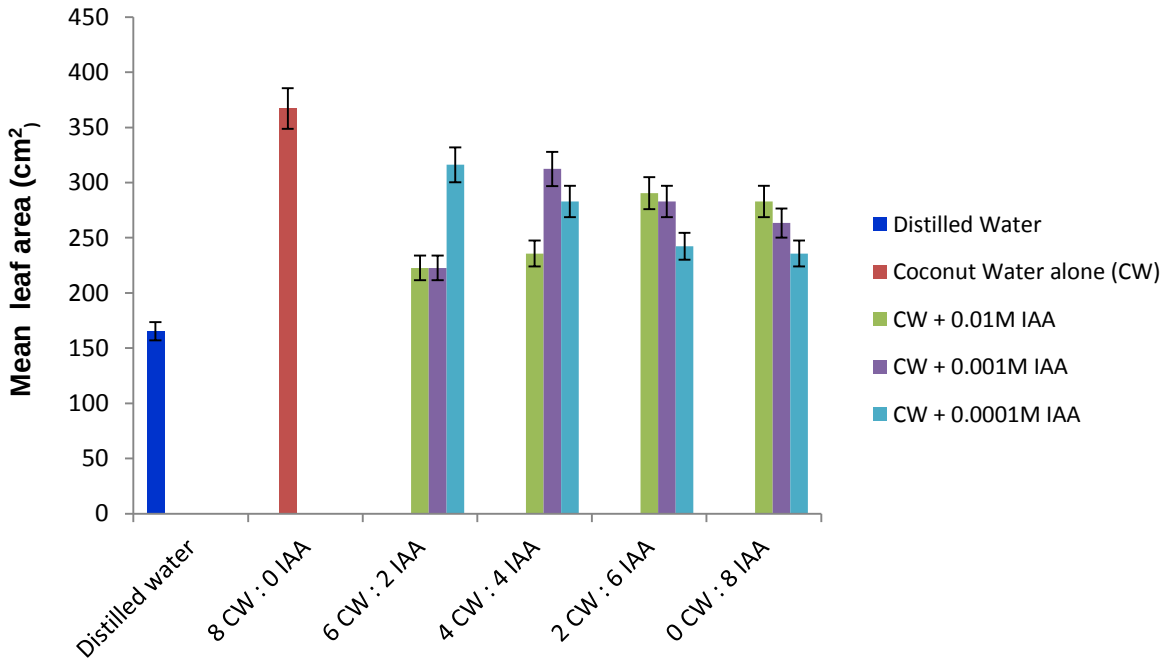


Figure 11. Mean leaf area of plantlets drived from 3-month old split corm-derived Asamienu suckersrms of suckers treated with varying ratios of coconut water (CW) and indole-3-acetic acid (IAA). Bars show standard deviation with n= 6.

4.1.5 Effects of varying ratios of coconut water and indole-3-acetic acid on proliferation of fully differentiated axillary buds and plantlets of 3-month old split corm-derived Asamienu suckers one month after the application of the bud manipulation technique.

Figure 12 shows the number of fully differentiated axillary buds developed from corms of 3-month old split corm-derived Asamienu suckers one month after the application of the bud manipulation technique.

The number of well-differentiated axillary buds increased as the IAA concentration and volume increased up to 6ml 10^{-2}M IAA and then declined as the volume increased further. The highest number of well-differentiated axillary buds (22.5) was produced with 2ml coconut water plus 6ml 10^{-2}M IAA. Coconut water alone or in combination with IAA produced higher number of well-differentiated axillary buds than the control treatment.

Figure 13 shows the number of plantlets developed from fully differentiated axillary buds of corms of 3-month old split corm-derived Asamienu suckers one month after the application of the bud manipulation technique. 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 4ml coconut water plus 4ml 10^{-2}M IAA. Coconut water alone or in combination with IAA produced higher number of plantlets than the control treatment.

I II

III IV

Plate 10. Effects of varying ratios of coconut water and indole-3-acetic acid on the number of fully differentiated axillary buds and plantlets of 3-month old split corm-derived Asamienu suckers one month after the application of the bud manipulation technique. I=Distilled water, II= Coconut water alone, III= CW: 10^{-2}M IAA (50:50 v/v), IV= CW: 10^{-2}M IAA (25:75v/v)

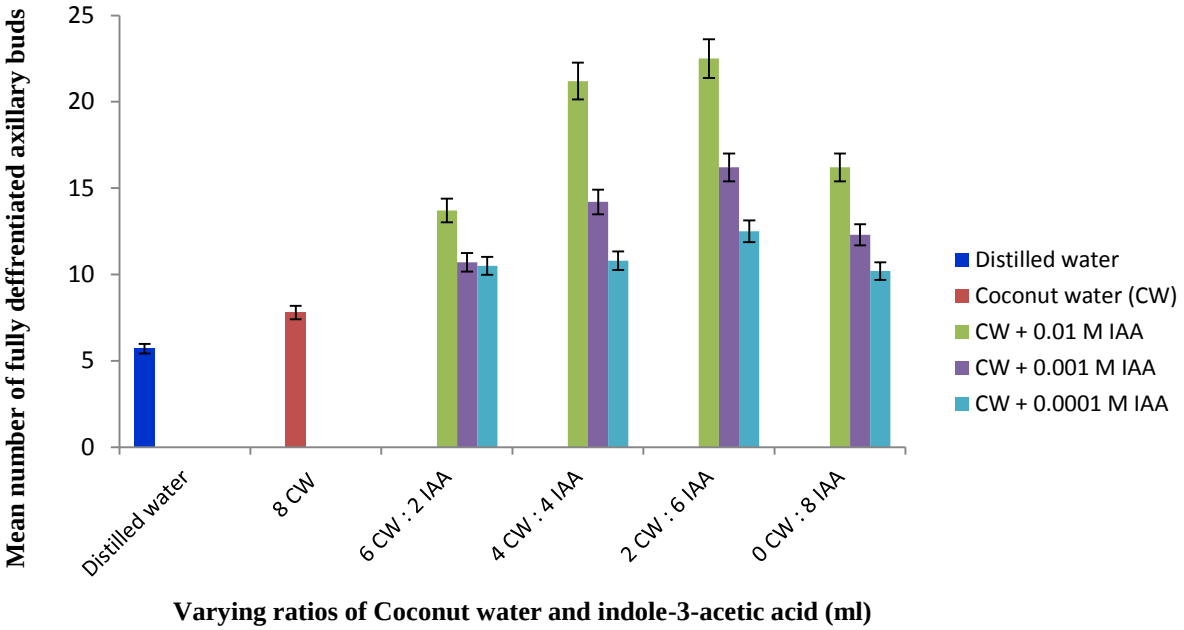


Figure 12 . Mean number of fully differentiated axillary buds development from corms of 3-month old split corm-derived Asamienu suckers one month after the application of the bud manipulation technique. Bars show standard deviation with n= 6.

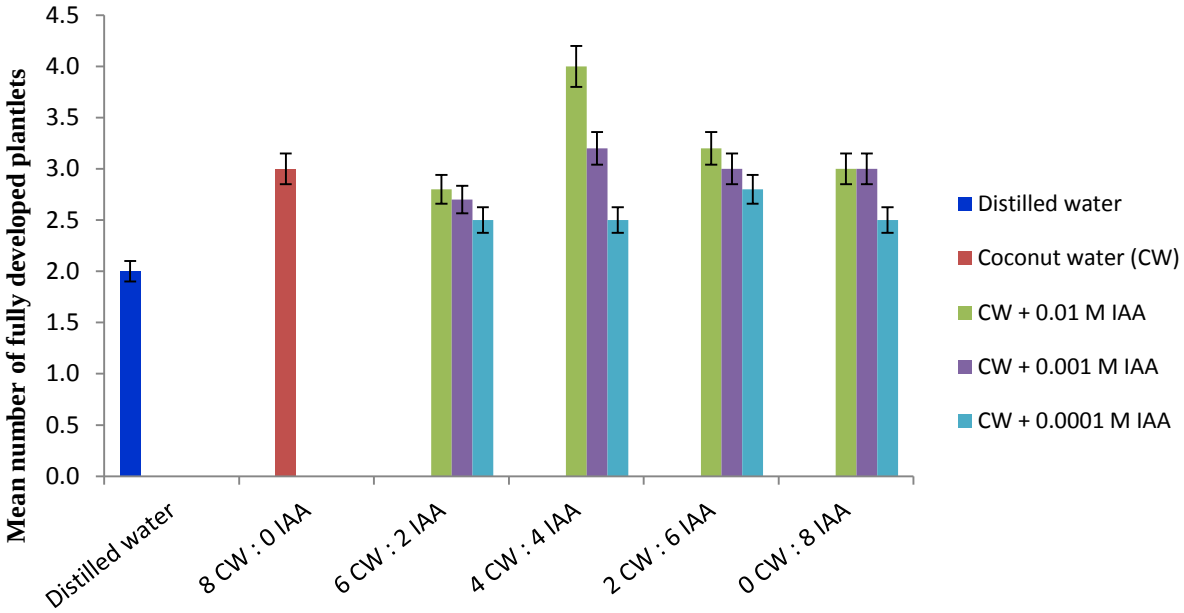


Figure 13. Mean number of fully developed plantlets from corms of 3-month old split corm-derived Asamienu suckers one month after the application of the bud manipulation technique. Bars show standard deviation with n= 6.

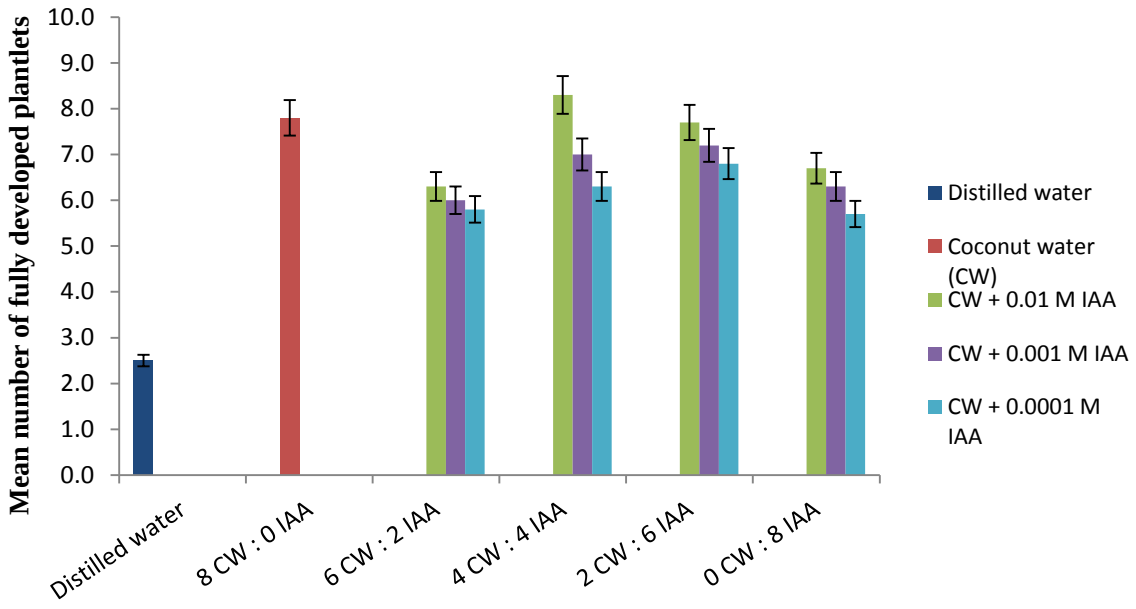


Figure 14. Total number of fully developed plantlets per palnt obtained from corms of 3-month old split corm-derived Asamienu suckers three weeks after the injection treatment and one month after the application of the bud manipulation technique. Bars show standard deviation with n= 6.

Figure 14 shows 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. Coconut water alone or in combination with all the various IAA 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 2ml coconut water plus 6ml 10^{-2}M IAA. The highest number of plantlets (8.3) produced was obtained with 4ml coconut water plus 4ml 10^{-2}M IAA and was significantly higher than that obtained from distilled water (2.5).

RESULTS

4.2 Experiment 2

4.2.1 Changes in the endogenous content of cytokinin (trans- zeatin riboside) and auxin (IAA) in coconut water at different fruit maturity stages.

Changes in endogenous contents of cytokinin (trans- zeatin riboside) and auxin (IAA) in coconut water at different fruit maturity stages is shown in Figure 15.

The endogenous content of trans- zeatin riboside and IAA in coconut water varied at the different fruit maturity stages. IAA content decreased from the liquid endosperm formation stage (1.51µg ml^{-1}) to fully matured dried stage (0.75 µg ml^{-1}). Thus the highest concentration of IAA is at the liquid endosperm formation stage (1.51µg ml^{-1}).

The trans- zeatin riboside content on the other hand increased from the liquid endosperm formation stage (0.44 µg ml^{-1}) to fully matured dried stage (1.01 µg ml^{-1}). Thus the highest concentration of trans- zeatin riboside is at the fully matured dried stage (1.01 µg ml^{-1}).

The correlation coefficient (r) between trans- zeatin riboside and IAA measured was highly significant ($P<0.05$). There was a strong negative linear relationship ($r= -0.92988$) between trans- zeatin riboside and IAA.

