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Supervisor: **Prof. Dr. Jens Norbert Wünsche**

Innovative propagation techniques in banana and plantain

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Examination committee:
Head of committee: Prof. Dr. Uwe Ludewig
1st examiner and reviewer: Prof. Dr. Jens Norbert Wünsche
2nd examiner and reviewer. Prof. Dr. Dominikus Kittemann
3rd examiner and reviewer: Prof. Dr. Georg Cadisch

Dedications

This doctoral thesis is dedicated to my parents, Mr. S.K. Opata and Mrs. Augusta Opata; and to my siblings for their encouragement and support during my studies.

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List of abbreviations[1]

ACP	African, Caribbean and Pacific
Ad	dominant alle
ANOVA	analysis of variance
BA	benzyl adenine
BAP	6-benzylaminopurine
BMBF	German Federal Ministry for Education and Research
CARBAP	African Research Centre into Banana and Plantain
CKs	cytokinins
CRI	Crops Research Institute
CSIR	Council for Scientific and Industrial Research
cv	cultivar
DEAE	Diethylaminoethyl
EPA	European Partnership Agreement
F_1	first filial generation
F_2	second filial generation
FAO	Food and Agriculture Organization
FSC	Food Security Centre
GA_3	gibberellic acid 3

[1] Standard abbreviation of units following the International System of Units (SI) are not listed.

IAA	indole-3- acetic acid
IBA	Indole-3-butyric acid
IITA	International Institute of Tropical Agriculture
iP	isopentenyl adenosine
ITC	Bioversity International Musa Germplasm Transit Centre
K	potassium
LSD	least significant difference (Fisher's protected)
MOFA-SRID	Ministry of Food and Agriculture / Statistics Research Information Directorate
N	nitrogen
NaOH	sodium hydroxide
PGRs	plant growth regulators
PIF	*Plants Issus de Fragments de tige*
PVPP	polyvinylpolypyrrolidone
RIA	Radio-Immuno-Assay
SI	International System of Units

1. General Introduction

1.1 Banana and plantain, classification and origin

Banana and plantain belong to the genus *Musa* and in the family Musaceae. Almost all cultivated *Musa* spp. have a chromosome number of 3n = 33, are sterile and develop fruits by parthenocarpy[2] (Vuylsteke et al., 1993). Most of the edible fruits have solely be maintained through persistent human activities (Ortiz, 2013). The cultivated varieties of bananas and plantains genomes are derived from the diploid species *Musa acuminata* (A genome) and *Musa balbisiana* (B genome), (Ortiz, 2013; Vuylsteke et al., 1993). The two ancestral diploid species through hybridization have resulted in over thousand cultivars contributing to a wide genetic diversity (Heslop-Harrison and Schwarzacher, 2007). *M. acuminata* and *M. balbisiana* coming from diverse environments have led to different agronomic traits towards the current genetic composition of the numerous *Musa* clones (Pillay et al., 2000). The East Africa Highland and the cultivated dessert bananas are classified as AAA whereas plantains are AAB and the cooking bananas are ABB (Pillay et al., 2000). However various important combinations such as AB, AAAB, AABB and ABBB also occur through natural means or occur as a result of hybridization programs. *M. balbisiana* genes are said to induce stronger disease resistant, higher nutritional value with increased starch content and further provide hybrids which are suitable for cooking when compared to the pure *M. acuminata* cultivars mainly suitable for dessert use (Robinson and Saúco, 2010). Both banana and plantain are giant monocotyledonous plants and can grow to

[2] Parthenocarpy is the development of the ovary of a flower into fruit without fertilization. Fruits thus formed are typically but not necessarily seedless.

a height of up to 3 m (Heslop-Harrison and Schwarzacher, 2007). Malaysia or Indonesia is being said as the centre of diversity of these crops (Daniells et al., 2001). However, these crops are grown in over 120 countries in the tropical and subtropical parts of the continents (Youssef et al., 2011). The crops are said to have been taken from Indonesia to Madagascar and later into East Africa, Zaire and then to West Africa (Robinson and Saúco, 2010). Over 116 cultivars of plantains with various morphological traits have been identified in West and Central Africa. However, prominent among the classification of plantain is based on bunch morphology and the number of leaves (Swennen, 1990). French plantains consist of many hands with several small fingers with a persistent large male bud at maturity (Fig. 1.1 a). The False horn plantain has no male bud at maturity but consists of large fingers followed with few neutral flowers. Horn plantain has few hands and large fingers but without neutral or male flowers (Swennen, 1990).

Figure 1.1. Plantains with different bunch morphologies: (A) French plantain with many hands and large persistent male bud; (B) True Horn plantain with few hands without any male bud; and (C) False horn plantain with few neutral flowers.

1.2 Morphology of banana and plantain

1.2.1 Pseudostem

Banana and plantain are giant herbaceous monocotyledon plants. The leaf petioles are well arranged spirally closely in the aerial shoots with long overlapping bases forming a formidable pseudostem (Ennos et al., 2000). The pseudostem can grow to height of 2 - 8 m based on the variety and conditions necessary for growth and development (Charles Kasoz and Zawedde, 2007). The stem lacks any woody tissue; however, they are able to support the huge widely opened leaves. The banana and plantain are endowed with the ability to store large amount of water, hence able to inure to extended period of drought conditions, however its growth rate slows down during this period (Nelson et al., 2006).

1.2.2 *Corm*

The corm which is the true stem of the banana and plantain plant is subterranean and produces aerial shoots which emanate from the lateral buds, develop into suckers and stay closely to the mother corm (Charles Kasoz and Zawedde, 2007; Karamura and Karamura, 1995; Swennen and Ortiz, 1997). The corm exhibits tremendous vegetative growth and at some point culminates into inflorescence under favourable conditions. Plantain in particular exhibit “high mat” which is a condition of the plant base to elevate above the soil as a result of ratooning which characterizes successive growth cycles (Vuylsteke et al., 1993), on the contrary bananas have their corms growing deep in the soil making them very stable. The corm is distinguished into two main regions, internally, consisting of a central cylinder made of starchy parenchyma surrounded by

a cortex. The outer part of the central cylinder consists of a network of huge horizontally oriented vascular bundles. Additionally, a cambium like meristematic tissue serves as region where roots emanate. In the corm apical parts lies the meristematic tissue where the vascular system develops (Price, 1995). On the corm of banana and plantain, which varies in sizes (Price, 1995), holds many latent buds. The corm is also the main point where adventitious primary axis of the root arises from (Draye et al., 1999).

1.2.3 *Roots*

Several roots emanate from the corm and most of these roots grow horizontally (Swennen, 1990). Root growth and development are all dependent on the soil temperature (Robinson and Saúco, 2010). Robinson and Alberts (1989) observed poor root growth and water extraction potential in a subtropical winter due to low soil temperatures. In general, the roots are long, which are rope like and could grow to a length of 4-5m with root diameter of 5-10mm, (Riopel and Steeves, 1964), however, their number is mostly dependent on the health conditions of the plant (Blomme et al., 2000). The distribution of the roots are mostly found in the top 40 cm layer of the soil with the soil type affecting the distribution as well (Irizarry et al., 1981). It is very imperative to mention that during the selection of field for banana and plantain cultivation, critical attention must be given to the soil type. The development of primary roots is persistent until flowering occurs (Draye et al., 1999). The roots of banana and plantain play significant role such as effective nutrient and water uptake and further provides anchorage for the plant (Blomme et al., 2000). In general roots are the main organs in plants responsible for cytokinin production (Shimizu-Sato et al., 2009) and

very critical as cytokinin plays a key role in banana and plantain towards lateral shoot development.

1.3 Cultivation and economic importance of banana and plantain

Plantains *Musa* AAB mostly referred to as cooking bananas are generally starchy in nature and can be eaten either ripe or unripe through boiling, frying or baking; whereas bananas are eaten raw (Kerbel, 2016; Singh et al., 2011; Swennen, 1990). They are crops which mature quickly and therefore can be harvested throughout the year (Arias et al., 2003).

Bananas and plantains rank as the fourth most important staples in developing countries (Gitonga et al., 2011; Heslop-Harrison and Schwarzacher, 2007) with an estimation of one-third of global production occurring in Sub-Saharan Africa. They provide more than 25% of the food energy requirements for millions of people living in the humid tropics of Africa (Eshetu and Tola, 2014; Tripathi et al., 2009).

They also serve as good sources of proteins, vitamins and minerals (Iqbal and Muhammad, 2013; Swennen, 1990; Vuylsteke et al., 1993; Wall, 2006). An estimation of 14.37% of the total world's production comes from India (Krishna and Chandrasekaran, 1996). The world average production in tons from 1998-2000 was estimated to be around 99 million in 2001 (Arias et al., 2003). Report indicates that almost 85% of the approximately 145 million tons of world annual harvest of banana and plantain come from plots and backyard gardens which are mainly situated in the developing world (Arias et al., 2003; Ortiz and Swennen, 2014).

Bananas and plantains have yielded greater significance both as cash and subsistence crops in various locations far from their primary centres of origin. This is as a result of high export mainly with dessert bananas specifically the Cavendish types coming from Central America and the Caribbean (Robinson and Saúco, 2010). However, in terms of export from Africa, Ivory Coast and Cameroon are very active in terms of quantities, nonetheless, their export volumes are not as big as those from Latin America, typically due to cultivation mainly on smaller plots (Robinson and Saúco, 2010). Plantains which are also very important crops, produced widely in tropical countries are almost entirely traded domestically, having about 1.6% of production exported mainly to the USA (Dzomeku et al., 2011; Robinson and Saúco, 2010). There has been report suggesting that 51% of plantain production globally in 2006 were from Africa countries such as Uganda, Ghana, Nigeria and Rwanda (Robinson and Saúco, 2010). In Nigeria, plantain occupies an important position in terms of food production. The last twenty years has seen a double increase in production, nonetheless, the production remains entirely in the hands of smallholder farmers (IITA, 2014). The significant contribution of plantains to rural household income in the main production areas in Nigeria has come as a result of research and extension activities undertaken by the International Institute of Tropical Agriculture (IITA, 2014). The estimated annual production of banana in Eastern and Southern Africa is about 20 million tons (Karamura et al., 1998). These crops form an integral part of the socio-economic settings of the people in regions where they are grown and gradually gaining importance in terms of income generation for resource poor farmers, aside serving as key staple food (Karamura et al., 1998; Nelson et al., 2006). Most people in the urban communities are notably known for the manufacture of banana fibre; and processing them into mats, baskets, ropes etc., which constitute a great economic activity. In Burundi, banana and plantain

are one of the major staple crops which are intercropped by smallholder farmers. They are however rated as the most important major cash crop in the country (Lepoint et al., 2013). Bananas are also very important food crops in Rwanda with an annual production of about two million tons covering a cultivation area of over 180, 000 hectares. It is estimated that per capita consumption of banana in Rwanda is around 197 kg counting it among the highest in the Great Lakes region (Nsabimana and Van Staden, 2007). Uganda in particular has the highest annual per capita consumption of about 243 kg in 1996, with Gabon and Cameroon recording between 100 and 200 kg respectively (Arias et al., 2003). In some East African countries such as Burundi and Rwanda, beer production from banana is one of the major economic activities. Banana beer takes about 64% of total annual beer production. There is also an increase in banana beer production in Uganda and Northern Tanzania (Karamura et al., 1998). The potential of these crops have enhanced food security in the production areas and has to a large extent, cut down on rural poverty (Adejoro et al., 2010; Lorenzen et al., 2010).

The crops are very important in most farming communities serving as shade plants for other important tree species such as coffee and cocoa (Albertin and Nair, 2004; Dzomeku et al., 2011). Higher economic benefit has been recorded for coffee-banana intercropping when compared to banana or coffee mono cropping in smallholder farming system in Uganda (van Asten et al., 2011). Apart from the food uses, banana leaves are used in some parts of the world for wrapping food. The pseudostem is also used for fibre and sometimes left on the field after harvesting the crop to serve as organic matter (Kumar et al., 2012). Furthermore, the sap of banana has been used in traditional medicine to treat different types of ailments such as leprosy, fever, digestive disorders and even insect bites (Kumar et al., 2012). In some fishing communities in

West Africa, the fibre obtained from the pseudostem of plantains and bananas are used to fabricate fishing lines (Abiodun-Solanke and Falade, 2011).