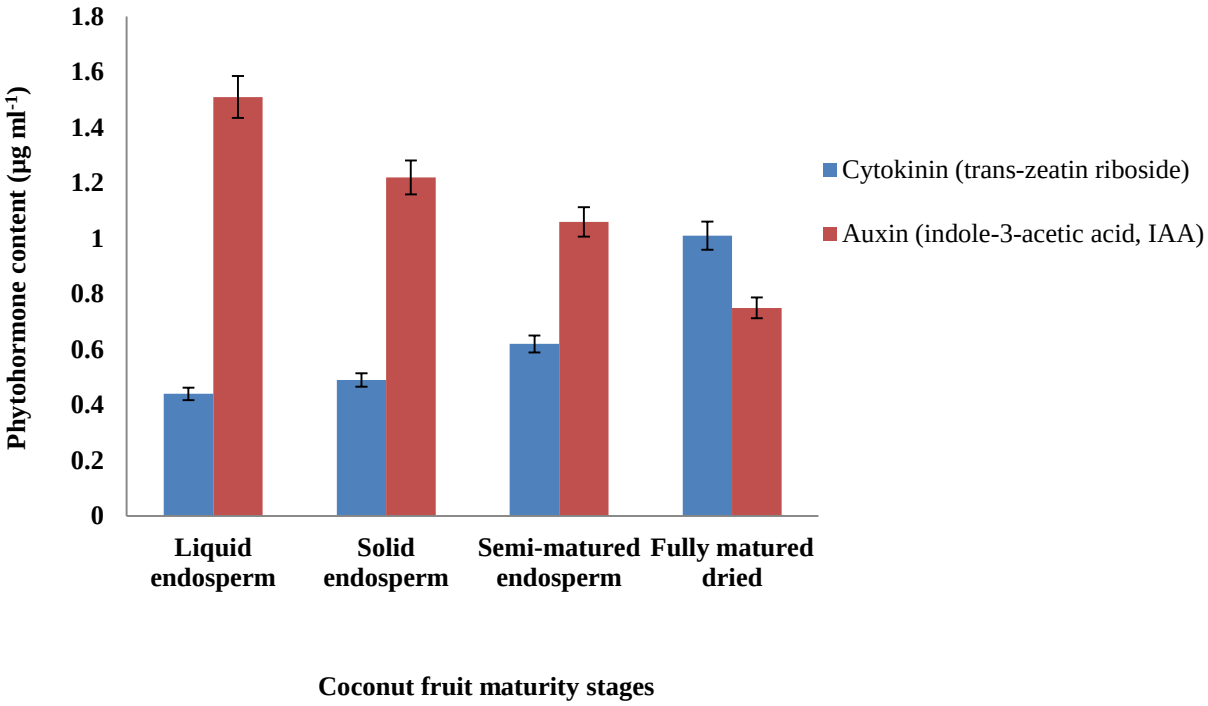


Figure 15. Changes in endogenous contents of cytokinin(trans- zeatin riboside) and auxin (IAA) in coconut water at different fruit maturity stages.Vertical bars represent the mean±SE (n=4). If not shown, error bars are smaller than the symbol size.

4.2.2 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.

Figures 16 and 17 respectively show 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 at 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 increased in plant girth (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 at the fully matured dried stage recorded the lowest percentage growth in height of 1.1 % (Figure 16) and also the highest increasing effect on the percentage growth in girth of 70.1% (Figure 17). In contrast, coconut water at the liquid endosperm formation stage had the highest percentage growth in height (4.5%) and also the lowest percentage increase in girth (58.9%) among the coconut water treatments. Coconut water from fruits at the various maturity stages recorded significantly increased percentage growth in girth compared with the distilled water treatment.

It was observed that plant height decreased as the fruit matured while plant girth inreased with increased fruit maturity.

Ia

IIa

Ib

IIb

Plate 11. Effects of coconut water from different maturity stages on the growth and suckering of 3-month old split corm-derived Asamienu suckers three weeks after the injection treatment. IA=Distilled water (Top part), Ib =Distilled water (Base part), IIa= Semi-matured endosperm stage (Top part), IIb= Semi-matured endosperm stage (Base part)

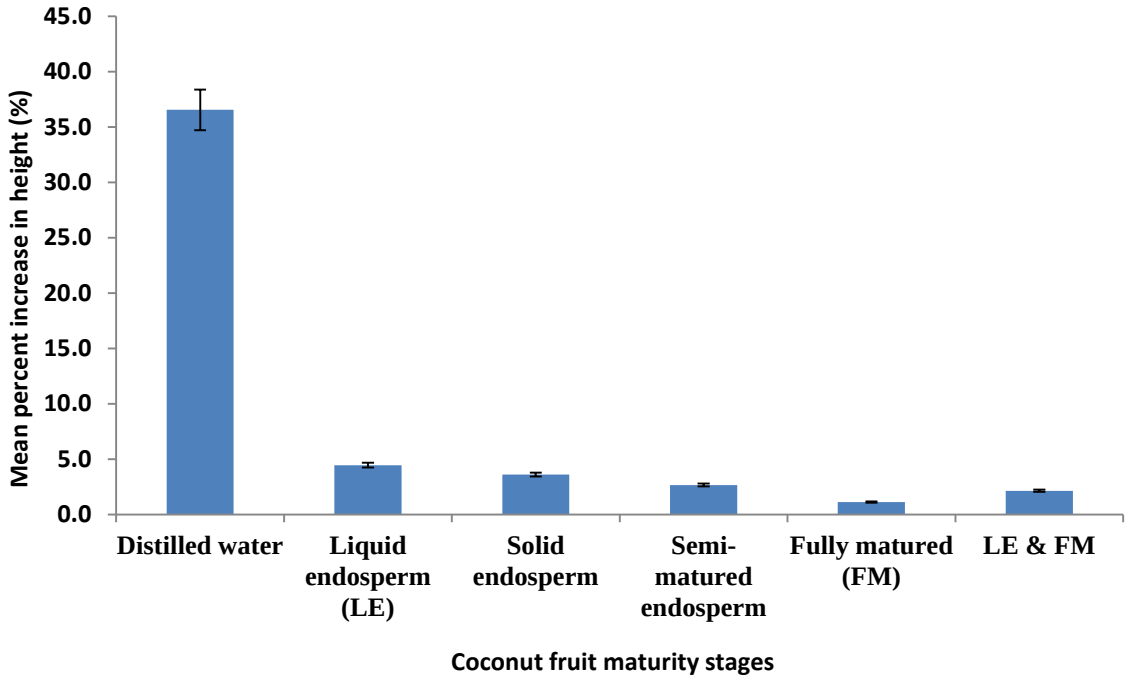


Figure 16. **Percentage increase in plant height of 3-month-old split corm derived Asamienu suckers three weeks after injection with coconut water from fruits at different maturity stages. Bars show standard deviation with n= 6.**

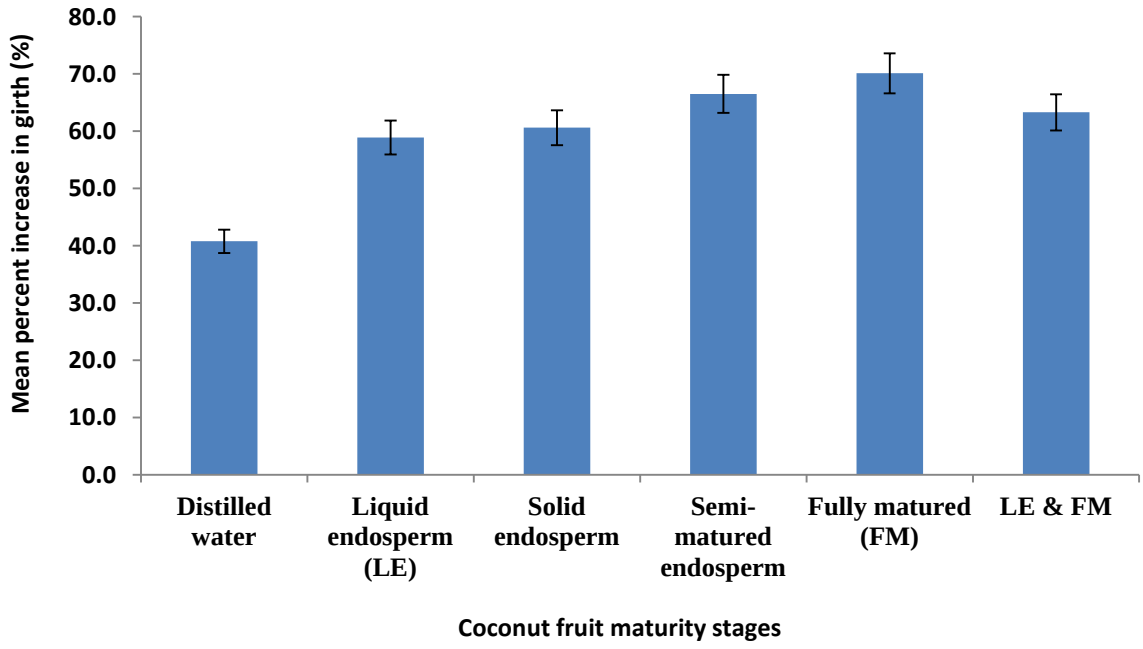


Figure 17. **Percentage increase in plant girth of 3-month-old split corm derived Asamienu suckers three weeks after injection with coconut water from fruits at different maturity stages. Bars show standard deviation with n= 6.**

4.2.3 Effects of coconut water from different maturity stages on production of fully differentiated axillary buds and plantlets of 3-month old split corm-derived Asamienu suckers

Plate 12. Effects of coconut water from different maturity stages on fully differentiated axillary buds and plantlets of 3-month old split corm-derived Asamienu suckers 3 weeks after the injection treatment. I=Distilled water, II= Liquid endosperm stage (T1), III= Solid endosperm stage (T2), IV= Semi-matured endosperm stage (T3), V= Fully matured dried stage (T4), VI= Mixed (T1 and T4).

4.2.4 Effects of coconut water from different maturity stages on root production of 3-month old split corm-derived Asamienu suckers.

Figures 18, 19 and 20, respectively show the mean root number, root diameter and root dry weight of 3-month old split corm-derived Asamienu suckers 3 weeks after the injection with coconut water from fruits at different maturity stages.

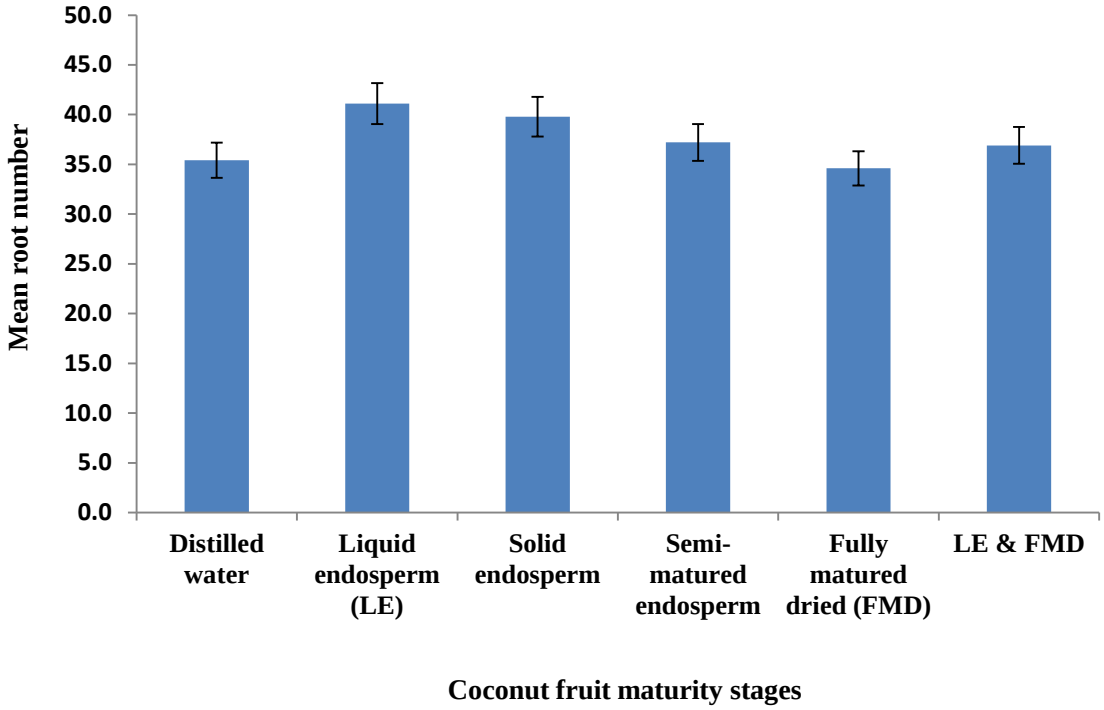


Figure 18. Mean root number of 3-month-old split corm derived Asamienu suckers three weeks after injection with coconut water from fruits at different maturity stages. Bars show standard deviation with n= 6.

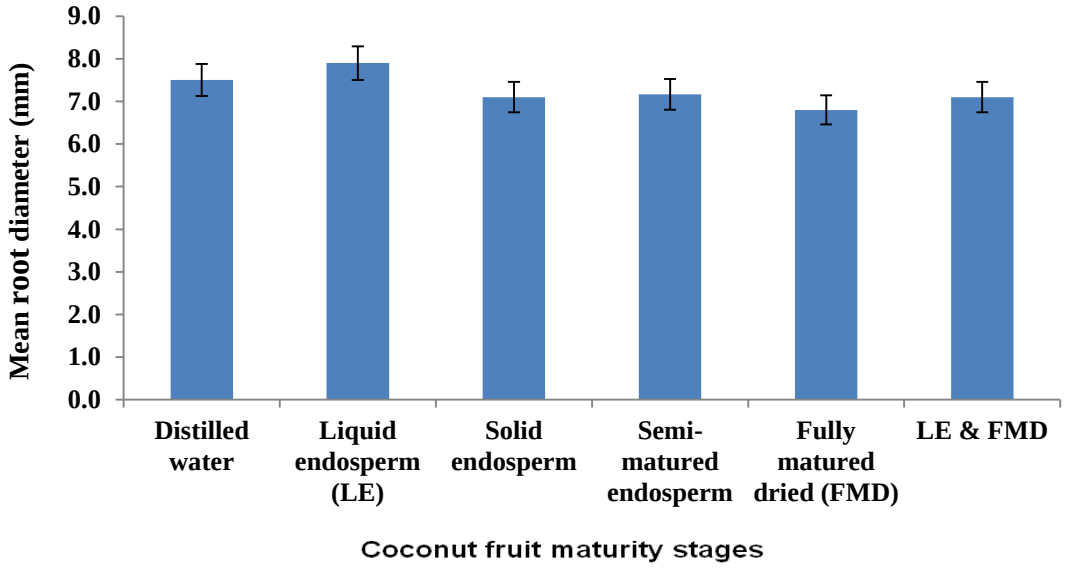


Figure 19. Mean root diameter of 3-month-old split corm derived Asamienu suckers three weeks after injection with coconut water from fruits at different maturity stages. Bars show standard deviation with n= 6.