Plantain cultivation in Ghana is mainly in the humid forest belt which receives annual rainfall of 1500-3600mm/year. The crop is mainly grown together with cocoa in order to provide shade for the seedling (Schill et al., 2000). Plantain is considered the most important starchy food crop in Ghana after the grains mainly maize (*Zea mays*) and other starchy crops such as cassava (*Manihot esculenta*) and yam (*Dioscorea esculenta*) in terms of area under cultivation (MOFA-SRID, 2013).

There is a high potential for the use of the banana and plantain sap in the dye industry in Ghana. A study carried out by Dzomeku and Boateng (2013) on the exploration of the sap of banana and stem bark of *Bridelia micratha* revealed that there is a huge potential for the use of sap of banana and *Bridelia micratha* in the dye industry specifically the 'Adinkra' industry in Ghana. Plantain cultivation has been identified to play an important socio-economic role in Ghana considering food security and employment. The yield of plantain in Ghana has risen steadily from 8 Mt/ha in 1996 to almost 10 Mt/ha since 2003 (Dzomeku et al., 2011).

Comparing the relevance of plantains to other food crops in the agricultural sector in Ghana, contribution of plantain to the Agricultural Gross Domestic Product is about 13.1% (Dzomeku et al., 2011). Currently in Ghana, the agro-industry has taken a key strategic position, processing large quantities of plantains into flour and also the production of plantain chips from the green fruits which are all meant for both local consumption and export (Dzomeku et al., 2011).

1.4 Requirements for banana and plantain cultivation

Banana and plantain are mainly cultivated in the tropical and subtropical regions, meaning they require hot and humid conditions to thrive very well. These growing areas are geographically located between the equator and the latitudes 20^{o} N and 20^{o} S, however, in the subtropics, they are located between 20^{o} and 30^{o} North or South of the equator (Robinson and Saúco, 2010). The average temperature of the air required by these crops should be around 30^{o}C. A critical balance between growth which is mainly assimilation and development which characterizes leaf development requires an optimum temperature of about 27^{o}C (Robinson and Saúco, 2010). Temperature thresholds are very critical for predicting production potential and also establishing factors which are limiting to banana production in various climatic conditions (Robinson and Saúco, 2010). Additional essential climatic condition which determines where banana and plantain could be grown aside temperature is the rainfall (Robinson and Saúco, 2010). Areas which cannot have an average annual rainfall of 2000-2500 mm well distributed throughout the year require external source of water supply through irrigation. van Asten et al. (2011) reported that the East Africa Highland bananas in particular, require annual rainfall of more than 1300 mm / annum for optimum production and therefore a decrease in 100 mm of rainfall per annum could reduce bunch yield by 9%. However, the relatively high humidity coupled with high rainfall in the tropics contributes to high infection of the black Sigatoka (*Mycospharella fijiensis*) disease which is extremely virulent (Robinson and Saúco, 2010).

Banana and plantain require soils which are deep, well-drained loams, very fertile and high in organic matter content. They also require soils which are devoid of compaction, absence of excessive clay as well as acidity or salinity. In large commercial fields,

irrigation and mineral fertilizers are additionally provided to ensure effective growth and development. Though the bananas and plantains require adequate water, excessive irrigation coupled with poorly drained sites may affect plants which have loosely root and further results in the mat floating (Nelson et al., 2006).

1.5 Prospect of cultivation of bananas and plantains

These crops in recent times have received immense improvement in production due to research activities and findings which have been disseminated through extension activities to these crops growers (Dzomeku et al., 2011). In Asia, the use of disease resistant varieties such as the Cavendish in place of Gros Michel and other important measures adopted such as efficient water management, integrated pest and disease management, harvest and postharvest handling have resulted in large yields of banana (Singh et al., 2011). Similar success stories are also reported elsewhere including Africa. Smallholder farmers in Kenya have resorted to the use clean tissue culture planting material (Singh et al., 2011). Global banana shipment from Africa grew by 2.4 percent in 2012, as export reached 649 000 tons. This actually accounted for about 3.9 percent of the global banana shipment of which Côte d'Ivoire, the largest banana exporter in the region shipped 339 000 tons in 2012 representing 6.0 percent more than it did in 2011, (FAO, 2014).

There is a high prospect for banana production in Africa and also the opportunity provided by the European Union to allow banana to enter the European market at a reduced tariff. Since 2008, African, Caribbean and Pacific (ACP) banana suppliers who initialled what is termed as Economic Partnership Agreement (EPA) have made

significant gains from duty and quota free access to the European Union market. Some African countries on the list include Cameroon, Ivory Coast and Ghana (European Commission, 2010).

1.6 Propagation of banana and plantain

1.6.1 Types of planting material

Several types of planting material in various sizes exist for the establishment of banana and plantain fields. Notably among them are peepers, sword suckers, water suckers, maiden suckers, mother corms (Fig. 1.2; Dzomeku et al., 2014; Staver and Lescot, 2015). Peepers are usually very little suckers which are just emerging from the soil surface. Whereas sword suckers are bigger than the peepers with leaves which are very pointed and not fully developed unlike maiden suckers which are bigger with leaves fully opened (Swennen, 1990). However, in terms of productivity, maiden and sword suckers are the preferred types (Dzomeku et al., 2014; Swennen, 1990). Heslop-Harrison and Schwarzacher (2007) indicated that the major source of planting material are the suckers and often remain true-to-type.

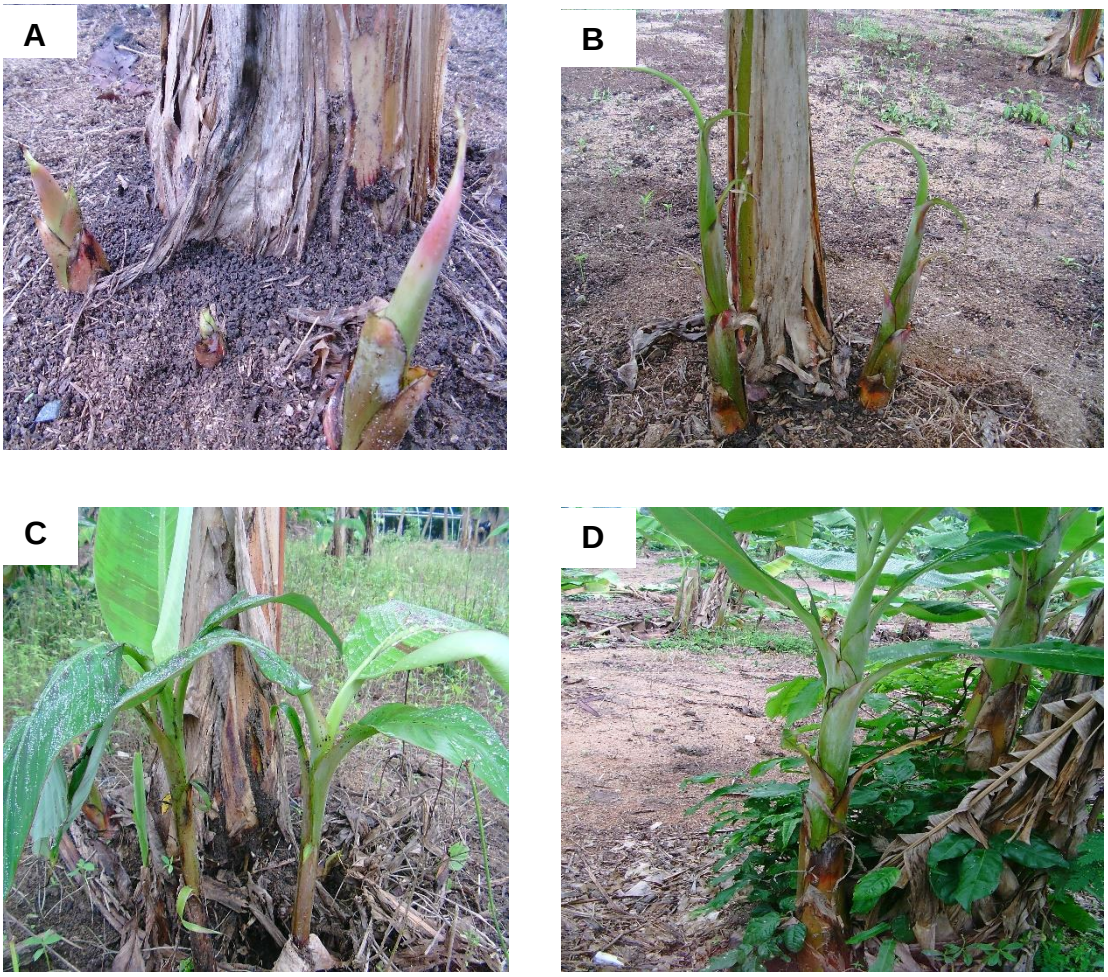

Figure 1.2. Various planting material of plantains: (A) peeper around the mother plant; (B) sword suckers around the mother plant; (C) water suckers; and (D) maiden suckers**.**

Relatively young shoots such as peepers, water suckers and buds which have been developed into new sprouts usually take long from the time of planting in the field to harvest (Staver and Lescot, 2015). The shortest interval from field planting to harvest could be obtained when large maiden corms are used for planting (Staver, and Lescot, 2015). However, it is always challenging getting adequate and uniform planting material when farmers want to rely on these planting material. This is because almost at every stage of growth of the plant, different stages in terms of age of the various planting material could be obtained around the mat, however, very scanty. This is

because the plant in its life cycle could produce 5-10 sucker based on the cultivar and also different internal and external factors (Bhende and Kurien, 2015; Rahman et al., 2004; Vora and Jasrai, 2012). Apical dominance which is hormone mediated is a major factor resulting in slow growth of suckers within the mat (Singh et al., 2011). It is a common phenomenon in many plant species where the intact shoot apex dominates growth and suppresses the outgrowth of axillary buds (Shimizu-Sato et al., 2009). However, farmers need large numbers of these planting material, sometimes in hundreds or even in thousands to establish their plantation. The non-accessibility of clean planting material contributes hugely to the inability to expand plantain fields beyond the backyards (Nkendah and Akyeampong, 2003).

1.6.2 Methods of propagation

In general, propagation of banana can be carried out in two ways, sexual, thus, through the use of seeds and in particular of the wild species which are diploid; and the asexual method by the use of suckers (Singh et al., 2011). Presently, propagating of edible plantains and bananas are carried out by macropropagation and micropropagation. However, there are several low cost methods of propagating bananas and plantains by smallholder farmers in the developing countries particularly Sub- Saharan Africa. These low cost options are mainly through macropropagation techniques. Since the edible cultivars are mainly triploid and seedless, propagation has mainly been by vegetative means (Dzomeku et al., 2014). Propagation can be carried out directly in the field (on-field technique) or the plant is completely taken from the field (detached corm technique) and established in a nursery (off-field technique).

On-field propagation practices involve either the complete or false decapitation of the pseudostem. Complete decapitation involves cutting down the pseudostem completely and destroying the existing meristem on the shoot stump remaining in the soil with any sharp object. This breaks down apical dominance and induces the growth of lateral buds into more or less uniform sizes shoots. Apical dominance in plantain and banana is phenomenon in which the mother plant suppresses the outgrowth of lateral bud into shoots. This is mainly due to hormonal imbalance in the plant. Segregation ratio of F_1 and F_2 plantain-banana hybrids reveals that apical dominance is controlled by a major recessive gene, *ad*, acquired through inheritance (Ortiz and Vuylsteke, 1994). Gibberellic acid (GA_3) has been reported to be the main factor for sucker growth rate and also the dominant allele, *Ad*, reported to have improved suckering of plantain-banana hybrid and was responsible for the regulation of GA_3.

The complete decapitation is relevant for farmers who do not have larger fields and therefore require few planting material to establish their farms (Singh et al., 2011). The method could produce about 9-15 shoots within a period of four weeks. In some complete decapitation methods, the meristem is fully bored out into cavity, with plant hormones mainly cytokinin, poured into the cavity, covered and this further enhances the rate of lateral shoot proliferation. The resulting outcome which is mainly young shoots are carefully excised from the mother plant and subsequently planted directly in the field or further hardened in the nursery and planted out later in the field. The use of plant hormone directly on the plant in the field through injection has been demonstrated by Osei (2006) with some positive responses.

False decapitation on the other hand involves the destruction of the meristem by piercing the pseudostem with a sharp object. This in a way breaks down or suppresses

apical dominance and induces lateral shoot growth. However, with this method, the foliage of the plant remains active for some time until it completely dies off.

The off-field propagation generally involves the harvesting of young suckers or the corm of a mother plant. These are subjected to paring where all the roots are removed, in some cases prior to the removal of the roots, hot water treatment of the corms is carried out which is very important for nematode and banana weevil control (Coyne et al., 2010). These prepared plants are then nursed in any good growth substrate such as saw dust or rice hull (Baiyeri, 2005) embedded in a greenhouse (Fig.1.3) or growth chamber, which has the potential to generate high heat and hold enough moisture to enhance shoot development (Dzomeku et al., 2014). The growth chamber is typically built with local materials and covered with transparent polyethylene sheet. The transparent polyethylene sheet is able to hold high heat and moisture in the chamber, which is a prerequisite for sprouting of buds into shoots (Dzomeku et al., 2014). Plantlets derived from this propagation technique are harvested and acclimatized under shade for a period of about three months before planted out in the field (Fig. 1.3).

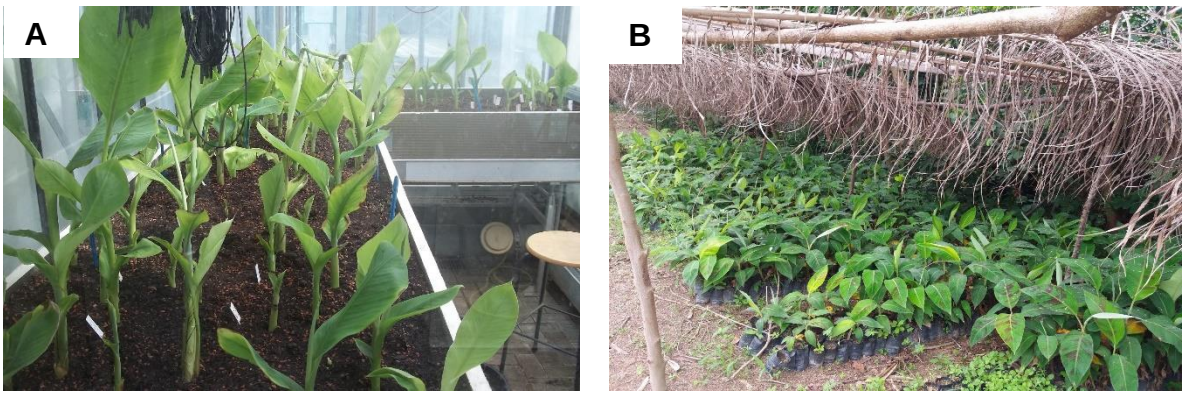

Figure 1.3. Sprouted corms in a greenhouse at the University of Hohenheim (A) and seedlings under shade, constructed with palm fronds during acclimatization (B).

Different techniques are employed with regard to the off-field propagation method. Prominent among them are split corm method, decorticated method and the *Plants Issus de Fragments de tige* (PIF) method or the bud manipulation method. The PIF technique in particular is hitherto exploited extensively by smallholder farmers to raise numerous and uniform banana and plantain seedlings in West and Central Africa (Tomekpe et al., 2011).

The PIF technique is an improved macropropagation technique which was worked on by the African Research Centre into Banana and Plantain (CARBAP) in the early nineties (CARBAP, 2014) and the International Institute of Tropical Agriculture (IITA). The technique has been extensively disseminated in the West Africa sub region and also in Central Africa (Tomekpe et al., 2011). The PIF method involves the use of young suckers, where the roots are first pared. After the paring process, the leaf sheaths are carefully removed with sterilized sharp knife, leading to the exposure of latent buds on the corm (Dzomeku et al., 2014; Kwa, 2003). The apical meristem of the young corm at some point in time is exposed and a crosswise incision is made through it to destroy it. This in so doing leads to apical meristem dominance suppression. The prepared corms termed as ‘explants’ are planted in a humidity chamber commonly filled with sterilized sawdust and sometime rice hull. Large uniform and healthy plantlets could be raised within 3-4 four months of applying the technique, however, the shoot numbers is based on the cultivars of banana and plantain used (CARBAP, 2014). The use of clean planting material as a starting material is very critical for this very technique. Largely, harvested corms are subjected to hot water

treatment to get rid of nematodes, eggs of banana weevils and other microorganisms (Hauser and Coyne, 2010). A healthy corm has the potential to stay longer in the germination bed and therefore harvesting of young shoots could be carried out several times unlike corms which are not healthy and highly prone to rotting.

The technique is relatively cheap, requires simple materials and no sophisticated skills. Due to the low investment cost with this approach, the cost of seedlings generated from this technique is affordable by resource poor farmers. This technique serves as an alternative to tissue culture by smallholder farmers in Africa. The technique has also been reported to provide jobs mainly through nursery operators who have adopted it especially in Côte d'Ivoire through a special program. Furthermore, with its simple application nature, the technique though developed in Africa, it has also been disseminated in the French West Indies particularly Haiti and Guadeloupe as well as the Pacific Islands (CARBAP, 2014).

1.7 Plant growth regulators

Plant growth regulators are organic substances other than nutrients that control plant physiological processes and are mostly active at minute concentrations (Harms and Oplinger, 1988). Their actions may either be at the site of production or are either transferred to long distances from where they are synthesized to effect a specific action (Santner et al., 2009). However, they may be synthetically produced. Different classes of phytohormones exist which are very crucial in the regulation of plant growth and development, notably among them and termed as 'Classical' are; auxins, cytokinins, gibberellins, abscisic acids and ethylene (Santner et al., 2009). However, and more

recently included are jasmonates, salicylic acid, brassinosteroids, and polyamines (Jiménez, 2005).

1.7.1 Specific roles of some PGRs

Auxins in general induce cell enlargement and enhances root initiation (Kamínek, 1992). Cytokinins are also known to stimulate growth of cultured plant cells and tissues, release buds from apical dominance and also enhances lateral growth of buds when applied (Kamínek, 1992; Kintzios et al., 2000; Tiainen, 1993). Furthermore, cytokinins are known to mediate and control responses to different extrinsic factors including light conditions in the shoots and nutrient availability and water in the root; and further plays a critical role to the responses of biotic and abiotic stress (Werner and Schmülling, 2009). Auxin and cytokinin play very important role in banana and plantain growth; and development especially in propagation. Auxin and cytokinin acts synergistically or antagonistically to control vital developmental process with regards to meristem formation and maintenance (Azizi et al., 2015; George, 2008; Su et al., 2011). Apical dominance which is a major challenge in axillary shoot development of banana and plantain and in other plants have been overcome by the use of different growth regulators. These in various combinations, relatively enhance shoot and root formation; and growth of buds (George, 2008; Madhulatha et al., 2004). Various combinations of auxin and benzylaminopurine in large concentrations have the potential to reduce phenolic compounds in an in-vitro medium for grapes (Sedighi et al., 2014). High auxin - cytokinin concentration ratio in a culture medium tends to stimulate root formation and vice versa (Kamínek, 1992). It has also been found that exogenous zeatin, iP and BA enhance bud formation and tend to inhibit the growth of

root in *Catasetum fimbriatum* (Lazaro et al., 1999). Despite the fact that cytokinin, particularly, benzylaminopurine, promotes shoot development, it has been reported that high concentrations might be deleterious to cultures with the consequences of high abnormality index (Najmeh et al., 2011). Phenolics are very important secondary metabolites which play significant role as modulators of plant development through the control of indole acetic acid catabolism (López Arnaldos et al., 2001). In general indole-3-acetic acid (IAA) an auxin; and benzylaminopurine which is a cytokinin have been used extensively during in-vitro culture to enhance root and shoot development in the multiplication of healthy plantlets of bananas and plantains. BAP and IBA have also been used in different combinations to enhance shoot and root growth (Ngomuo et al., 2013). The effect of this has been demonstrated through various experiments by different authors (Arinaitwe et al., 2000; Bhosale et al., 2011; Ngomuo et al., 2013). The understanding of the phenomenon that auxins inhibit the development of axillary buds growth into shoots and which is direct contrast of the role of cytokinin as demonstrated by Doerte and Leyser (2011) has been reported many decades ago. Again, the phenomenon has contributed to the various manipulation in tissue culture propagation largely resulting in desired features of plantlets during each step of operation. Naturally derived cytokinins such as isopentenyl adenosine (iP) and zeatin are used in research yet in most cases commercial laboratories hardly use them due to the cost involved (George, 2008).

1.8 Coconut

Coconut (*Cocos nucifera*) is one of the most important fruit trees found in tropical regions with the fruit being used for diverse foods (Yong et al., 2009). The naturally

available coconut water which is the liquid endosperm of the fruit contains various hormones which are used as growth promoting supplements mainly in tissue culture (Ma et al., 2008; Yong et al., 2009). Coconut water is also reported to be an important source of isoprenoid and aromatic cytokinin as well as important source of nutrients (Appaiah et al., 2014; Ma et al., 2008). The fruits mostly mature after 11 months of emergence of the inflorescence, though cultivar differences exist. However, the fruits could be left in the tree for close to two years. Studies have shown that coconut fruits of 9 months old tend to have more water, nonetheless, the quantity of fat, protein soluble solids etc. also increase with maturity (Jackson et al., 2004; Tanqueco et al., 2007). The water to flesh ratio decreases with increases of fruit age (Appaiah et al., 2014; Jackson et al., 2004). Most researches on the availability of essential growth hormones have been on the coconut water. Moreover, it has been reported that purified liquid extract from the coconut meat has a growth factor that has the potential in promoting growth of tissue culture plants (Mauney et al., 1952). Various methods have extensively been employed to identify various classes of phytohormones such as auxins, gibberellins, abscisic acid and cytokinin as well as important mineral elements in coconut water (Ma et al., 2008; Tan et al., 2014; Vigliar et al., 2006). Coconut water is known to be characterized with a complex biological matrix that holds several endogenous organic compounds (Ge et al., 2006). Kinetin and kinetin riboside which are important cytokinin, have been recently found in coconut water (Ge et al., 2005; Kobayashi et al., 1997). Important cytokinin conjugates in coconut water such as zeatin-O-glucoside and dihydrozeatin- O-glucoside when made readily available could further enhance shoot development especially in the propagation of bananas and plantains.

1.9 Problem Statement, Justification and Objectives of the study

The current macropropagation approach for banana and plantain, widely employed using the PIF method is relevant in the farming communities of smallholder farmers mainly in the tropics. The approach has contributed to the propagation of healthy plantlets for the establishment of productive plantations, subsequently resulting in the improvement of livelihood of many small banana and plantain growers. Tissue culture which is able to generate many uniform, disease and pest-free plantlets appears to be no option for smallholder growers in Africa due to the high capital investment which makes the approach expensive. This is obviously reflected in the cost of acquisition of banana and plantain seedlings raised through tissue culture approach. Against this backdrop, macropropagation techniques on banana and plantain plants with the introduction and application of plant growth regulators have been carried out. This involves either direct application of hormonal solution to plants in the field or by treating harvested suckers with the aim of generating many plantlets with similar characteristics as those of tissue culture plants. The major treatment application method of plant growth regulators in macropropagation of banana and plantain has been through soaking of corms in various concentrations of hormonal solutions. This to a large extent has recorded some limited success (Kindimba and Msogoya, 2014; Langford et al., 2017; Sajith et al., 2014). Moreover, the approach practically makes use of large quantities of hormonal solution which to a large extent appears to be exorbitant. Additionally, the cost of acquisition of synthetic plant growth hormone is expensive for smallholder growers. For effective solution uptake, long hours of soaking duration are required. Therefore, an alternative to submergence approach which require less quantities of hormonal solution with swift application could be vacuum infiltration

technique. Hypothesized to make use of less quantities of hormonal solution, it is further estimated that the solution uptake through this approach will be significantly high to facilitate higher shoot proliferation of corms. Moreover, the approach will also ensure that minimum quantities of plant growth regulators are required during the application process in relation to the volume of prepared solution. With various trials employing synthetic growth hormones mainly 6-benzylaminopurine (BAP) and indole-3- acetic acid (IAA) in the multiplication of banana and plantain, it is often expensive and not easily accessible by smallholder farmers.