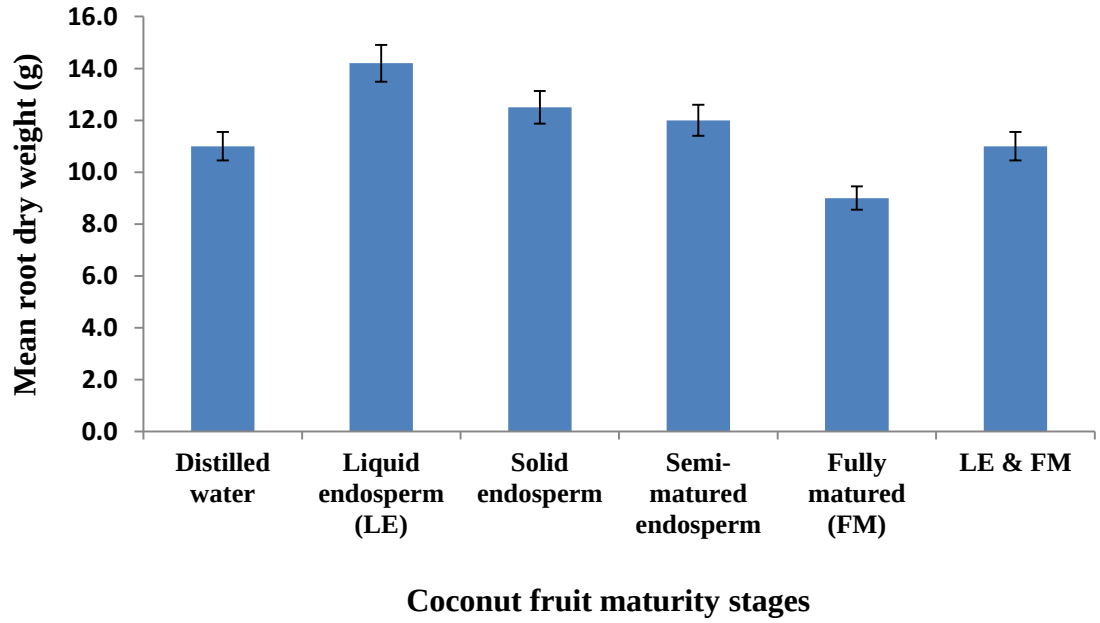


Figure 20. Root dry weight of 3-month-old split corm derived Asamienu suckers three weeks after injection with coconut water from fruits at different maturity stages. Bars show standard deviation with n= 6.

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).

4.2.5 Effects of coconut water from fruits at different maturity stages on fully differentiated axillary buds and plantlets of 3-month old split corm-derived Asamienu suckers.

Figure 21 shows the number of fully differentiated axillary buds 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 from 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.

Figure 22 shows the number of plantlets that developed from the well-differentiated axillary buds with the various injection treatments. The highest (5.5) and the lowest (0.3) number of fully developed plantlets was produced from treatments with coconut water from semi-endosperm formation stage and distilled water respectively.

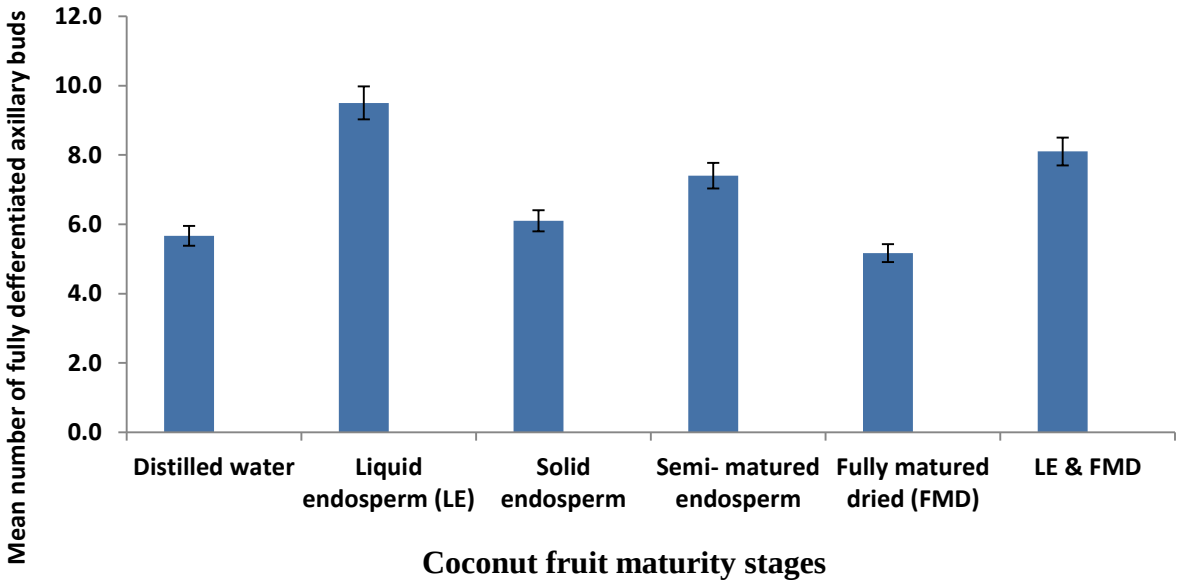


Figure 21. Mean number of fully defferentiated axillary buds of 3-month old split corm-derived Asamienu suckers three weeks after the injection with coconut water from fruits at different maturity stages. Bars show standard deviation with n= 6.

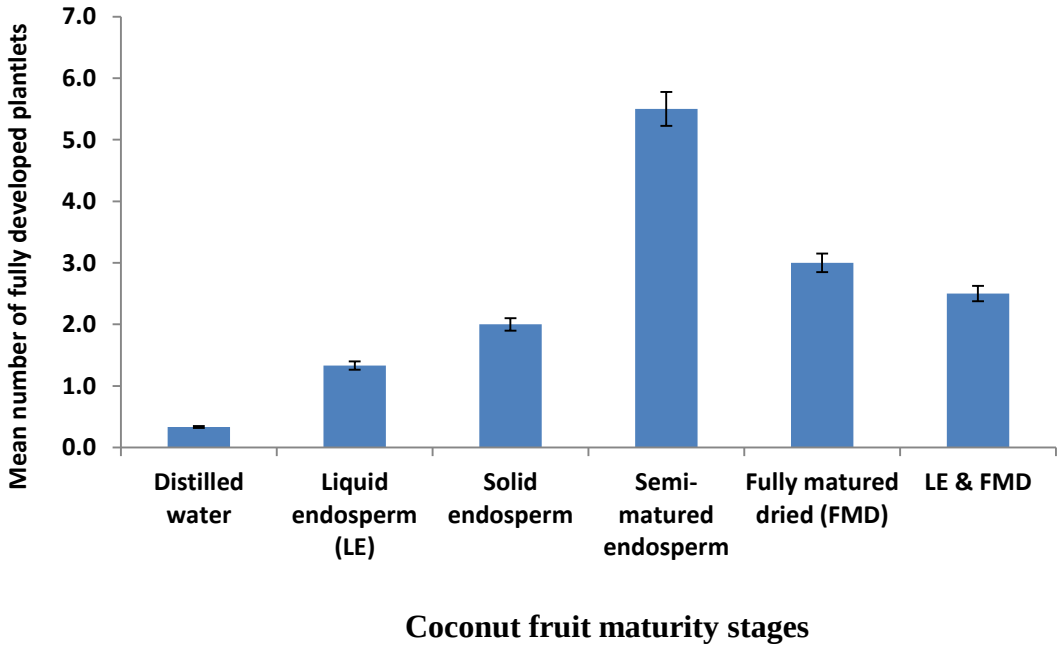


Figure 22. Mean number of fully developed plantlets of 3-month old split corm derived Asamienu suckers three weeks after injection with coconut water from fruits at differernt maturity stages . Bars show standard deviation with n= 6.

4.2.6 Effects of coconut water at different fruits maturity stages on plant height, girth, number of leaves and total leaf area of plantlets derived from 3-month old split corm-derived Asamienu suckers.

Figures 23, 24, 25, and 26 respectively show 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. Plantlets generated from suckers treated with coconut water at all the different stages were bigger than that obtained from the distilled water treatment.

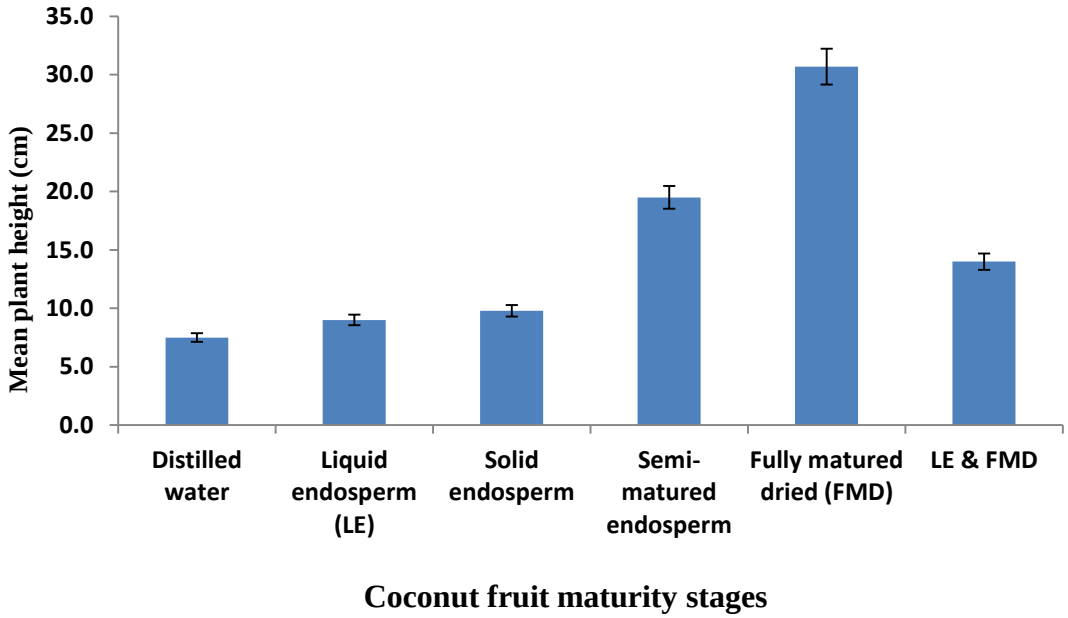


Figure 23. Mean plant height of plantlets derived from 3- month old split corm derived Asamienu suckers three weeks after injection with coconut water from fruits at different maturity stages. Bars show standard deviation with n= 6.

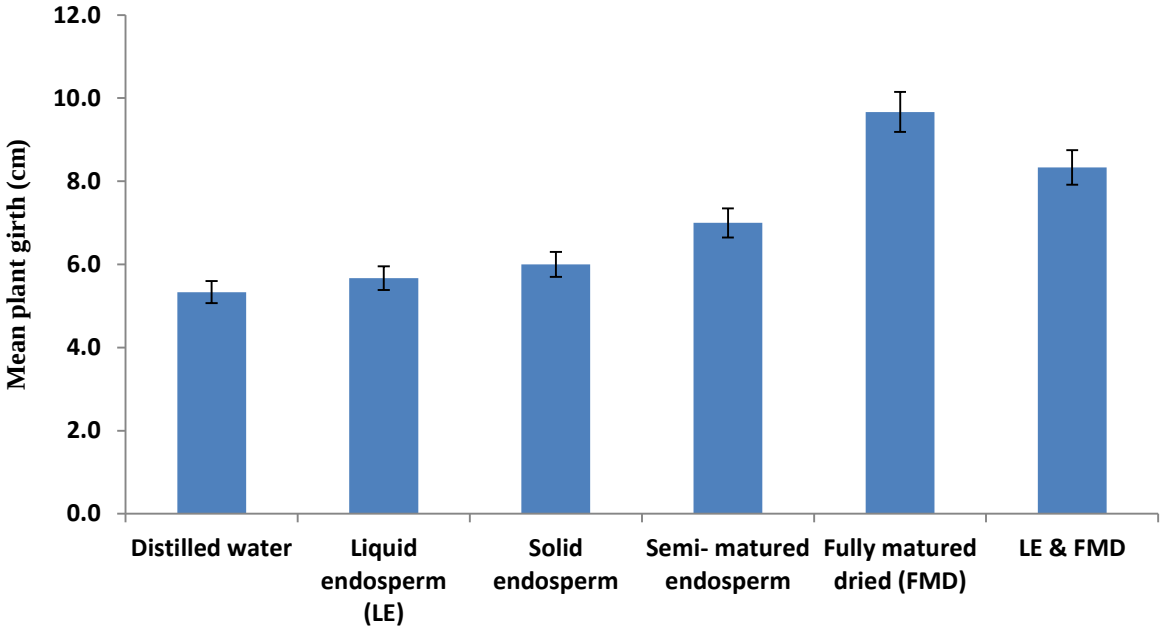


Figure 24. Mean plant girth of plantlets derived from 3-month old split corm derived Asamienu suckers three weeks after injection with coconut water from fruits at different maturity stages. Bars show standard deviation with n= 6.

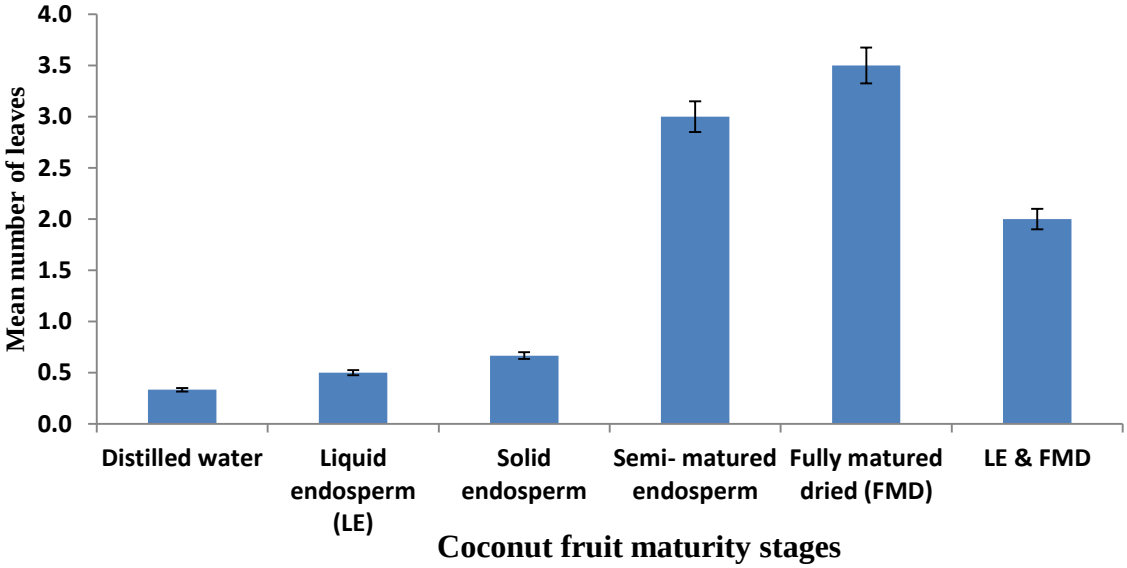


Figure 25. Mean number of leaves of plantlets drived from 3-month old split corm derived Asamienu suckers three weeks after injection with coconut water from fruits at diffrent maturity stages . Bars show standard deviation with n= 6.

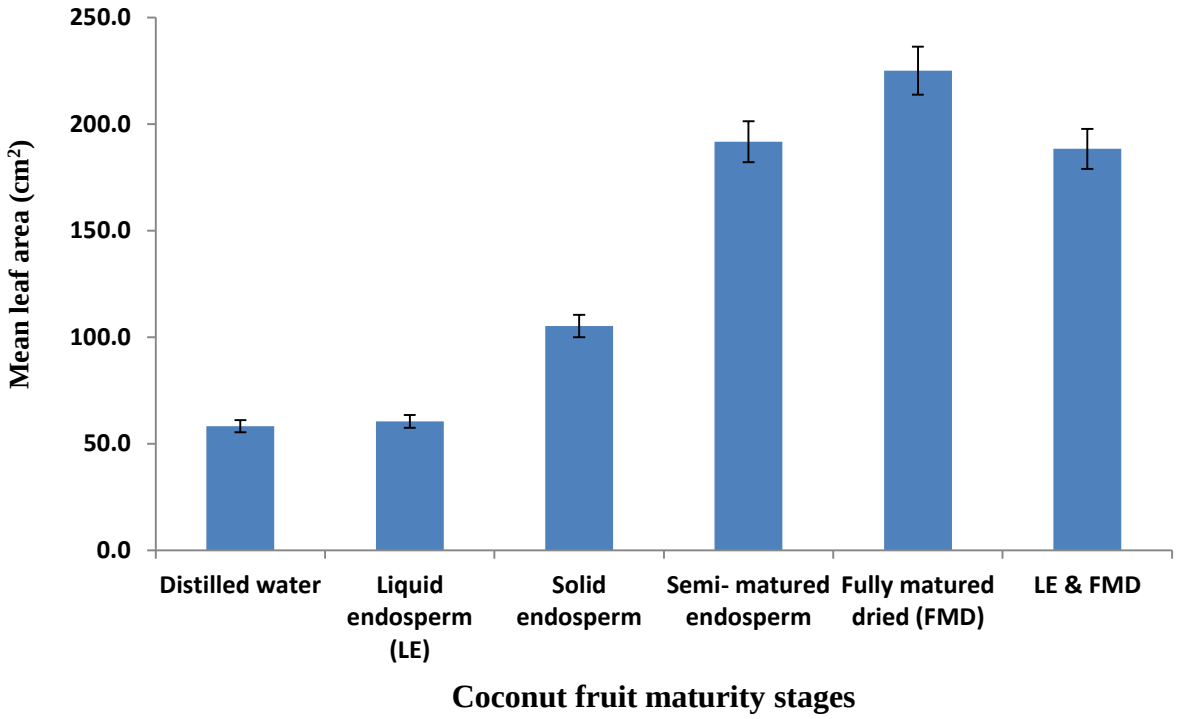


Figure 26. Mean leaf area of plantlets derived from 3-month old split corm-derived Asamienu suckers three weeks after injection with coconut water from fruits of different maturity stages. Bars show standard deviation with n= 6.

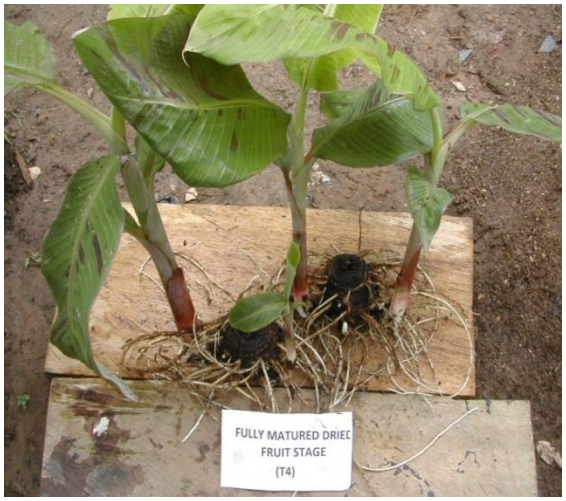
4.2.7 Effects of coconut water from different maturity stages on fully differentiated axillary buds and plantlets of 3-month old split corm-derived Asamienu suckers three weeks after the injection treatment and one month after the application of the bud manipulation technique.

Figure 27 shows the number of fully differentiated axillary buds produced by 3-months old split corm derived Asamienu suckers three weeks after injection with coconut water from fruits at different maturity stages and one month after application of the bud manipulation technique.

The number of well-differentiated axillary buds decreased from treatments with coconut water at liquid endosperm formation stage to fully matured dried stage. The highest number of well-differentiated axillary buds (18.0) was obtained from suckers treated with coconut water at liquid endosperm formation stage whilst the lowest number was obtained with distilled water treatment (4.7).

Figure 28 shows the number of fully developed plantlets produced by 3-months old split corm derived Asamienu suckers three weeks after injection with coconut water from fruits at different maturity stages and one month after application of the bud manipulation technique. The highest number of fully developed plantlets (5.7) was produced from treatments with coconut water at liquid endosperm formation stage. The rresults also indicated that the number of fully developed plantlets decreased from treatments with coconut water at liquid endosperm formation stage to fully matured dried stage.

I II



III IV

Plate 13. Effects of coconut water at different fruit maturity stages on fully differentiated axillary buds and plantlets 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. I= Liquid endosperm stage, II=Solid endosperm stage, III= Fully matured dried stage, IV= Distilled water

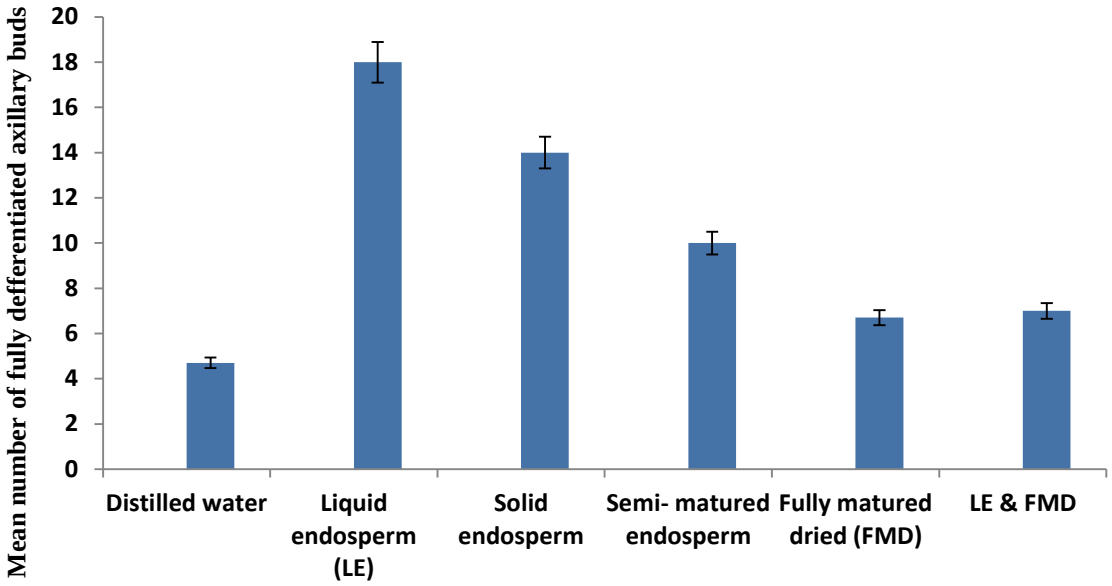


Figure 27. Mean number of of fully defferentiated axillary buds produced three weeks after injection with coconut water from fruits at different maturity stages and one month after the application of bud manipulaton technique. Bars show standard devia

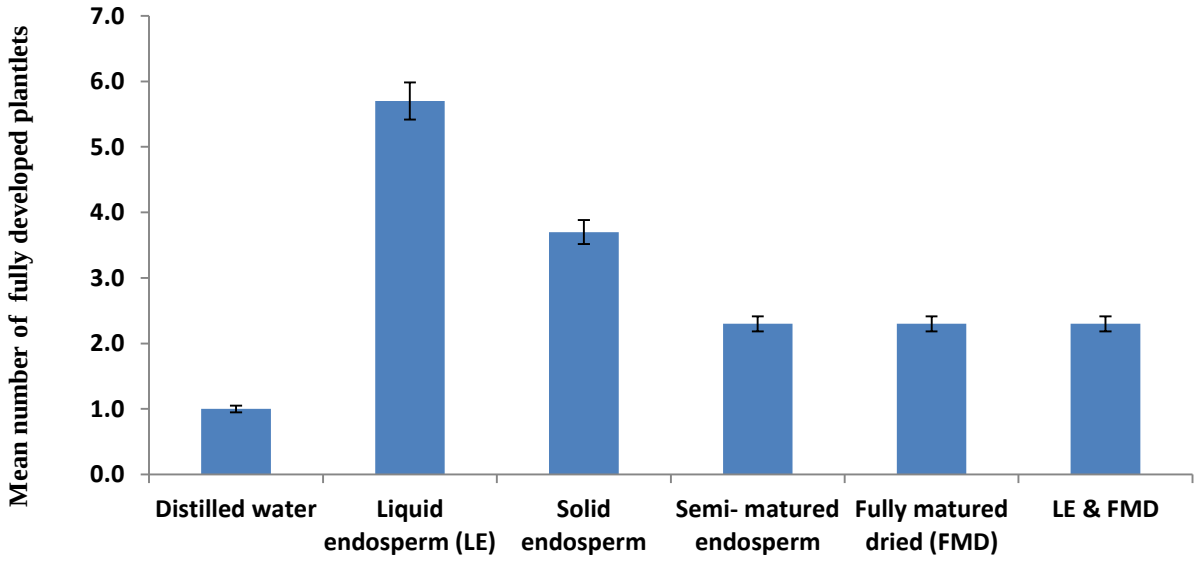


Figure 28. Mean number of fully developed plantlets of 3-month old split corm derived Asamienu suckers three weeks after injection with coconut water from fruits at differernt maturity stages and one month after application of the bud manipulation tec

4.2.8 Effects of coconut water at different fruit maturity stages on plant height, girth, number of leaves and total leaf area of plantlets derived from 3- month old split corm derived Asamienu suckers three weeks after injection treatment and one month after the application of the bud manipulation technique.

Figures 29, 30, 31, and 32 respectively show 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 and the subsequent application of the bud manipulation technique.

Plant height, girth, leaf number and total leaf area of plantlets obtained from suckers treated with coconut water from fully matured dried fruits were numerically higher than that derived from all other treatments. Thus treatments with coconut water from fully matured dried fruits produced vigorously growing plantlets than the other treatments. Plantlets generated from suckers treated with coconut water at all the different stages were more than that obtained from the distilled water treatment.

The highest plant height (35.0 cm), girth (9.3 cm), number of leaves (4.4) and leaf area (1236.1 cm^2) were obtained with treatments with coconut water at fully matured dried stage. However, the lowest plant height (17.5 cm), girth (5.0 cm) and leaf area (375.6 cm^2) were obtained with treatments with coconut water at liquid endosperm formation stage. The results showed that coconut water at fully matured dried stage significantly produced vigorously growing plantlet than plantlets produced with coconut water at liquid endosperm formation stage.