Huge opportunity exists in the tropics and subtropics where most smallholder banana and plantain farmers are centered, with abundant of coconut fruit growing almost at the backyard of these farmers. The exploitation of coconut water as an alternative to benzlyaminopurine (BAP) will be very relevant. Coconut water is naturally rich in cytokinins, auxins and gibberellins which are key elements in shoot and root proliferation in plants, including bananas and plantains. Additionally, resource poor farmers will be able to assess coconut water at a minimum cost (Buah and Agu-Asare, 2014) or even freely obtained from their backyards without much difficulty since it is highly abundant in the tropics and subtropics. Since coconut water has been reported to have some cytokinin conjugates, it was very important to exploit various strategies with the aim of ensuring that these cytokinin conjugates are deconjugated, rendering them freely available in the treatment solutions. Therefore, the present study hypothesized that through autoclaving to precipitate proteins and fats in young coconut water, the process will trigger the release of cytokinin conjugates into free forms, with high cytokinin activities, eventually resulting in high shoot proliferation of treated corms. Apart from the treatment of coconut through autoclaving, the study also focuses on the biological properties of papain on its effects on proteins in coconut water. Papain is a

proteolytic enzyme mainly for the cysteine proteinase family which is able to break down organic molecules constituting of amino acids known as polypeptides (Amri and Mamboya, 2012).

The rationale for the use of papain is to look for alternative cheap options of enhancing the breakdown of protein molecules in coconut water. The process was expected to enhance effective activities of coconut water contributing to high shoot proliferation in banana and plantain corms employing macropropagation techniques. Subsequent reactions of coconut water with papain was also expected to ensure the free availability of other plant growth hormones in the treated coconut water. The ultimate aim of every farmer is to obtain high yield from their farms. Despite, the use of some improved macropropagation techniques in raising plantlets at the nursery stage, growth and yield performances of these plantlets have not been critically assessed in the field. It is therefore important to further study the growth and yield performances of the healthy and robust seedling raised through the improved macropropagation techniques as well as the plantlets derived through the PIF technique.

Consequently, the overall objective of the study was to improve shoot proliferation in banana and plantain by employing innovative macropropagation techniques. Specifically, the research focus was on:

1. investigating the effectiveness of vacuum infiltration and soaking of corms with varying BAP concentrations and coconut water and
2. assessing the growth and yield performance of macropropagated seedlings under field conditions.

It is hypothesized that vacuum infiltrating corms with BAP or coconut water will increase solution uptake, enhance shoot proliferation of corms subjected to

mechanical preparation and perform better in terms of growth and yield than plantlets solely derived from the PIF-technique.

Publications

This doctoral thesis consists of two published articles (I and II) and one submitted article (III) in peer reviewed academic journals. Each publication is presented in a chapter.

Articles I and II have been reproduced in this thesis with the kind permission of International Society for Horticultural Science (ISHS).

Article I

J. Opata, J. Skala, M. Hegele, B.M. Dzomeku and J.-N. Wünsche. 2020. Macropropagation of banana (*Musa* AAA): Responses to hormonal and mechanical corm manipulation. Fruits, The International Journal of Tropical and Subtropical Horticulture, 75 (2), 78-83. (https://doi.org/10.17660/th2020/75.2.3).

Article II

J. Opata, P.-F. Melichar, M. Hegele, S. Hegele B.M. Dzomeku and J.-N. Wünsche. (2020). Macropropagation of plantain (*Musa* AAB): Responses to hormonal and mechanical corm manipulation. Fruits, The International Journal of Tropical and Subtropical Horticulture, 75 (3), 123-129. (https://doi.org/10.17660/th2020/75.3.4).

Article III

J. Opata, B.M Dzomeku, S.K Darkey and J.-N Wünsche. (Submitted to the Journal of Horticulture and Plant Research)

Field performance of False Horn Plantain (*Musa* AAB) corms treated mechanically and with hormone solutions.

2. Macropropagation of banana (*Musa* AAA): Responses to hormonal and mechanical corm manipulation

2.1 Summary

Introduction – The cultivation of banana (*Musa* spp.) by smallholder farmers in the humid forest zones of Africa is impaired by the low availability of quality planting material due to the insufficient sucker regeneration from the mother plant. Consequently, different low cost macropropagation techniques have been exploited to enable growers to increase the number of rooted suckers per corm for new plantings. *Materials and methods* - Young banana plants were harvested and leaf sheaths were carefully excised. The exposed apical meristems of the corms were either destroyed by crosswise incisions or left intact. These corms were then subjected to vacuum infiltration or soaking in different concentrations (0, 2.25 and 225.25 mg L^{-1}) of the cytokinin 6-benzylaminopurine (BAP). Treated corms were then planted in a heated germination bed filled with plant growth substrates to evaluate the treatment effects on number and growth characteristics of lateral shoots. *Results and discussion* - Breaking the apical dominance by destroying the meristem induced an earlier shoot emergence; however, did not result in a higher number of shoots per corm when compared to corms with intact meristems. Vacuum-infiltrated corms absorbed more hormonal solution than soaked corms and thus produced more and thicker shoots. Corms treated with BAP had a greater number of strong shoots with more roots than untreated controls, an effect that was independent of the applied concentration. *Conclusion* - Although infiltrating corms with hormone solution requires the procurement and use of a simple vacuum pump, this minor cost should not prevent resource-poor farmers, particularly

when organized in cooperatives, from adopting this method for producing more planting material.

Keywords

apical meristem, 6-benzylaminopurine, crosswise incision, infiltration, soaking

2.1.2 Significance of the study

2.1.2.1 What is already known on this subject?

Soaking banana corms in synthetic plant hormones is a widely adopted method by smallholder farmers in Africa for raising new planting material.

2.1.2.2 What are the new findings?

Corms absorbed greater amounts of 6-benzylaminopurine by vacuum-infiltration than by soaking and consequently produced more and stronger shoots.

2.1.2.3 What is the expected impact on horticulture?

Application of 6-benzylaminopurine solution by vacuum infiltration of banana corms can be easily adopted by smallholder farmers in Africa to raise large quantities of rooted shoots as source material for new plantations.

2.2 Introduction

Banana (*Musa* spp.) is an important fruit crop that is grown mainly in the tropical and subtropical regions. Annually, over 31 million tons of banana are produced mainly by smallholders in developing countries (Lescot and Ganry, 2010; Singh et al., 2011). The crop is a major export commodity to many countries (Buah and Agu-Asare, 2014; Hauser, 2010; Hauser and Coyne, 2010) and generates substantial income for family-based farms in rural communities (Ortiz and Vuylsteke, 1994). The crop has been continually improved, especially through breeding and agronomic practices, including pests and diseases management (Lescot and Ganry, 2010).

All cultivated bananas are triploid and do not produce viable seeds. Therefore, propagation is carried out by vegetative procedures (Buah and Agu-Asare, 2014; Ortiz and Vuylsteke, 1994; Rahman et al., 2004); however, apical dominance is a major constraint to the sprouting of new lateral shoots from the mat. Consequently, various techniques have been developed to break the apical dominance and to induce multiple shoot proliferation around the mother plant (Baiyeri and Aba, 2007; Dzomeku et al., 2014; Kwa, 2003; Singh et al., 2011). Complete decapitation in the field requires the cutting-down of the pseudostem just above the ground level and then destroying the growing point in the middle of the remaining stem attached to the corm (Singh et al., 2011). Other techniques commonly rely on harvesting the corm, removing roots (paring), taking off leaf sheaths to expose the meristem and to scarify lateral buds and then planting the prepared corms or excised buds in sawdust inside a germination bed. The PIF (*Plants Issus de Fragments de tige*) technique is also frequently applied to destroy with a crosswise incision the apical meristem. In addition, harvested corms are frequently subjected to hot water treatments prior to paring to produce pest-free

plantlets (Tenkouano et al., 2006). However, these propagation methods require further improvements to raise high quality rooted shoots in sufficient numbers for the establishment of new plantations by smallholder farmers.

Plant growth hormones are also increasingly employed in macropropagation techniques to promote lateral shoot growth (Thiemele et al., 2015; Kindimba and Msogoya, 2014). To induce multiple shoots, spray applications of the synthetic cytokinin 6-benzylaminopurine (BAP) are commonly considered by smallholder banana farmers. Moreover, corms can be soaked with hormonal solutions at various concentrations (Osei, 2007); however, large volumes of solutions are required to ensure complete submergence and a long soaking duration of corms for an effective uptake.

Consequently, exploring alternative approaches that facilitate a greater amount of solution uptake to regenerate more shoots are needed. Vacuum infiltration in combination with different concentrations of BAP is hypothesized to increase solution uptake and thus shoot proliferation of corms. Consequently, the objective of this study was to investigate the effectiveness of vacuum infiltration and soaking of banana corms with varying BAP concentrations on shoot regeneration under greenhouse conditions.

2.3 Materials and methods

2.3.1 Experimental site and corm preparation.

The research was conducted at the University of Hohenheim, Germany, during the summer months of 2015 and 2016. Tissue cultured plantlets of the banana cultivar ‘Khai Thong Ruang’ (KTR), a dessert banana (*Musa* AAA), were obtained from the

Bioversity International Musa Germplasm Transit Centre (ITC) at Leuven, Belgium. The plantlets were further multiplied by tissue culture, acclimatized in a growth chamber for six weeks and then cultivated under controlled greenhouse conditions at 25/ 20 °C day and night temperature, respectively, and 700 µmol m^{-2} s^{-1} light intensity above the plant stand.

The plants were harvested ten months after planting when they had developed sizeable corms appropriate for hormonal and mechanical manipulation. First, roots were removed with a sterilized knife, followed by rinsing under tap water to remove all substrate remnants. Thereafter, all leaf sheaths along the collar of the corms were carefully excised to expose the latent buds and the apical meristem. The meristems of half of the randomly selected corms was destroyed with a crosswise incision by employing the PIF technique while the others were left with intact meristems.

2.3.2 Hormonal treatments.

Three concentrations (0, 2.25 and 225.25 mg L^{-1}) of the synthetic plant hormone 6-benzylaminopurine (Carl Roth, GmbH, Germany) were prepared, using 1N NaOH as solvent and water as diluent. Corms with destroyed or intact apical meristem were then either infiltrated or soaked with the three hormonal solutions. The concentration of 2.25 mg L^{-1} served as a benchmark since it is the most commonly applied BAP treatment for macropropagation of banana by smallholder farmers. The high BAP concentration was justified since 25.25 mg L^{-1} did not result in higher shoot numbers per corm in a preliminary experiment.

Initially weighed corms were placed inside a glass desiccators and fully submerged in the hormonal solutions (Figure 2.1a). A vacuum pump (Leybold Heraeus, Trivac, D8A, Germany) applied pressure of 40 kPa for 5 min to outgas the intercellular spaces of the corm tissue. Thereafter, the pressure was gradually released to normal atmospheric conditions over a 5 min period during which the corms were infiltrated with the respective hormonal solution. The corms were then removed from the solution, dried with paper towels and weighed to get an estimate of how much solution has been taken up during the infiltration process. The operation times of the vacuum pump were experimentally determined to ensure effective outgassing and solution uptake of the corm.