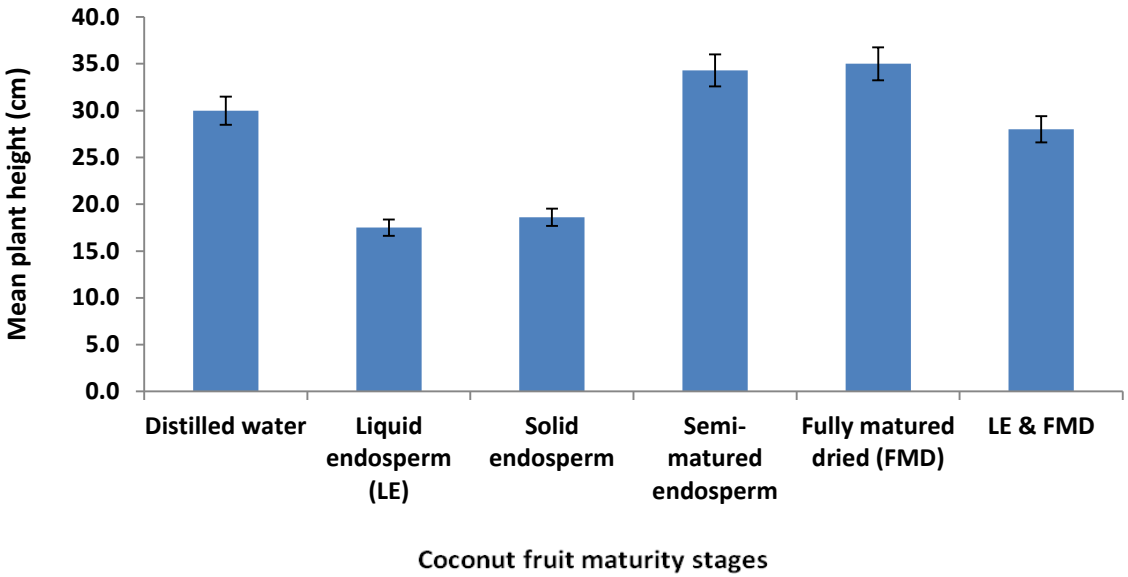


Figure 29. Mean plant height of plantlets derived from produced three weeks after injection with coconut water from fruits at different maturity stages and one month after the application of the bud manipulation technique. Bars show standard deviation

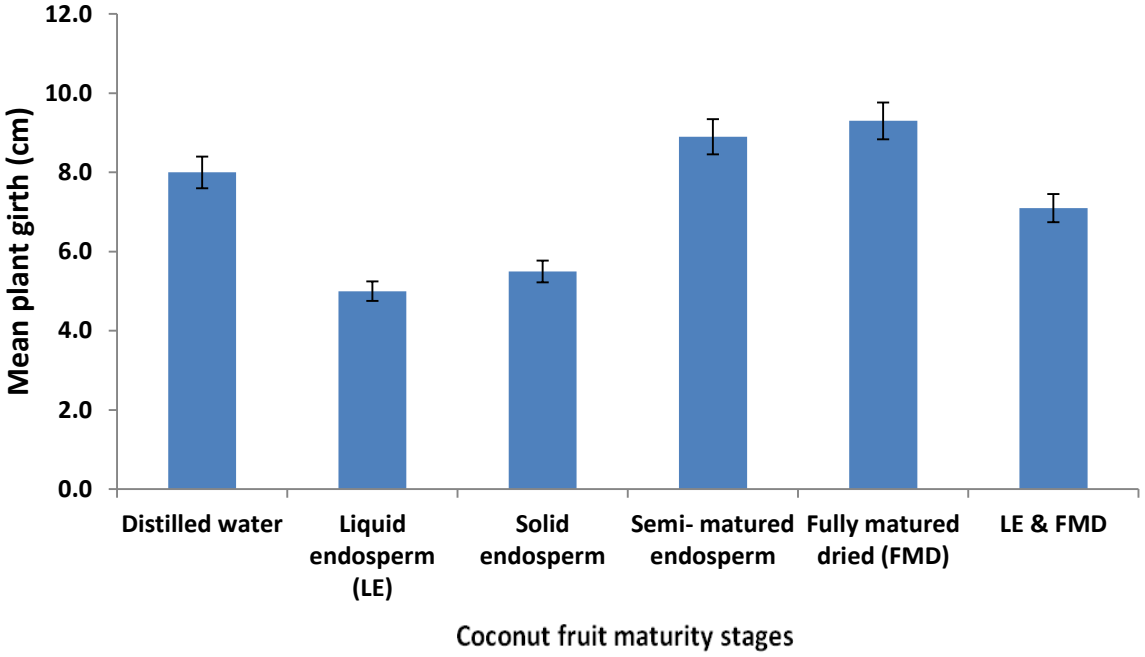


Figure 30. Mean plant girth of plantlets produced three weeks after injection with coconut water from fruits at different maturity stages and one month after the application of the bud manipulation technique.

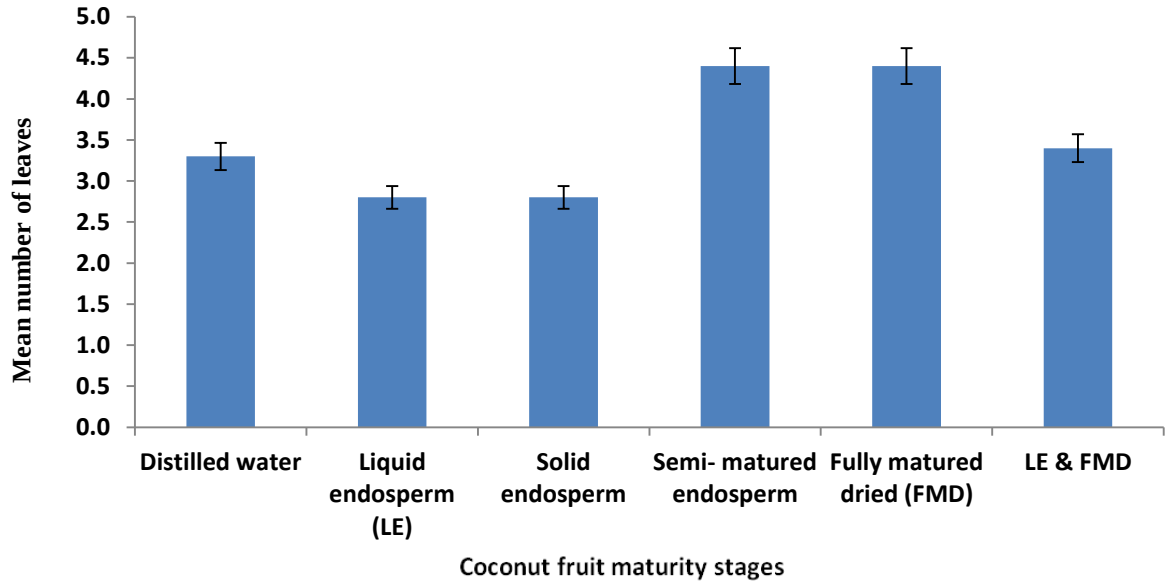


Figure 31. Mean number of leaves of plantlets produced three weeks after injection with coconut water from fruits at different maturity stages and one month after the application of the bud manipulation technique. Bars show standard deviation with n= 6.

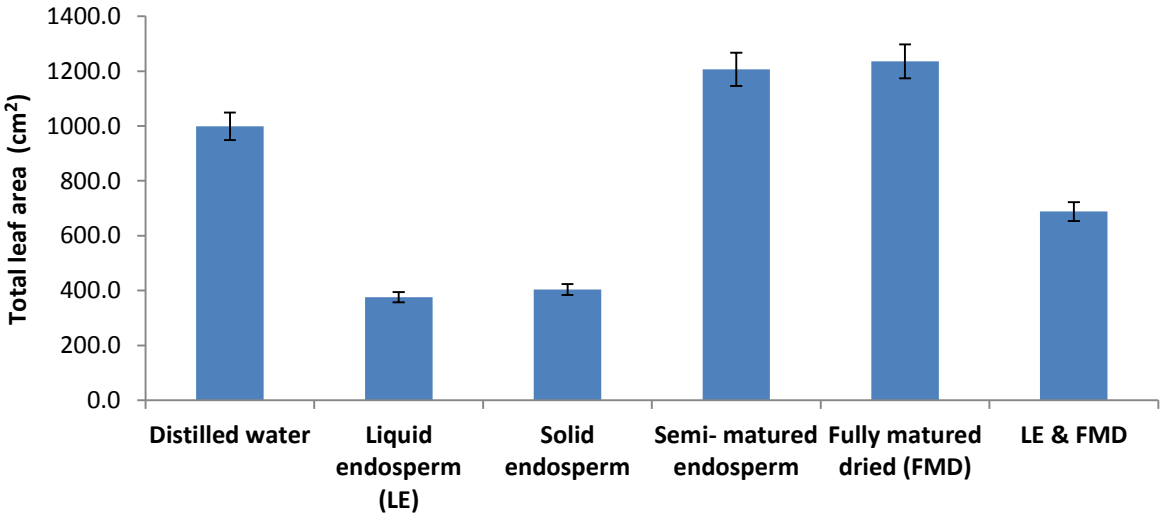


Figure 32.Total leaf area of plantlets produced three weeks after injection with coconut water from fruits at different maturity stages and one month after the application of the bud manipulation technique. Bars show standard deviation with n= 6.

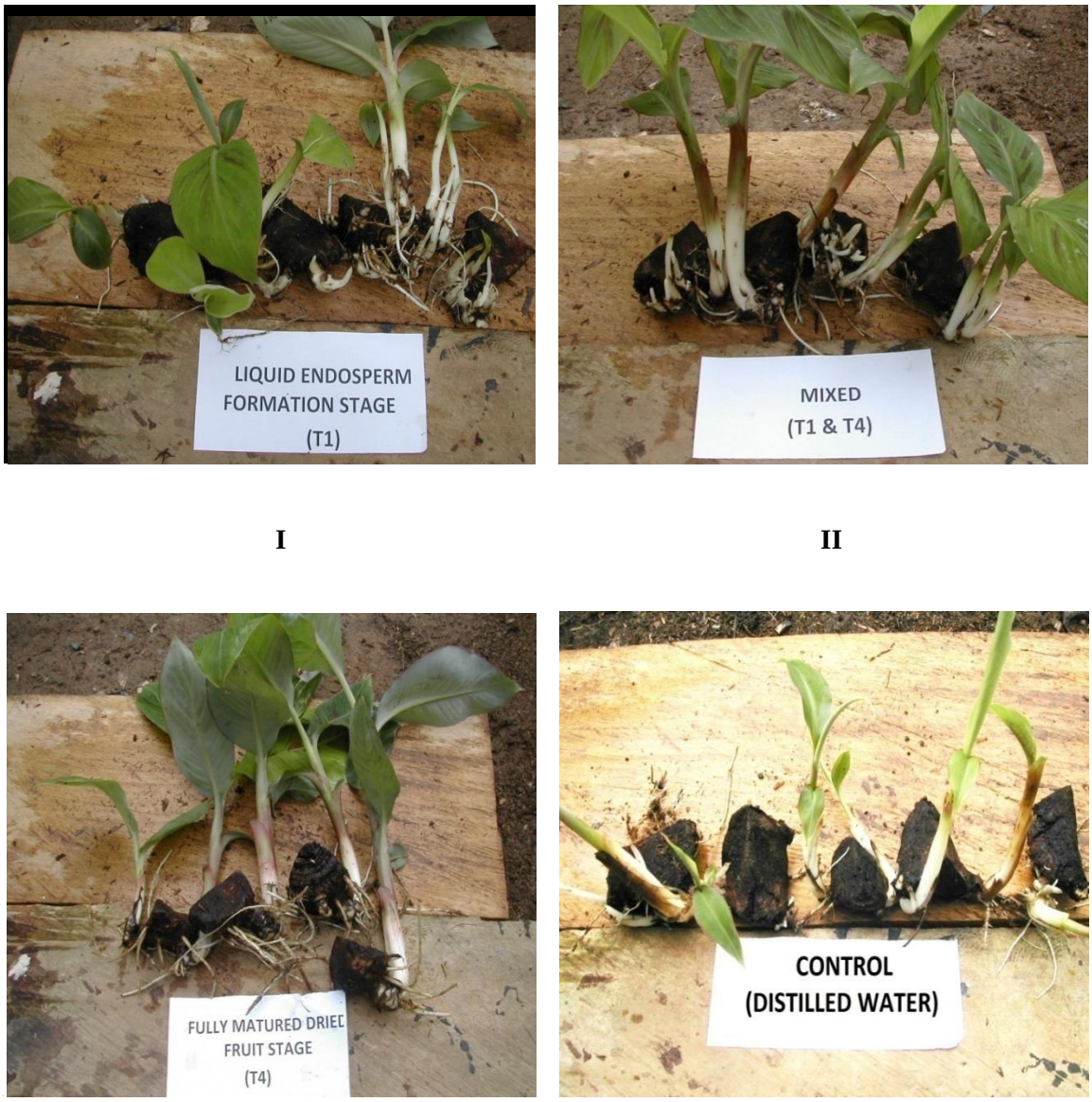
4.2.9 Effects of coconut water at different fruit maturity stages on fully differentiated axillary buds and plantlets of 3-month old split corm-derived Asamienu suckers three weeks after the injection treatment and one month after the application of the split corm technique.

Figure 33 shows the number of fully differentiated axillary buds produced by 3-months old split corm derived Asamienu suckers three weeks after injection with coconut water from fruits at different maturity stages and one month after application of the split corm technique.

Treatments with coconut water at liquid endosperm formation stage significantly produced a higher number of fully differentiated axillary buds (34.5) than the distilled water (12.0). Distilled water treatment produced the lowest number of well-differentiated axillary buds (12.0) than any of the coconut water treatments (15.2- 34.5).

Figure 34 shows the number of fully developed plantlets produced by 3-months old split corm derived Asamienu suckers three weeks after injection with coconut water from fruits at different maturity stages and one month after application of the split corm technique.

The highest number of fully developed plantlets (7.0) was produced by combination of coconut water at liquid endosperm formation stage and fully matured dried stage. Distilled water treatment produced the lowest number of fully developed plantlets (5.0) than any of the coconut water treatments (5.5- 7.0).



I **II**

III **IV**

Plate 14. Effects of coconut water at different fruit maturity stages on fully differentiated axillary buds and plantlets of 3-month old split corm-derived Asamienu suckers three weeks after the injection treatment and one month after the application of the split corm technique. I= Liquid endosperm stage, II= Mixed (T1 and T4), III= Fully matured dried stage, IV= Distilled water

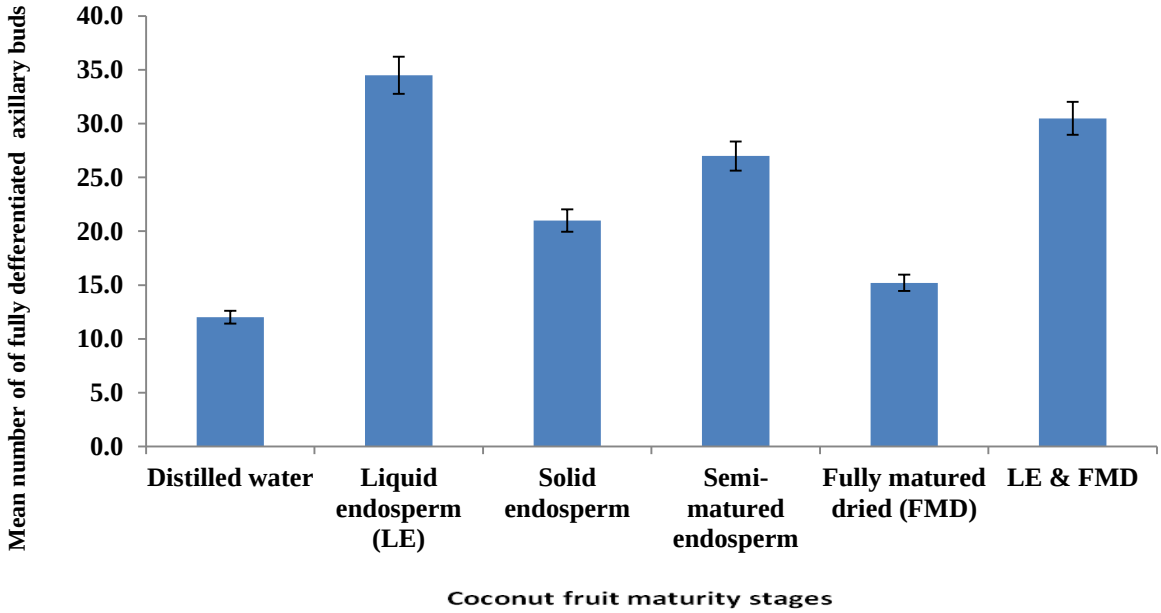


Figure 33. Mean number of fully defferentiated axillary buds produced three weeks after injection with coconut water from fruits at different maturity stages and one month after application of the split corm technique. Bars show standard deviation

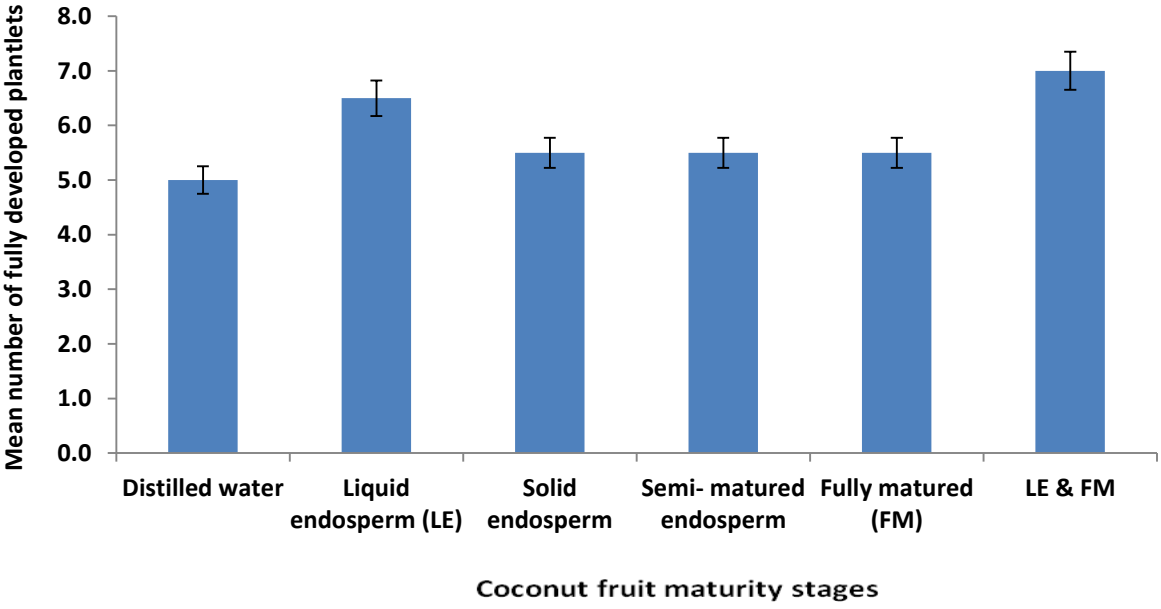


Figure 34. Mean number of fully developed plantlets from 3- month old split corm derived Asamienu suckers three weeks after injection with coconut water from fruits at different maturity stages and one month after application of the split corm technique

4.2.10 Effects of coconut water at different fruit maturity stages on plant height, girth, number of leaves and total leaf area of plantlets derived from 3- month old split corm derived Asamienu suckers three weeks after injection treatment and one month after the application of the split corm technique.

Figures 35, 36, 37, and 38 respectively show the mean plant height, girth, leaf number and total leaf area of plantlets generated from corms of Asamienu suckers treated with coconut water from fruits at different maturity stages and the subsequent application of the split corm technique.

Generally, plant height, girth, leaf number and total leaf area of plantlets obtained from suckers treated with coconut water from fully matured dried fruits were higher than that derived from all other treatments.

The mixture of coconut water from liquid endosperm formation stage and fully matured dried fruits produced plantlets with the highest number of leaves. Plantlets from treatments with coconut water from liquid endosperm formation stage had the lowest number of leaves (1.6) as well as the lowest total leaf area (100.0 cm^2) among the treatments.

The highest plant height (22.4 cm), girth (5.8 cm) and leaf area (340.1 cm^2) were obtained with treatments with coconut water at fully matured dried stage. However, the lowest plant height (14.1 cm), number of leaves (1.6) and leaf area (100.0 cm^2) were obtained with treatments with coconut water at liquid endosperm formation stage.

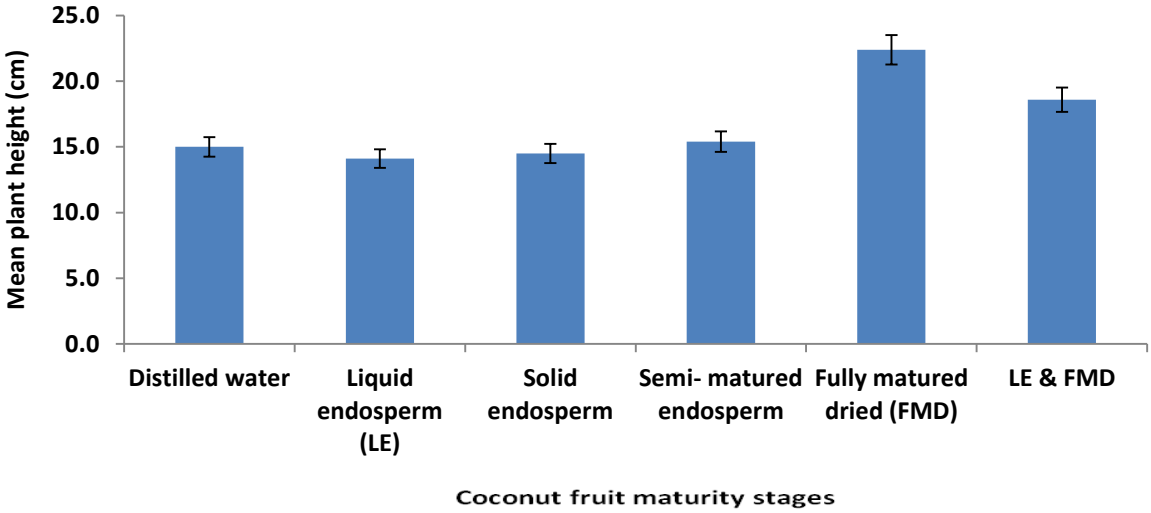


Figure 35. Mean plant height of plantlets produced three weeks after injection with coconut water from fruits at different maturity stages and one month after the application of the split corm technique. Bars show standard deviation with n= 6.

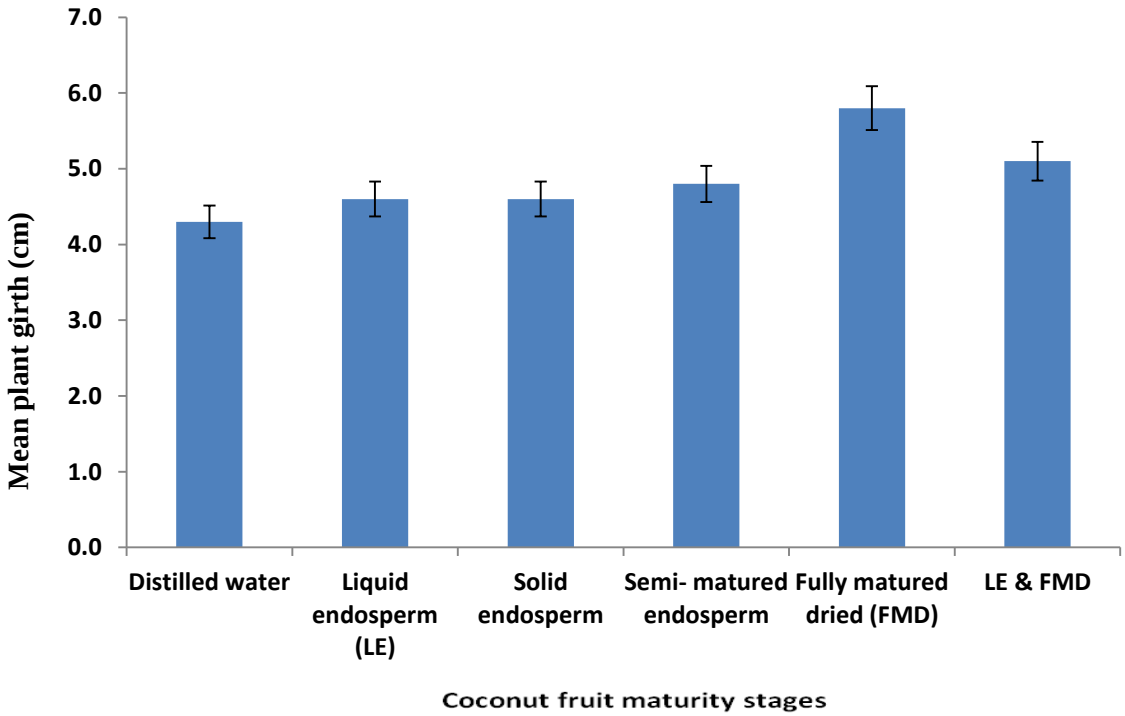


Figure 36. Mean plant girth of plantlets produced three weeks after injection with coconut water from fruits at different maturity stages and one month after the application of the split corm technique. Bars show standard deviation with n= 6.

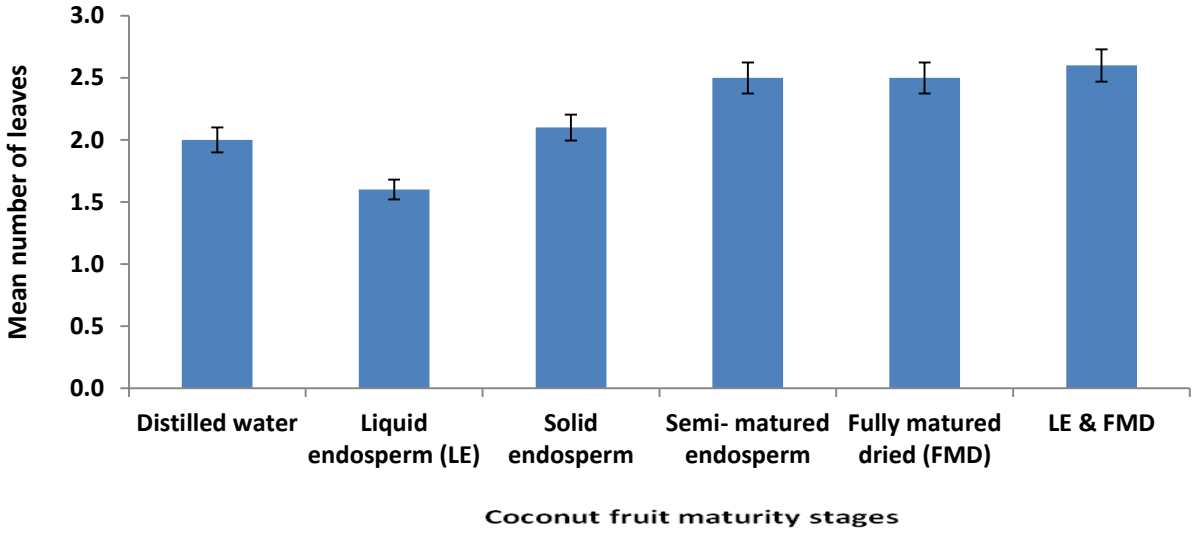


Figure 37. Mean number of leaves of plantlets produced three weeks after injection with coconut water from fruits at different maturity stages and one month after the application of the split corm technique. Bars show standard deviation with n= 6.