For soaking, corms were placed in plastic barrels and submerged in the three hormonal solutions for 12 hours, respectively (Figure 2.1b). To avoid flotation, a ceramic plate kept the corms fully immersed in the solution during treatment. The solution was stirred for about 10 min after four and eight hours of soaking, respectively, to ensure that the BAP remained in solution and to facilitate uptake by the corms (Muhammad et al., 2007). After soaking, the surfaces of the corms were dried with paper towels and weighed to determine the amount of solution uptake.

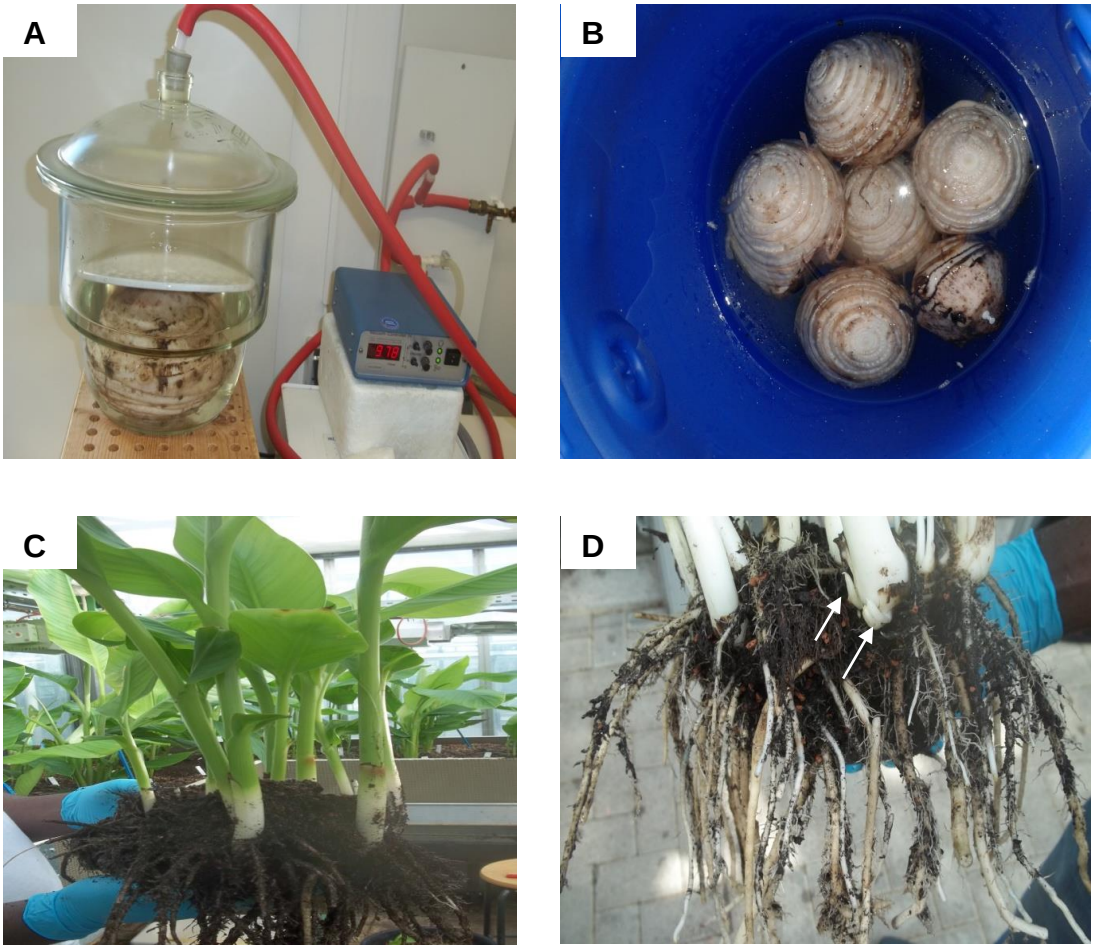

Figure 2.1. Corm under (**A**) vacuum infiltration; (**B**) corm under soaking condition; (**C**) sprouted corm with multiple shoots and roots in the greenhouse; and (**D**) arrows indicate shoot formation at the base of a mature shoot grown on 225.25 mg L^{-1} 6-benzylaminopurine.

2.3.3 Experimental design, data collection and analysis.

Twelve treatment combinations, each with six replicates (treated in 4 L of solution), were planted in a heated (25°C) germination bed filled with a plant growth substrate (Seramis Anzucht Bioerde, Germany), which has similar properties to what is commonly used in West Africa (Baiyeri and Aba, 2005; Dzomeku et al., 2014; Kindimba

and Msogoya, 2014). Throughout the trial, the air temperature inside the greenhouse chamber was set to 30°C during the day and to 20-25°C during the night.

Sprouting of latent buds commenced two weeks after planting and again two weeks later, lateral shoots were decapitated at 3 cm above the point of attachment to the mother corm. The leaf sheaths of the shoot stumps were removed until the apical meristem was exposed, which subsequently was destroyed by crosswise incision. This procedure repressed apical meristem growth and facilitated the growth of multiple rooted shoots (Figure 2.1c, 2.1d). The shoots were harvested fortnightly for three consecutive periods at which time the corms had terminated shoot regeneration.

The experiment was laid out in a factorial design, consisting of three factors (corm manipulation technique, hormone application method, BAP concentration) with 12 treatment combinations in each of six blocks. Data collection included (i) amount of solution uptake during infiltration and soaking; (ii) time (in days) to first shoot emergence; (iii) harvested shoots per corm; (iv) shoot diameter measured with a caliper 2 cm above the base; (v) shoot length measured from the base of the harvested shoot to the shoot tip; and (vi) the number of roots per shoot. Data were analysed using Genstat (18th Edition, Rothamsted, United Kingdom) and displayed graphically with Origin (version 19, Wellesley Hills, MA, USA).

2.4 Results

2.4.1 Solution uptake.

The amount of solution uptake by corms was significantly affected by the application method. Corms subjected to vacuum infiltration absorbed 33 % more solution than

those to soaking (Figure 2.2). The initial weight of corms prior to soaking and infiltration was similar with averagely 1,726 g and therefore had no effect on the solution uptake. Moreover, there was no significant difference in solution uptake between corms with intact and destroyed meristem (data not shown).

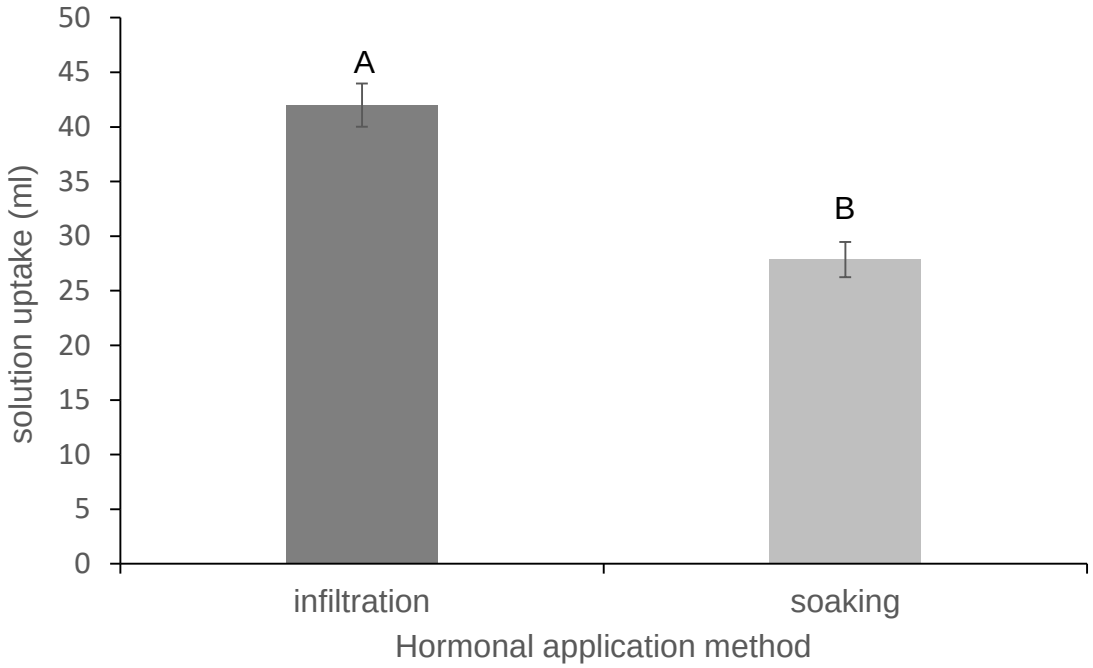

Figure 2.2. Solution uptake of cv. 'Khai Thong Ruang' corms by soaking and infiltration. Vertical bars indicate standard error of the means (n=36) and different letters represent significant difference using LSD test at P≤0.05.

2.4.2 Days to lateral shoot emergence.

Days to first lateral shoot emergence was significantly affected by the corm manipulation technique. Irrespective of the two hormone application methods, lateral shoots of PIF-treated corms emerged averagely 11 days after planting, whereas shoot

emergence of control corms was approximately 2 days later (Figure 2.3). The soaked corms with an intact meristem needed significantly longer to first lateral shoot emergence than any other treatment (Figure 2.3). The various concentrations of BAP applications had no effect on the number of days to lateral shoot emergence (data not shown).

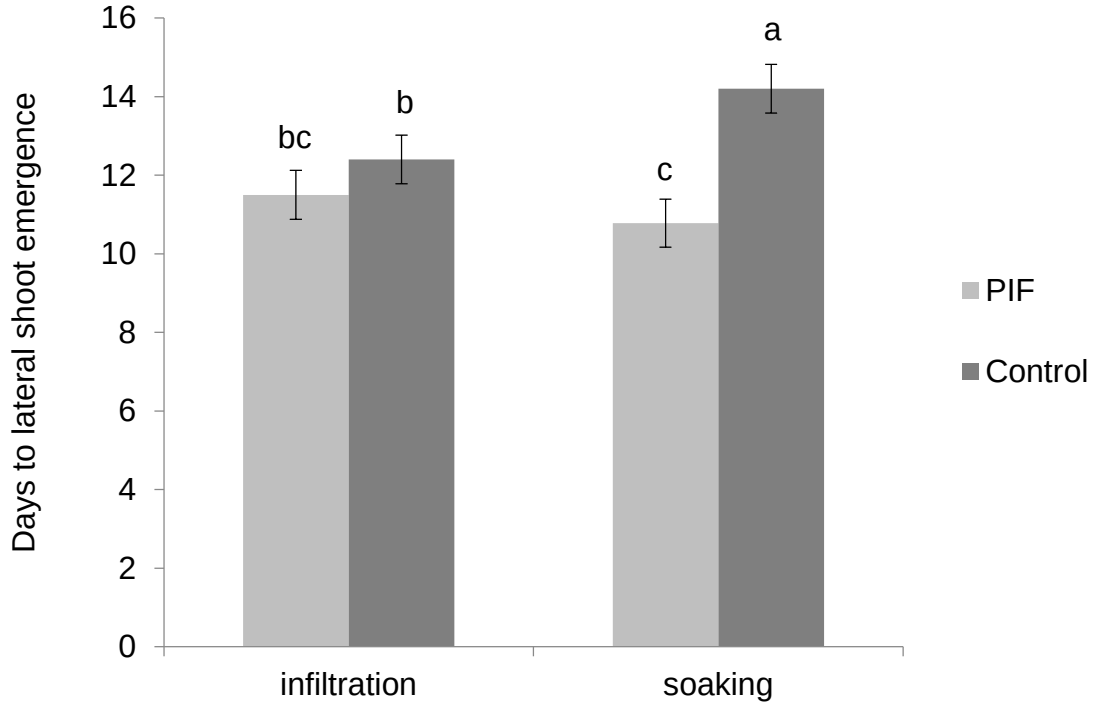

Figure 2.3. Effects of corm manipulation (PIF - crosswise incision and control – non-crosswise incision) and hormonal solution application (infiltration and soaking) on days to shoot emergence of the banana cv. 'Khai Thong Ruang'. Vertical bars indicate standard error of the means (n=18) and different letters represent significant difference using LSD test at P≤0.05.