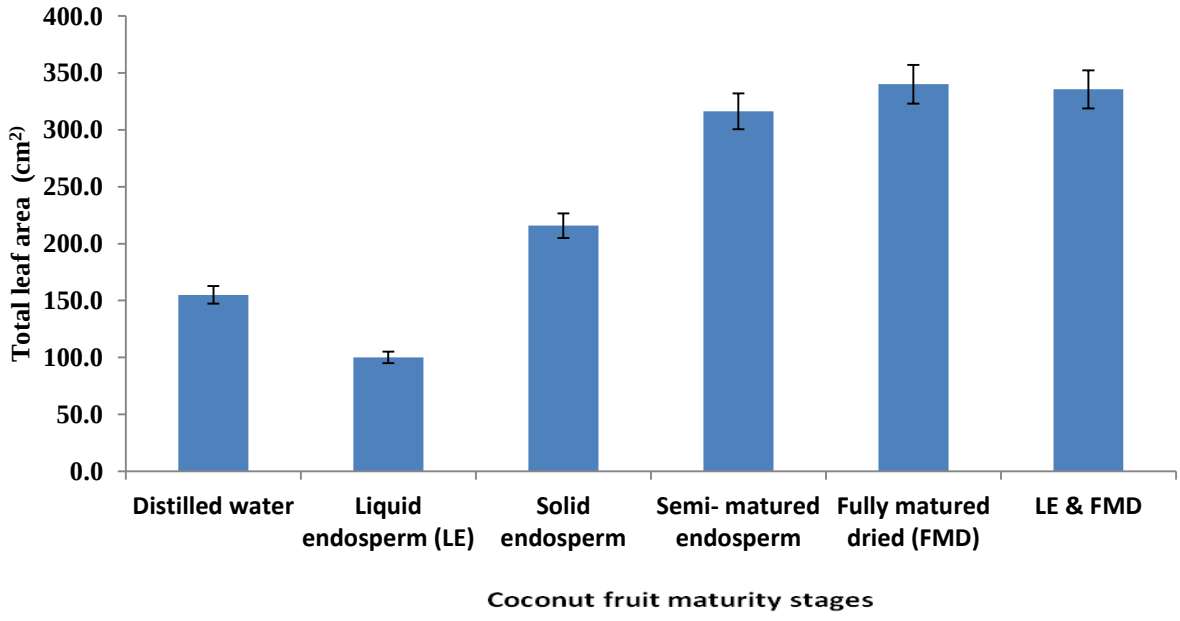


Figure 38. Total leaf area of plantlets derived from 3- month old split corm derived Asamienu suckers three weeks after injection with coconut water from fruits at different maturity stages and one month after the application of the split corm technique

CHAPTER 5

DISCUSSION

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. Similar results have been achieved *in vitro* by several reseachers (Sangwan and Harada, 1975; Cronauer and Krikorian, 1984; Jain *et al*., 1988; Daniells, 1997; Hirimburegama and Gamage, 1997; Kadota and Niimi, 2003; Haq and Dahot, 2007). Hence the application of *in vitro* micro-propagation principles to develop an efficient rapid field multiplication technique of plantain that will allow for production of many vigorous and uniform growing suckers has been achieved.

Preliminary studies carried out at the University of Ghana, Forest and Horticultural Crops Research Centre, Kade, with three local plantain cultivars namely Apem, Apantu and Oniaba indicated that their response to coconut water alone or in combination with indole-3-acetic acid (IAA) for axillary bud formation and growth were consistent with all the treatments. Thus the response to coconut water alone or in combination with indole-3-acetic acid (IAA) was not cultivar specific and could be general for all the major plantain cultivars. Osei (2006) made a similar observation when he used coconut water from different batches yet the results were consistent with all the major local cultivars.

The observation that there was a decrease in growth in height and significant increase in growth in girth within the 3-week period after the injection with IAA or coconut water in both Experiments 1 and 2 indicates that the activity of the apical meristem of the growing suckers was suppressed by the injected IAA and/ or coconut water, but there was increased lateral cellular activity (Salisbury and Ross, 1985). 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. Osei (2006) also observed a similar response when three months old split corm-derived plantain suckers were treated with benzyl adenine or coconut water from fully matured dried fruits.

In Experiment 1, the results clearly showed that proliferation of fully differentiated axillary buds can be induced in plantain by injecting 3-months old split corm-derived well-developed and actively growing suckers with 2ml or 4ml coconut water from fully matured dried fruits combined with 6ml or 4ml 10^{-2} M IAA respectively three weeks after the injection treatment. Amdadul *et al*., (2012) reported that the highest percentage of shoot regeneration (90%) and maximum number of shoots (10) per explant were observed when meristematic stem cuttings explants were cultured on MS medium containing 4.0 mg/l BAP plus 2.0 mg/l IAA plus13% (v/v) coconut water. 2ml or 4ml coconut water from fully matured dried fruits combined with 6ml or 4ml 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 (2ml or 4ml coconut water from fully matured dried fruits combined with 6ml or 4ml 10^{-2} M IAA respectively).

Treatments with coconut water from fully matured dried fruits alone produced larger and vigorously growing plantlets than the other treatments. This observation can be attributed to the fact that coconut water from fully matured dried fruits contains higher amount of cytokinin especially trans-zeatin riboside (Ge *et al.,* 2007; Ma *et al.,* 2008) which promotes shoot growth. Wickson and Thimann, (1958) reported that direct application of cytokinin to axillary buds promotes axillary buds outgrowth even in intact plants.

In Experiment 2, the analysis of the coconut water showed major changes in the level of endogenous content of cytokinin (trans- zeatin riboside) and auxin (IAA) at different fruit maturity stages. The strong negative correlation between trans- zeatin riboside and IAA contents indicated that as the coconut fruit matures the IAA content in the coconut water decreases and *vice versa* for the trans- zeatin riboside. This finding agrees with studies by a number of researchers (Ge *et al.,* 2007; Ma *et al.*, 2008**,** Wu and Hu, 2009**)**. These observations resulted in varied effects of coconut water at different fruit maturity stages on the proliferation and development of axillary buds from the treated plantain suckers.

Coconut water at the liquid endosperm formation stage significantly promoted root production and also induced proliferation of fully differentiated axillary buds over the control treatment**.** Again, coconut water at the liquid endosperm formation stage numerically produced more roots and fully differentiated axillary buds than the other coconut water treatments. This response can be attributed to higher content of auxin (IAA) in coconut water at the liquid endosperm formation stage (Ge *et al*., 2007; Ma *et*

al., 2008). IAA is known to promote root development (Krikorian *et al*., 1987; Dodds and Roberts, 1988). However, since cytokinin (trans- zeatin riboside) which promotes formation of axillary buds is produced in the roots (Arteca, 1996; Mauseth, 1991, Raven, 1992, 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 cytokinin (trans- zeatin riboside) due to the increased root production enhanced proliferation of axillary buds. This study confirmed the observations made by Letham (1994) and Shimizu-Sato and Mori (2001) that cytokinin derived from roots promotes axillary buds outgrowth after decapitation and auxin derived from a shoot apex regulates cytokinin transport in plants. It can also be stated from this study that the optimum ratio of IAA and trans- zeatin riboside to enhance maximum stimulation of axillary buds proliferation was at the liquid endosperm formation stage.

Coconut water at the liquid endosperm formation stage also showed a carryover effect for proliferation of fully differentiated axillary buds and plantlets after the application of the bud manipulation and the split-corm techniques on the treated corms. Thus the effects of IAA and trans- zeatin riboside contents in coconut water at the liquid endosperm formation stage on proliferation of axillary bud formation continued after 3 weeks of the initial injection treatment.

Coconut water at the fully matured dried stage vigorously enhanced growth of the axillary buds into suckers. Thus suckers produced from treatments with coconut water at the fully matured dried stage were significantly bigger than those obtained from the other treatments. This response can be attributed to the higher content of cytokinin (trans-zeatin riboside) at the fully matured dried stage (Ge *et al*., 2007; Ma *et al*., 2008)**.** The

results obtained show that as the exogenous content of trans- zeatin riboside increases and *vice versa* for IAA in coconut water at the fully matured dried stage, the outgrowth of axillary buds into shoots or suckers is improved. Thus, higher content of trans- zeatin riboside plays a major role in the development of axillary buds into shoots. Shimizu-Sato and Mori (2001) reported that direct application of cytokinin to intact plants promotes outgrowth of axillary buds.

On the other hand, 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 trans- zeatin riboside to enhance maximum number of axillary buds to growth into plantlets or shoots is at the semi-matured endosperm stage.

The number of fully developed plantlets produced per plant declined at the fully matured dried fruits stage as a result of increased endogenous content of basipetally flow IAA due to increased leaf production. Shimizu-Sato *et al*., (2009) suggested that apically derived-auxin transported basipetally inhibits outgrowth of axillary buds.

CHAPTER 6

CONCLUSION

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 alone or in combination with IAA.

Treatments with coconut water from fruits at liquid endosperm formation stage containing higher levels of IAA and lower level trans-zeatin riboside produced higher numbers of fully differentiated axillary buds.

Formation of axillary buds was 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 (as in coconut water from fully matured dried fruits) enhanced growth of axillary buds into suckers.

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

It can be concluded from the studies that:

- 2ml or 4ml coconut water from fully matured dried fruit combined with 6ml or 4ml 10^{-2} M IAA respectively induced proliferation of axillary bud and shoot formation of plantain significantly over the control treatment.

- Coconut water from fruits at the liquid endosperm formation stage and semi-matured endosperm formation stage significantly induced proliferation of axillary bud and shoot formation of plantain respectively three weeks after the injection treatments.
- Coconut water from fruits at the fully matured dried stage significantly enhanced axillary bud growth and development into suckers.
- Coconut water from fruits at the liquid endosperm formation stage induced proliferation of axillary bud development from the corms of the treated suckers one month after the application of the bud manipulation and the split corm techniques.

RECOMMENDATIONS

Further investigations need to be carried out to determine whether there will be any carry over effect of coconut water alone or in combination with IAA on the growth, yield and quality of fruits of the derived plantlets (suckers) under field conditions.

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APPENDICES

APPENDIX 1. Analysis of variance for correlation matrix for the relationship between trans-zeatin riboside (t-ZR) and indole -3-acetic acid (IAA) in coconut water from fruits at different maturity stages.

Variable	N	Mean	Std Dev	Sum	Minimum	Maximum	Label
t-ZR	4	0.64000	0.25807	2.56000	0.44000	1.01000	TZR
IAA	4	1.13625	0.31510	4.54500	0.75500	1.51000	IAA

Pearson Correlation Coefficients, N = 4
Prob > |r| under H0: Rho=0

	TZR	IAA
t-ZR	1.00000	-0.92988
t-ZR		0.0701
IAA	-0.92988	1.00000
IAA	0.0701	

APPENDIX 2. Analysis of variance of percentage increase in plant girth of 3-month old split corm-derived Asamienu suckers three weeks after injection with varying ratios of coconut water (CW) and indole-3-acetic acid (IAA).

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replicates stratum	2	230.81	115.41	1.79	
Replicates.*Units* stratum					
TREATMENT	13	3722.04	286.31	4.44	<.001
Residual	26	1677.63	64.52		
Total	41	5630.48			

Rep.	3
D.f.	26
S.e.d.	6.56
L.s.d.	13.48

APPENDIX 3. Analysis of variance of percentage increase in plant height of 3-month old split corm-derived Asamienu suckers three weeks after the injection with varying ratios of Coconut water (CW) and indole-3-3acetic acid (IAA).

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Repl stratum	2	13.72	6.86	0.52	
Repl.*Units* stratum Trmnt	13	2094.37	161.11	12.32	<.001
Residual	26	339.98	13.08		
Total	41	2448.07			

Rep.	3
D.f.	26
S.e.d.	2.741
L.s.d.	5.635

APPENDIX 4. Analysis of variance of mean root number of 3-month old split corm derived Asamienu suckers three weeks after treatment with varying ratios of coconut water (CW) and indole-3-acetic acid (IAA).

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	4.8057	2.4029	6.03	
Rep.*Units* stratum trnt	13	138.5850	10.6604	26.77	<.001
Residual	26	10.3543	0.3982		
Total	41	153.7450			

Rep.	3
D.f.	26
S.e.d.	0.5153
L.s.d.	1.0591

APPENDIX 5. Analysis of variance of mean root diameter of 3- month old split corm derived Asamienu suckers three weeks after treatment with varying ratios of coconut water (CW) and indole-3-acetic acid (IAA).

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	9.1429	4.5714	30.19	
Rep.*Units* stratum trnt	13	5.5800	0.4292	2.83	0.011
Residual	26	3.9371	0.1514		
Total	41	18.6600			

Rep.	3
D.f.	26
S.e.d.	0.3177
L.s.d.	0.6531

APPENDIX 6. Analysis of variance of mean root dry weight of 3-month old split corm derived Asamienu suckers three weeks after treatment with varying ratios of coconut water (CW) and indole-3-acetic acid (IAA).

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.3333	0.1667	0.27	
Rep.*Units* stratum trnt	13	142.1667	10.9359	17.96	<.001
Residual	26	15.8333	0.6090		
Total	41	158.3333			

Rep.	3
D.f.	26
S.e.d.	0.637
L.s.d.	1.310

APPENDIX 7. Analysis of variance of mean number of fully differentiated axillary buds of 3-month old split corm-derived Asamienu suckers three weeks after the injection with varying ratios of coconut water (CW) and indole-3-acetic acid (IAA).

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.3333	0.1667	0.27	
Rep.*Units* stratum trnt	13	138.1250	10.6250	17.27	<.001
Residual	26	16.0000	0.6154		
Total	41	154.4583			

Rep.	3
D.f.	26
S.e.d.	0.641
L.s.d.	1.317

APPENDIX 8. Analysis of variance of mean number of fully developed plantlets of 3-month old split corm-derived Asamienu suckers three weeks after the injection with varying ratios of coconut water (CW) and indole-3-acetic acid (IAA) concentrations.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.4405	0.2202	1.35	
Rep.*Units* stratum trnt	13	40.4524	3.1117	19.14	<.001
Residual	26	4.2262	0.1625		
Total	41	45.1190			

Rep.	3
D.f.	26
S.e.d.	0.3292
L.s.d.	0.6767

APPENDIX 9. Analysis of variance of mean number of fully differentiated axillary buds development from corms of 3-month old split corm-derived Asamienu suckers one month after the application of the bud manipulation technique.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Repl stratum	2	5.726	2.863	1.90	
Repl.*Units* stratum Trmnt	13	852.000	65.538	43.57	<.001
Residual	26	39.107	1.504		
Total	41	896.833			

Rep.	3
D.f.	26
S.e.d.	1.001
L.s.d.	2.058

APPENDIX 10. Analysis of variance of mean number of fully developed plantlets from corms of 3-month old split corm-derived Asamienu suckers one month after the application of the bud manipulation technique.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Repl stratum	2	0.2500	0.1250	0.71	
Repl.*Units* stratum Trmnt	13	9.8095	0.7546	4.28	<.001
Residual	26	4.5833	0.1763		
Total	41	14.6429			

Rep.	3
D.f.	26
S.e.d.	0.3428
L.s.d.	0.7047

APPENDIX 11. Analysis of variance of 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.