2.4.3 Shoot production.

The number of shoots produced per corm was not affected by the corm manipulation technique; however, significant effects were found for the method and concentration of hormone application, respectively. Corms subjected to infiltration resulted in 16% more shoots compared to soaked corms (Table 2.1). Moreover, corms treated with BAP produced averagely 37% more shoots than the untreated control, but the concentration was not having a significant effect on the number of shoots per corm (Table 2.1). Moreover, there were significant interactions between corm manipulation technique, hormone application method and BAP concentration on shoot production. In general, control corms infiltrated with BAP had the greatest shoot production, whereas controls and PIF-treated corms without BAP application, respectively, had the lowest number of shoots per corm (Table 2.1).

2.4.4 Root production.

Root production of the banana cultivar was neither significantly affected by the corm manipulation technique nor the hormone application method (Table 2.1). However, corms treated with either of the two BAP concentrations produced 4 roots per shoot, which was about 34% greater than the number of roots grown on shoots of untreated controls. Again, there were significant interactions between the three treatments; however, BAP applications resulted always in the largest number of roots per shoot, an effect that was independent of hormone application method and corm manipulation technique, respectively Table 2.1).

Table 2.1. Main effects and interaction of corm manipulation (PIF - crosswise incision and control – non-crosswise incision), hormonal application (infiltration and soaking) and 6-benzylaminopurine concentrations on mean numbers of shoots per corm and roots per shoot for the banana cv. 'Khai Thong Ruang'

Effects	Number of	
	shoots per corm	roots per shoot
Corm manipulation		
PIF	28.9^{a}	3.6^{a}
Control (Co)	30.5^{a}	3.5^{a}
Hormone application		
Infiltration (I)	31.9^{a}	3.6^{a}
Soaking (S)	27.6^{b}	3.5^{a}
BAP Concentration		
0 mg L^{-1}	23.9^{b}	2.9^{b}
2.25 mg L^{-1}	33.0^{a}	3.8^{a}
225.25 mg L^{-1}	32.3^{a}	4.0^{a}
Man. x App. x Conc.		
PIF+I+0	25.8^{f}	3.2^{cd}
PIF+S+0	20.2^{g}	2.8^{de}
Co+I+0	23.7^{fg}	2.4^{e}
Co+S+0	26.0^{f}	3.2^{cde}
PIF+I+2.25	31.5^{cd}	3.4^{bcd}
PIF+S+2.25	31.2^{cde}	3.9^{abc}
Co+I+2.25	39.2^{a}	4.0^{ab}
Co+S+2.25	30.0^{de}	3.7^{abc}
PIF+I+225.25	34.3^{bc}	4.1^{ab}
PIF+S+225.25	30.7^{cde}	4.2^{ab}
Co+I+225.25	36.8^{ab}	4.3^{a}
Co+S+225.25	27.5^{ef}	3.6^{abc}
P-value		
Corm manipulation	0.056	0.666
Hormone application	<0.001	0.853
BAP concentration	<0.001	<0.001
man. x app. x conc.	<0.001	0.013

Means with different letters within columns and statistical effects are significantly different at P≤ 0.05.

2.4.5 Shoot characteristics.

There were significant main effects on the average shoot length (Table 2.2). Control corms compared to PIF-treated corms had 12% longer shoots, shoots of infiltrated corms were 7% longer than those of soaked corms and BAP-treated corms had 16% longer shoots than those treated with water. Shoot girth was not affected by the corm manipulation technqiue; however, it was significantly affected by the hormone application method and BAP concentrations (Table 2.2). Shoots that emerged from corms subjected to vacuum infiltration had averagely 9% greater shoot girths when compared to those produced from soaked corms. BAP treated corms had shoots with a 16% bigger girth than that of shoots from corms treated with water.

Table 2.2. Main effects of corm manipulation (PIF - crosswise incision and control – non-crosswise incision), hormone application and 6-benzylaminopurine concentrations on shoot growth of the banana cv. 'Khai Thong Ruang'

Effects	Shoot length (cm)	Shoot girth (cm)
Corm manipulation		
PIF	21.4[b]	2.1[a]
Control (Co)	24.4[a]	2.1[a]
Hormone application		
Infiltration (I)	23.7[a]	2.2[a]
Soaking (S)	22.1[b]	2.0[b]
BAP concentration		
0 mg L^{-1}	20.7[b]	1.9[b]
2.25 mg L^{-1}	24.1[a]	2.2[a]
225.25 mg L^{-1}	24.0[a]	2.2[a]
P-value		
Manipulation	<0.001	0.995
Application	0.03	<0.001
Concentration	<0.001	<0.001

Means with different letters within columns are significantly different at p≤ 0.05.

2.5 Discussion

The study demonstrated a higher uptake of hormonal solution by corms subjected to vacuum infiltration in comparison to the method of soaking. The differential solution uptake induced a greater number of shoots in infiltrated corms compared to soaked corms, an effect that was independent of the applied BAP concentration (Table 2.1). Despite this result, shoot proliferation of vacuum infiltrated corms may have been to some extent adversely affected by the vacuum pressure applied at the infiltration stage, possibly leading to cell disintegration and a reduced longevity of the corm. The twelve hours of soaking banana corms in hormonal solutions was previously proposed as an appropriate treatment duration (Thiemele et al., 2015; Kindimba and Msogoya, 2014; Msogoya and Mwakisitu, 2014) and soaking durations of less than 30 min had only limited success (Dayarani et al., 2013; Langford et al., 2017).

The PIF-technique applied for suppressing the growth of the apical meristem led, in agreement with findings of Dayarani et al. (2013), to an early shoot emergence; however, did not result in a higher number of shoots per corm, which is contrary to previous findings (e.g. Kindimba and Msogoya, 2014). This might be due to the observed slight degree of decomposition of PIF-treated corms in the germination bed that in turn affected the total number of shoots produced per corm.

In contrast, a significantly increased multiple shoot proliferation over the untreated controls was achieved by treating corms with BAP. This cytokinin is known to reduce apical dominance and thus promotes the formation of lateral shoots and adventitious root growth (Cronauer and Krikorian, 1984; Devendrakumar et al., 2013; Muhammad et al., 2007; Najmeh et al., 2011). The stimulating effect of various BAP concentrations on shoot multiplication rate has been studied and demonstrated previously in field

experiments (Thiemele et al., 2015; Kindimba and Msogoya, 2014; Osei, 2007). In addition, seed priming with plant growth regulators and mineral elements have also been reported to enhance germination and shoot growth of some plants (Ajouri et al., 2004; Sedghi et al., 2010; Shah et al., 2011).

Msogoya and Mwakisitu (2014) demonstrated that relatively low concentrations of thidiazuron, a diphenyl urea-based cytokinin, effectively induced multiple shoots in banana. Similar shoot proliferation responses to BAP were reported by Arinaitwe et al. (2000) and Muhammad et al. (2007), conducting in-vitro experiments with several banana cultivars. Moreover, Madhulatha et al. (2004) found that cytokinin concentrations over 200 mg L^{-1} in tissue culture media resulted in reduced number of banana shoots per explants. Indeed, and consistent with this result, a BAP concentration independent effect on shoot proliferation was shown in the present study. Contrary to our findings, an increased shoot regeneration of plantain corms treated with increasing BAP concentrations was reported by Thiemele et al. (2015). The different shoot proliferation responses to BAP of plantain and banana cultivars may be attributed to some genetic variability as well as the constituent content of auxins and cytokinins in the plant tissue (Arinaitwe et al., 2000).

Both BAP concentrations produced shoots with more roots compared to shoots from the control treatment. In contrast, Kindimba and Msogoya (2014) reported no BAP effect on root production, irrespective of the applied concentrations. The higher BAP application rate may have increased the cytokinin content in the corms to such an extent that the required auxin-cytokinin ratio needed for root initiation was too low (Kamínek, 1992). Applications of 2.25 mg L^{-1} of BAP for propagating banana could be an efficient and cost effective approach to produce well-rooted shoots, which would

also lead to a greater survival rate during the acclimatization phase prior to planting (Baiyeri and Aba, 2005; Baiyeri and Aba, 2007).

2.6 Conclusions

The present study demonstrated, in line with the stated hypothesis, that vacuum infiltration in comparison to soaking facilitated a greater uptake of BAP solution by banana corms, which in turn resulted in higher shoot and root proliferation. The shoot regeneration performance was not dependent on the applied BAP concentration, implying that resource poor farmers can continue to use cost-effectively the standard low cytokinin concentrations during the macropropagation process of banana. Further research is needed to define the most appropriate vacuum pressure, thus to avoid any potential adverse effects (e.g. cell disintegration, rotting) and to prolong the survival duration of the corms. This method could further be tested on corms of banana plants after fruit harvest, which might be able to withstand greater vacuum pressures due to hardened, woodier tissue. Though robust cost-benefit ratios for the various treatment combinations cannot be calculated due to site-dependent, largely varying production costs and potential incomes per plantlet, vacuum infiltration is recommended to resource-poor banana farmers for raising planting material. The additional cost compared to the standard farmer practice is caused by the need to invest in a simple vacuum pump and more BAP because of the greater amount of solution uptake during the infiltration process. These costs should be well compensated for by the increase in sales of rooted shoot.

2.7 Acknowledgements

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3. Macropropagation of plantain (*Musa* AAB): Responses to hormonal and mechanical corm manipulation

3.1 Summary

Introduction – The availability of plantain plantlets in sufficient numbers for the establishment of new plantations is a major challenge to smallholder farmers in Sub-Saharan Africa. Therefore, applications of a plant growth regulator or natural hormonal solutions in combination with the mechanical manipulation of the excised mother corm were evaluated to enhance shoot proliferation in plantain. *Materials and methods* – Pared corms of plantain suckers were subjected to vacuum infiltration with either autoclaved natural (cytokinin, seaweed extract) or synthetic (6-benzylaminopurine; BAP) hormonal solutions prior to mechanically destroying the meristem with a crosswise incision known as "*Plant Issus de Fragments de tige*" (PIF). These treatments were compared to only PIF-treated corms and untreated controls. All corms were planted in a germination bed filled with sawdust inside a humidity chamber. *Results and discussion* – Corms infiltrated with autoclaved coconut water and then treated with PIF developed lateral shoots at least two days earlier than any other treatments. With a few exemptions, this treatment produced also about 10% longer and thicker shoots, respectively, and 25% more roots than corms treated with other natural hormonal solutions. Moreover, PIF-treated corms, infiltrated with either autoclaved coconut water or BAP, produced at least 10% more shoots compared to other treatments. *Conclusion* – The results indicate a beneficial effect of treating plantain corms with autoclaved coconut water on shoot proliferation. This specific

measure is in support for small-scale farming, especially for low-income and resource-poor farmers.

Keywords
6-benzylaminopurine, coconut water, infiltration, meristem, phytohormones

3.1.2 Significance of this study.

3.1.2.1 What is already known on this subject?

Natural plant hormones have been exploited to enhance the propagation of several important horticultural crops, including plantain and banana.

3.1.2.2 What are the new findings?

Vacuum infiltration of plantain corms with autoclaved coconut water, followed by destroying the apical meristem of the corm prior to planting and of two-week old lateral shoots emerging from the mother corm resulted in a high number of quality lateral shoots.

3.1.2.3 What is the expected impact on horticulture?

Smallholder farmers in Sub-Saharan Africa may use coconut water as an available source of plant hormone to enhance the production of young plantain plantlets. This will allow the regular establishment of plantain fields when the replacement of old and unproductive fields become necessary.

3.2 Introduction

Plantains and bananas (*Musa* spp.) are very important food crops as well as income generating crops in most humid countries in Africa (Arinaitwe et al., 2000; Hauser, 2010; Tomekpe et al., 2011). Both *Musa* species rank as the fourth most important staple crop in developing countries after rice, wheat and maize (Gitonga et al., 2011; Heslop-Harrison and Schwarzacher, 2007) with one-third of its global production occurring in Sub-Saharan Africa. Plantain as a source of proteins, vitamins, and minerals (Adamu et al., 2017; Iqbal and Muhammad, 2013) provides more than 25% of the food energy requirement (Tripathi et al., 2009) for the majority of people in Sub-Saharan Africa. The crop also serves as shade plant for other important tree species such as coffee and cocoa and therefore is an integral part of the agroforestry farming system (Albertin and Nair, 2004; Dzomeku et al., 2011; Ortiz and Vuylsteke, 1994; Schill et al., 2000).

The crop was subject of much production improvement research and dissemination activities (Dzomeku et al., 2011), yet there are still major production constraints. Most plantain cultivars are triploid with the formation of parthenocarpic fruit without viable seeds. Therefore, propagation is conventionally performed by using suckers or corms; however, both source tissues are frequently infested with pests and diseases (Rahman et al., 2004). The resulting outcome of using such unhealthy material is poor plant stand, yield reduction and thus low income to the farmer. The mother plant is typically able to produce between 5-10 suckers within the year after planting (Rahman et al., 2004; Vora and Jasrai, 2012). Even so, the suckering ability in plantain and banana mother plants, which exhibit similar botanical characteristics (Heslop-Harrison and Schwarzacher, 2007), is suppressed by apical dominance due to a high level of auxin

synthesis and a basipetal auxin transport (Arinaitwe et al., 2000). However, the apical dominance is reduced at flowering, allowing daughter suckers to sprout. It is not recommended to remove daughter suckers from the mother plant during flowering as this will weaken the base and subsequently result in lodging. However, these young suckers are best suited for establishing new plantations (Dzomeku et al., 2014).

Studies on macropropagation to enhance shoot proliferation of plantain and banana cultivars described either the application of synthetic plant growth regulators (Kindimba and Msogoya, 2014; Langford et al., 2012), mainly with the cytokinin, 6-benzylaminopurine (BAP), or mechanical techniques such as the *Plant Issus de Fragments de tige* (PIF) that destroy the apical meristem. The mechanical manipulation of the excised mother corm has been widely used by smallholder farmers, especially in Africa (Tomekpe et al., 2011) and has been described as one of the affordable techniques that can be employed for obtaining new planting material (Kwa, 2003). Trials where field-grown plantains were injected with both synthetic and natural plant hormones resulted in multiple shoots (Osei, 2006). Other techniques, which involve the submergence of excised and mechanically treated corms in synthetic hormones such as BAP, have also been studied; however, with limited success (Kindimba and Msogoya, 2014; Langford et al., 2012).

Coconut (*Cocos nucifera* L.) is one of the most important perennial fruit crops in tropical and subtropical regions and is well-known for its multiple uses in beverages and medicine (Jackson et al., 2004; Moore, 1948). The wide applications of coconut water can be justified by its unique chemical composition of sugars, vitamins, minerals, amino acids and the rich source of phytohormones, mainly cytokinins and auxins (Agampodi and Jayawardena, 2009; Ma et al., 2008; Prades et al., 2012; Tan et al., 2014; Vigliar

et al., 2006; Yong et al., 2009). The cytokinins found in coconut water support cell division and thus promote rapid plant growth. Coconut water has traditionally been exploited as one of the growth supplements in culture media for the in-vitro propagation of banana and plantain (Buah and Agu-Asare, 2014; Iqbal and Muhammad, 2013; Khawaj et al., 2015; Yong et al., 2009). However, there is a need to investigate the potential use of coconut water in macropropagation procedures of plantain. It is therefore hypothesized that the introduction of natural hormonal solutions into the corm of plantain is an effective method to induce multiple shoots. The objective of this study was to vacuum-infiltrate corms with coconut water to enhance shoot proliferation in plantain.

3.3 Materials and methods

3.3.1 Experimental site.

The research was carried out in the rainy season between April to June 2016 at the Crops Research Institute (CRI) in Kumasi (1°38'W, 6°43'N), Ghana, located within a semi-deciduous forest region and characterised by a sandy-loam soil (Arenosol), a bimodal rainfall of 1,500 mm annually and an annual mean air temperature of 25.6 °C. The 2016 experiment followed a preliminary trial in the dry season between February and April 2015.

3.3.2 Corm preparation.

Sword and ratoon suckers of the False Horn plantain cultivar ‘Apantu’ were harvested from an experimental field at the CRI. These two types of suckers are considered as the most productive source material for establishing new plantings (Dzomeku et al., 2014). Apantu’ is a False Horn belonging to the plantain subgroup and AAB genome group. It is characterised by the male bud degenerating at maturity, retaining only a few neutral flowers, large fingers and different plant size categories. The harvested, 4-5 months old plantain suckers were kept under shade prior to mechanical preparation (Buah et al., 2010). The corms were then subjected to paring, which involved cleaning the corms by cutting off the roots. This paring process also ensured that soil-borne microorganisms, especially root nematodes and banana stem borers, were also eliminated (Swennen, 1990). The pared corms were then subjected to mechanical manipulation by carefully removing all leaf sheaths around the collar with a sharp knife that was frequently sterilized with 70% ethanol to reduce microbial contamination of the corms. When the apical meristem of the corm was exposed, the corm was washed under running tap water to further remove any foreign material. The air-dried corms were first infiltrated with hormonal solution (as described below under vacuum infiltration) and then subjected to mechanical treatment by destroying the apical meristem with a crosswise incision using the PIF technique (Figure 3.1a) or remained unchanged (untreated control). Preliminary trials revealed that first destroying the meristem with crosswise incision and then subjecting it to vacuum infiltration shortens the survival period of corms in the germination bed.

3.3.3 Hormonal solutions.

Mature coconut fruits were harvested from a farmer's field near Kumasi. The fresh coconuts were thoroughly cleaned with tap water, broken and water was extracted from the nuts. The coconut water was sieved to remove suspended materials, kept in clean plastic containers and used to prepare different treatments solutions: fresh coconut water (CW_f); autoclaved coconut water at 121^oC for 15 min (CW_a); coconut water with 0.1% (w/v) pulverized papain, incubated at 40^oC for 40 min and stirred every 10 min for one minute throughout the incubation time and then autoclaved at 121^oC for 15 min (CWP_a) and autoclaved coconut water with 0.5% (v/v) of seaweed extract (Tecamin Raiz, AgriTecno, Spain) as root growth bio-stimulant (Calvo et al., 2014; CW_aSW). Additional solutions were 0.5% (v/v) seaweed extract dissolved in distilled water (SW); 2.25 mg L^{-1} 6-benzylaminopurine (Carl Roth, GmbH, Germany) and four drops of 1N NaOH as solvent (BAP); distilled water (W_d). These seven solutions were compared to only PIF-treated corms and untreated controls (UTC).

3.3.4 Determination of phytohormones in coconut water

The free cytokinins (CKs) zeatin/zeatinriboside (Z/[9R]Z) and N6(Δ2-isopentenyl) adenine /N6(Δ2-isopentenyl) adenosine (iP/[9R]iP) in the coconut water were determined by Radio-Immuno-Assay (RIA) according to Weiler (1984). Prior to analysis, coconut water samples were homogenized and extracted overnight with 50 ml of 80% (v/v) methanol at 4°C in darkness. The extracts were purified using a combination of polyvinylpolypyrrolidone (PVPP; Sigma), DEAE Sephadex TM A-25 (GE Healthcare) and SepPak C18 (Waters) columns, following the procedure previously described by Jiménez et al. (2001). Papain (Amri and Mamboya, 2012) was

used as a treatment component to stimulate the de-conjugation of conjugated inactive cytokinins into active forms by enzymatic hydrolysis. The papain used in the trial was obtained from immature papaya fruit still attached to the plant and collecting latex flow into small plastic cups. The latex was then freeze-dried for 72 hours at the CRI. The freeze-dried latex was pulverized into fine powder with a hammer mill (A11, IKA-Werke, Germany).

3.3.5 Vacuum infiltration.

The prepared corms were weighed and completely submerged in glass desiccators which contained the respective hormonal solutions (Figure 3.1b). To prepare 4 liters of solution for the infiltration of 10 corms, 9 mg of BAP or 20 fruits that contained averagely about 200 ml of coconut water were used. An electric vacuum pump (Vacuubrand 1, model 100, Germany) was operated at a pressure of 40 kPa to outgas the intercellular spaces of the corm tissue without damaging the cellular structure. Preliminary trials showed that higher pressure resulted in rapid rotting of the corm tissue. After 5 min of vacuum, the pressure was gradually released to normal atmospheric conditions to facilitate the uptake of the respective solution by the corms for 5 min. The corms were then taken out of the solutions, dried with paper towels and weighed. The meristem of the corm was then destroyed as described earlier.

3.3.6 Planting of corms.

Corms, subjected to the nine treatments, were planted in 10 replications in a completely randomized design under a germination bed filled with sterilized sawdust

(Figure 3.1c). This was located inside a shade house, clad with transparent polyethylene sheets. The corms were planted 20 cm apart and buried 3 cm below the surface of the sawdust. Watering of corms in the germination bed was carried out every three days to ensure high relative humidity inside the shade house.

Two-week-old lateral shoots were further subjected to meristem manipulation by decapitating the shoot 3 cm above the point of attachment to the mother corm. The leaf sheaths of the shoot stump still attached to the mother corm were peeled off until the apical meristem of the young shoot was exposed. The exposed apical meristem was destroyed with crosswise incision, using a sharp knife, and then again covered with sawdust. This practice further enhances the number of shoots (Figure 3.1d) emerging from the corm (Dayarani et al., 2013). Shoots were harvested every two weeks from the mother corm by which time they had obtained robust stems with sufficient numbers of roots and leaves to ensure a good survival rate at the acclimatization stage. Harvesting discontinued when the corms were exhausted and did no longer produce new shoots.

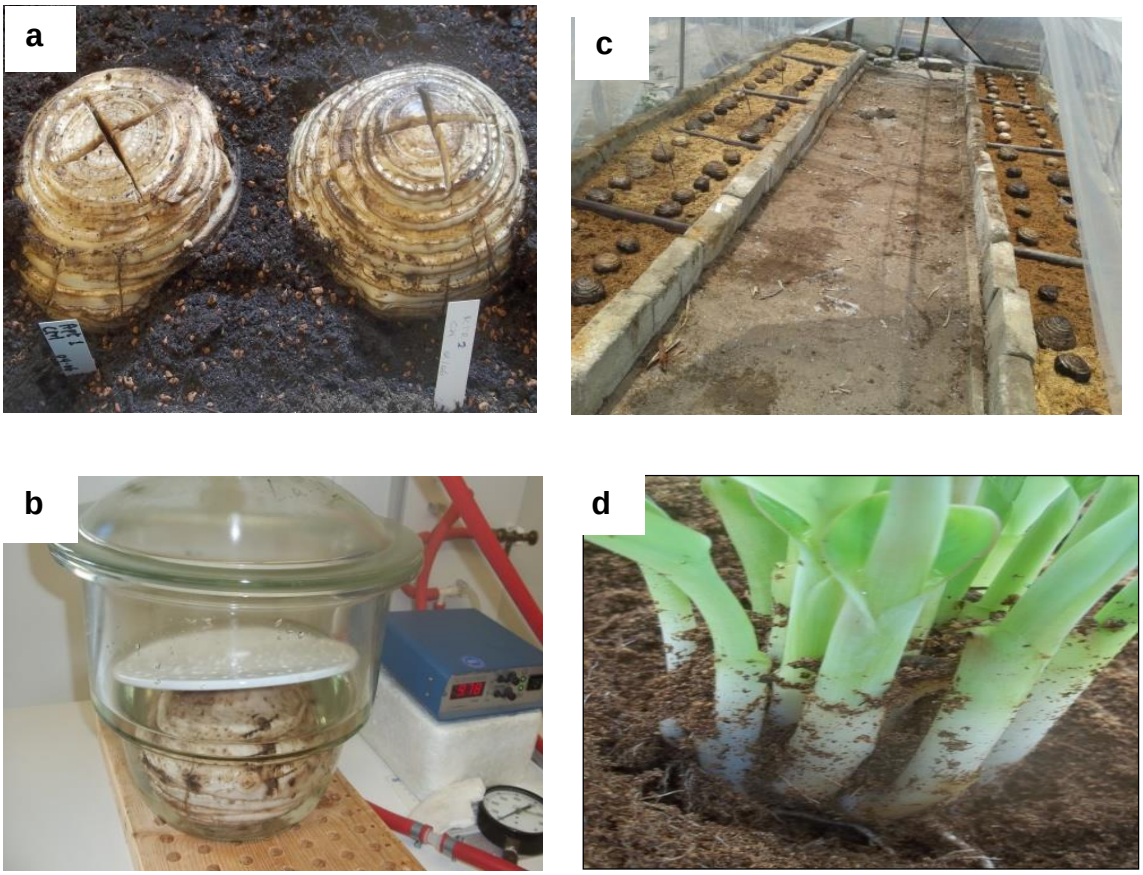

Figure 3.1. Corms with the crosswise incision known as *Plant Issus de Fragments de tige* (PIF) technique (a); a corm under vacuum infiltration (b); arrangement of corms inside a shade house prior to covering them with sawdust (c); and cluster of shoots after destroying the apical meristem of two-week old lateral shoot using the PIF technique (d).