Variate: Total_RP

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Repl stratum	2	0.7262	0.3631	1.08	
Repl.*Units* stratum Trmnt	13	103.1012	7.9309	23.50	<.001
Residual	26	8.7738	0.3375		
Total	41	112.6012			

Rep.	3
D.f.	26
S.e.d.	0.4743
L.s.d.	0.9750

APPENDIX 12. Analysis of variance of mean plant height of plantlets derived from corms treated with varying ratios of coconut water (CW) and indole-3-acetic acid (IAA), one month after the application of the bud manipulation technique.

Variate: Height

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replicates stratum	2	60.64	30.32	2.69	
Replicates.*Units* stratum TREATMENT	13	2095.07	161.16	14.30	<.001
Residual	26	293.05	11.27		
Total	41	2448.76			

Rep.	3
D.f.	26
S.e.d.	2.741
L.s.d.	5.635

APPENDIX 13. Analysis of variance of mean plant girth of plantlets derived from corms treated with varying ratios of coconut water (CW) and indole-3-acetic acid (IAA), one month after the application of the bud manipulation technique.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Repl stratum	2	8.143	4.071	3.97	
Repl.*Units* stratum Trmnt	13	53.077	4.083	3.98	0.001
Residual	26	26.690	1.027		
Total	41	87.911			

Rep.	3
D.f.	26
S.e.d.	0.827
L.s.d.	1.700

APPENDIX 14. Analysis of variance of mean number of leaves of plantlets derived from corms treated with varying ratios of coconut water (CW) and indole-3-acetic acid (IAA), one month after the application of the bud manipulation technique

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Repl stratum	2	0.1548	0.0774	0.31	
Repl.*Units* stratum Trmnt	13	20.7381	1.5952	6.37	<.001
Residual	26	6.5119	0.2505		
Total	41	27.4048			

Rep.	3
D.f.	26
S.e.d.	0.4086
L.s.d.	0.8399

APPENDIX 15. Analysis of variance of mean leaf area of plantlets derived from corms of suckers treated with varying ratios of coconut water (CW) and indole-3-acetic acid (IAA), one month after the application of the bud manipulation technique.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Repl stratum	2	57332.	28666.	6.65	
Repl.*Units* stratum					
Trmnt	13	100660.	7743.	1.80	0.099
Residual	26	112151.	4313.		
Total	41	270143.			

Rep.	3
D.f.	26
S.e.d.	53.6
L.s.d.	110.2

APPENDIX 16. Analysis of variance of percentage increase in plant height of 3-month-old split corm derived Asamienu suckers three weeks after injection with coconut water from fruits at different maturity stages.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	9.75	4.87	0.37	
Rep.*Units* stratum					
TREATMENT	5	2866.17	573.23	44.04	<.001
Residual	10	130.16	13.02		
Total	17	3006.07			

Rep.	3
D.f.	10
S.e.d.	2.946
L.s.d.	6.563

APPENDIX 17. Analysis of variance of percentage increase in plant girth of 3-month-old split corm derived Asamienu suckers three weeks after injection with coconut water from fruits at different maturity stages.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.41	0.20	0.00	
Rep.*Units* stratum TREATMENT	5	903.32	180.66	2.87	0.073
Residual	10	629.32	62.93		
Total	17	1533.04			

Rep.	3
D.f.	10
S.e.d.	6.48
L.s.d.	14.43

APPENDIX 18. Analysis of variance of mean root number of 3-month-old split corm derived Asamienu suckers three weeks after injection with coconut water from fruits at different maturity stages.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	198.78	99.39	4.18	
Rep.*Units* stratum TREATMENT	5	650.28	130.06	5.47	0.011
Residual	10	237.89	23.79		
Total	17	1086.94			

Rep.	3
D.f.	10
S.e.d.	3.98
L.s.d.	8.87

APPENDIX 19. Analysis of variance of mean root diameter of 3-month-old split corm derived Asamienu suckers three weeks after injection with coconut water from fruits at different maturity stages.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	1.4444	0.7222	6.84	
Rep.*Units* stratum TREATMENT	5	2.2361	0.4472	4.24	0.025
Residual	10	1.0556	0.1056		
Total	17	4.7361			

Rep.	3
D.f.	10
S.e.d.	0.2653
L.s.d.	0.5911

APPENDIX 20. Analysis of variance of root dry weight (20cm from corm) of 3-month-old split corm derived Asamienu suckers three weeks after injection with coconut water from fruits at different maturity stages.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Repl stratum	2	1.1548	0.5774	2.25	
Repl.*Units* stratum trmnt	13	101.2857	7.7912	30.33	<.001
Residual	26	6.6786	0.2569		
Total	41	109.1190			

Rep.	3
D.f.	26
S.e.d.	0.4138
L.s.d.	0.8506

APPENDIX 21. Analysis of variance of mean number of fully differentiated axillary buds of 3-month old split corm-derived Asamienu suckers three weeks after the injection with coconut water from fruits at different maturity stages.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	13.861	6.931	2.24	
Rep.*Units* stratum					
TREATMENT	5	47.944	9.589	3.10	0.060
Residual	10	30.972	3.097		
Total	17	92.778			

Rep.	3
D.f.	10
S.e.d.	1.437
L.s.d.	3.202

APPENDIX 22. Analysis of variance of mean number of fully developed plantlets of 3-month old split corm derived Asamienu suckers three weeks after injection with coconut water from fruits at different maturity stages.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.583	0.292	0.20	
Rep.*Units* stratum					
TREATMENT	5	46.125	9.225	6.40	0.006
Residual	10	14.417	1.442		
Total	17	61.125			

Rep.	3
D.f.	10
S.e.d.	0.980
L.s.d.	2.184

APPENDIX 23. Analysis of variance of mean plant height of plantlets derived from 3-month old split corm derived Asamienu suckers three weeks after injection with coconut water from fruits at different maturity stages.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	34.75	17.38	1.42	
Rep.*Units* stratum TREATMENT	5	1156.79	231.36	18.87	<.001
Residual	10	122.58	12.26		
Total	17	1314.12			

Rep.	3
D.f.	10
L.s.d.	6.370

APPENDIX 24. Analysis of variance of mean plant girth of plantlets derived from 3-month old split corm derived Asamienu suckers three weeks after injection with coconut water from fruits at different maturity stages.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	1.0000	0.5000	0.81	
Rep.*Units* stratum TREATMENT	5	43.3333	8.6667	14.05	<.001
Residual	10	6.1667	0.6167		
Total	17	50.5000			

Rep.	3
D.f.	10
L.s.d.	1.429

APPENDIX 25. Analysis of variance of mean number of leaves of plantlets derived from 3-month old split corm derived Asamienu suckers three weeks after injection with coconut water from fruits at different maturity stages.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	1.0833	0.5417	4.33	
Rep.*Units* stratum TREATMENT	5	28.1667	5.6333	45.07	<.001
Residual	10	1.2500	0.1250		
Total	17	30.5000			

Rep.	3
D.f.	10
S.e.d.	0.2887
L.s.d.	0.6432

APPENDIX 26. Analysis of variance of mean leaf area of plantlets derived from 3-month old split corm-derived plantlets three weeks after injection with coconut water from fruits of different maturity stages.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	7525.0	3762.5	6.23	
Rep.*Units* stratum TREATMENT	5	21583.3	4316.7	7.14	0.004
Residual	10	6041.7	604.2		
Total	17	35150.0			

Rep.	3
D.f.	10
S.e.d.	20.07
L.s.d.	44.72

APPENDIX 27. Analysis of variance of mean number of fully differentiated axillary buds from 3- month old split corm derived Asamienu suckers three weeks after injection with coconut water from fruits at different maturity stages and one month after the application of the bud manipulation technique.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.2133	0.1067	0.13	
Rep.*Units* stratum					
trnt	5	383.8600	76.7720	90.68	<.001
Residual	10	8.4667	0.8467		
Total	17	392.5400			

Rep.	3
D.f.	10
S.e.d.	0.751
L.s.d.	1.674

APPENDIX 28. Analysis of variance of mean number of fully developed plantlets of 3-month old split corm derived Asamienu suckers three weeks after injection with coconut water from fruits at different maturity stages and one month after the application of the bud manipulation technique.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.0033	0.0017	0.00	
Rep.*Units* stratum					
trnt	5	39.5050	7.9010	21.61	<.001
Residual	10	3.6567	0.3657		
Total	17	43.1650			

Rep.	3
D.f.	10
S.e.d.	0.494
L.s.d.	1.100

APPENDIX 29. Analysis of variance of mean plant height of plantlets derived from 3-month old split corm derived Asamienu suckers three weeks after injection with coconut water from fruits at different maturity stages and one month after the application of the bud manipulation technique.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.333	0.167	0.04	
Rep.*Units* stratum					
trnt	5	863.320	172.664	41.44	<.001
Residual	10	41.667	4.167		
Total	17	905.320			

Rep.	3
D.f.	10
S.e.d.	1.667
L.s.d.	3.714

APPENDIX 30. Analysis of variance of mean plant girth of plantlets derived from 3-month old split corm derived Asamienu suckers three weeks after injection with coconut water from fruits at different maturity stages and one month after the application of the bud manipulation technique.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	2.2533	1.1267	2.06	
Rep.*Units* stratum					
trnt	5	46.8600	9.3720	17.14	<.001
Residual	10	5.4667	0.5467		
Total	17	54.5800			

Rep.	3
D.f.	10
S.e.d.	0.604
L.s.d.	1.345

APPENDIX 31. Analysis of variance of mean number of leaves of plantlets derived from 3- month old split corm derived Asamienu suckers three weeks after injection with coconut water from fruits at different maturity stages and one month after the application of the bud manipulation technique.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.01333	0.00667	0.29	
Rep.*Units* stratum					
trnt	5	7.94500	1.58900	70.10	<.001
Residual	10	0.22667	0.02267		
Total	17	8.18500			

Rep.	3
D.f.	10
S.e.d.	0.1229
L.s.d.	0.2739

APPENDIX 32. Analysis of variance of total leaf area of plantlets derived from 3- month old split corm derived Asamienu suckers three weeks after injection with coconut water from fruits at different maturity stages and one month after the application of the bud manipulation technique.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	1677.	838.	0.07	
Rep.*Units* stratum					
trnt	5	2239466.	447893.	36.01	<.001
Residual	10	124376.	12438.		
Total	17	2365519.			

Rep.	3
D.f.	10
S.e.d.	91.1
L.s.d.	202.9

APPENDIX 33. Analysis of variance of mean number of fully defferentiated axillary buds from 3- month old split corm derived Asamienu suckers three weeks after injection with coconut water from fruits at different maturity stages and one month after application of the split corm technique.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	18.2533	9.1267	33.23	
Rep.*Units* stratum					
trnt	5	1168.6000	233.7200	850.92	<.001
Residual	10	2.7467	0.2747		
Total	17	1189.6000			

Rep.	3
D.f.	10
S.e.d.	0.428
L.s.d.	0.953

APPENDIX 34. Analysis of variance of mean number of fully developed plantlets from 3- month old split corm derived Asamienu suckers three weeks after injection with coconut water from fruits at different maturity stages and one month after application of the split corm technique.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	1.2033	0.6017	1.07	
Rep.*Units* stratum					
trnt	5	8.5000	1.7000	3.03	0.064
Residual	10	5.6167	0.5617		
Total	17	15.3200			

Rep.	3
D.f.	10
S.e.d.	0.612
L.s.d.	1.363

APPENDIX 35. Analysis of variance of mean plant height of plantlets derived from 3-month old split corm derived Asamienu suckers three weeks after injection with coconut water from fruits at different maturity stages and one month after the application of the split corm technique.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.3333	0.1667	0.39	
Rep.*Units* stratum					
trnt	5	156.8200	31.3640	73.51	<.001
Residual	10	4.2667	0.4267		
Total	17	161.4200			

Rep.	3
R.f.	10
S.e.d.	0.533
L.s.d.	1.188

APPENDIX 36. Analysis of variance of mean plant girth of plantlets derived from 3-month old split corm derived Asamienu suckers three weeks after injection with coconut water from fruits at different maturity stages and one month after the application of the split corm technique

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.9633	0.4817	1.71	
Rep.*Units* stratum					
trnt	5	4.1800	0.8360	2.97	0.067
Residual	10	2.8167	0.2817		
Total	17	7.9600			

Rep.	3
D.f.	10
S.e.d.	0.433
L.s.d.	0.966

APPENDIX 37. Analysis of variance of mean number of leaves of plantlets derived from 3- month old split corm derived Asamienu suckers three weeks after injection with coconut water from fruits at different maturity stages and one month after the application of the split corm technique.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	2	0.12000	0.06000	2.14	
Rep.*Units* stratum					
trnt	5	2.24500	0.44900	16.04	<.001
Residual	10	0.28000	0.02800		
Total	17	2.64500			

Rep.	3
D.f.	10
S.e.d.	0.1366
L.s.d.	0.3044

APPENDIX 38. Analysis of variance of total leaf area of plantlets derived from 3- month old split corm derived Asamienu suckers three weeks after injection with coconut water from fruits at different maturity stages and one month after the application of the split corm technique.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	8797.	4398.	2.47	
Rep.*Units* stratum					
trnt	5	157019.	31404.	17.67	<.001
Residual	10	17776.	1778.		
Total	17	183592.			

Rep.	3
D.f.	10
S.e.d.	34.42
L.s.d.	76.70