3.3.7 Data collection and analysis.

Data were collected for the number of days to first lateral shoot emergence and the total number of lateral shoots of each corm. In addition, shoot length from the base of the excised shoot to the shoot tip, the number of fully opened leaves per shoot, shoot

girth at 2 cm above the base and root number per shoot were determined from fifty randomly selected lateral shoots of each treatment. Analysis of variance (ANOVA) was used to evaluate the effect of hormonal solutions and mechanical manipulation on shoot emergence, total number of shoots per corm and shoot parameters. Data were analysed using Genstat (18th Edition, Rothamsted, United Kingdom) and displayed graphically with Origin (version 19, Wellesley Hills, MA, USA).

3.4 Results

3.4.1 Concentration of cytokinins in coconut water.

Both extractable free CKs, Z/[9R]Z) and iP/[9R]iP, had a concentration of averagely 31.5 ng mL^{-1} of fresh coconut water, which is similar to that presented by Yong et al. (2009). Although the concentration of the two extractable free CKs is considerably less compared to the applied BA concentration, the total concentration of all cytokinins and their derivatives in coconut water is greater (Yong et al., 2009). Moreover, inactive CKs were likely converted to active free forms as indicated by the increase of total extractable CKs of about 5% through the addition of papain.

3.4.2 Number of days to first lateral shoot emergence.

Lateral shoot emergence on corms treated with CW_a was averagely 2 days earlier than in any other hormonal treatment (Figure 3.2) and they emerged almost four days earlier than those from the W_d and PIF treatments, respectively. Moreover, corms treated with CW_f, SW and CW_aSW had lateral shoots that also emerged earlier than those from W_d

and PIF, respectively. It is interesting to note that lateral shoot emergence for BAP was only 1.5 days earlier than for W_d and PIF (Figure 3.2). Lateral shoots from UTC had not emerged within the two weeks monitoring period. What was observed was the continuous growth of the intact apical meristem, which emerged 9 days after planting the corm.

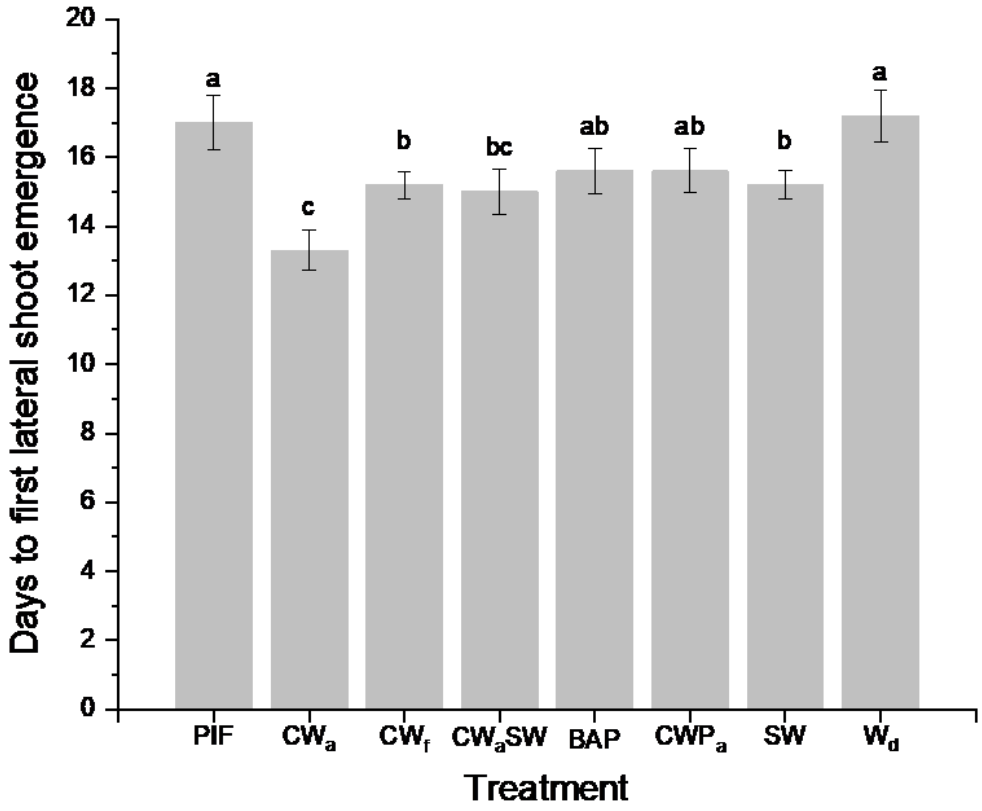

Figure 3.2. Effects of treatments (PIF - *Plant Issus de Fragments de tige*; CW_a - autoclaved coconut water; CW_f - fresh coconut water; CW_aSW - autoclaved coconut water with seaweed extract; BAP - 6-benzylaminopurine; CWP_a - coconut water with pulverized papain; SW - seaweed extract; W_d - distilled water) on number of days to first lateral shoot emergence of plantain corms, cv. 'Apantu'. Vertical bars indicate

standard error of the means (n=10) and different letters represent significant difference using LSD test at P≤0.05. Untreated control plants had no lateral shoot growth.

3.4.3 Number of lateral shoots.

Significant treatment differences were found for the number of lateral shoots after two weeks of corm sprouting (Figure 3.3). In general, the hormonally treated corms produced a greater number of lateral shoots in comparison to both W_d and PIF technique. For example, CW_a treated corms had 85% and 48% more laterals shoots than W_d and PIF corms, respectively (Figure 3.3). The number of lateral shoots obtained from BAP treated corms were 8.5% lower compared to those found on SW and CW_aSW treated corms. On average, there was only one lateral shoot that had emerged from the untreated corms (Figure 3.3).

There were significant treatment differences in the accumulated total number of shoots per corm that were harvested every two weeks (Figure 3.3). The highest number of shoots were produced in the BAP and CW_a treatments with averagely 38 shoots per corm, which were siginificantly higher than in any other treatment. Moreover, BAP and CW_a treated corms produced about 16% more shoots than PIF-treated corms, which in turn had 12% and 2.2-fold more shoots than W_d and UTC, respectively (Figure 3.3). The least number of shoots was produced by UTC corms, which was about 60% less compared to BAP and CW_a, respectively.

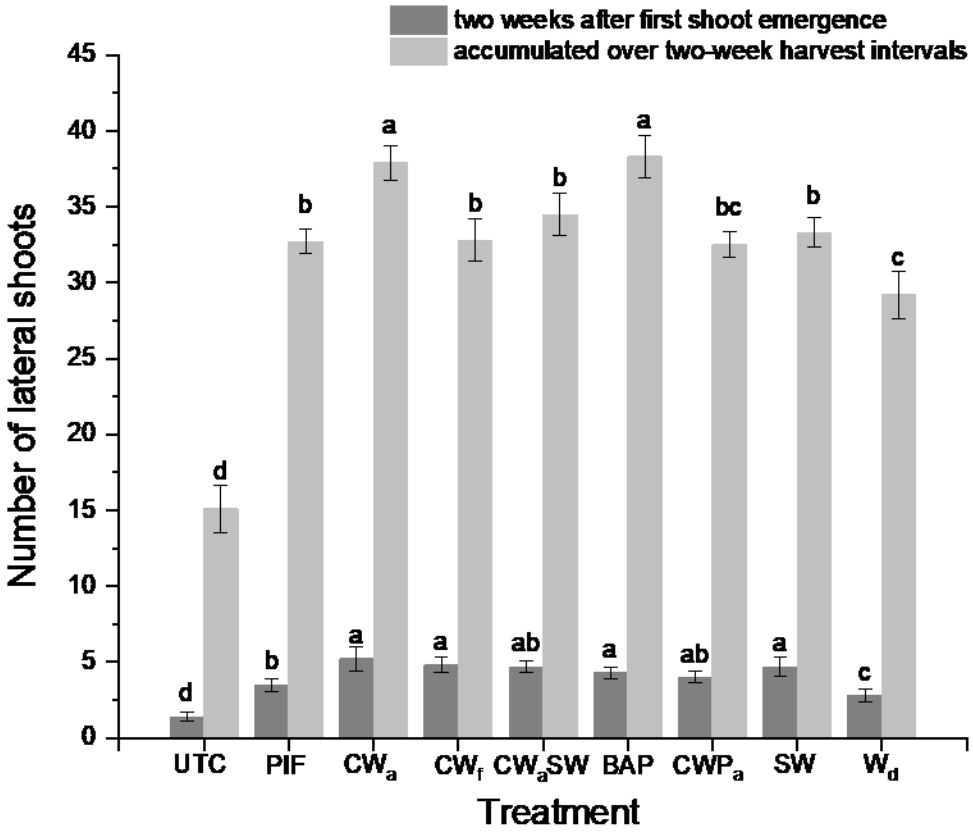

Figure 3.3. Effects of treatments (UTC - untreated control; PIF - *Plant Issus de Fragments de tige*; CW_a - autoclaved coconut water; CW_f - fresh coconut water; CW_aSW - autoclaved coconut water with seaweed extract; BAP - 6-benzylaminopurine; CWP_a - coconut water with pulverized papain; SW - seaweed extract; W_d - distilled water) on number of lateral shoots per corm of plantain, cv. 'Apantu', at two-weeks after first shoot emergence and accumulated over two-week harvest intervals until corms were exhausted. Vertical bars indicate standard error of the means (n=10) and different letters represent significant difference using LSD test at $P \leq 0.05$.

3.4.4 Growth parameters of lateral shoots at harvest.

The longest shoots were produced by both the CW_a and SW treated corms, which were about 9% longer than those from BAP and PIF treated corms, respectively, and 16% longer than the shortest shoots from the W_d treatment (Table 3.1). Interestingly, the UTC produced shoot length that were similar to those produced in the PIF and BAP treatments. Leaf number per shoot at harvest was also affected by treatment and ranged between three (UTC) and four leaves (all other treatments; Table 3.1). The UTC shoots had close to 20% stronger girth compared to the average shoot girth from corms treated with BAP and CW_a, which in turn was significantly greater than those from other hormonal or mechanical treatments (Table 3.1). Shoots from corms treated with CW_a had the highest number of roots (Table 3.1), which was 1.2- and 3.3-fold more than the number of roots on shoots from BAP treated and UTC corms, respectively. The PIF shoots had almost twice as many roots than those on shoots from the UTC and about 40% more roots than those on shoots from W_d corms. The average number of roots per shoot from corms treated with the natural plant hormones CW_f, CW_aSW, and SW, respectively, was about 22% higher compared to the PIF treatment (Table 3.1).

Table 3.1. Effects of treatments (UTC - untreated control; PIF - *Plant Issus de Fragments de tige*; CW_a - autoclaved coconut water; CW_f - fresh coconut water; CW_aSW - autoclaved coconut water with seaweed extract; BAP - 6-benzylaminopurine; CWP_a - coconut water with pulverized papain; SW - seaweed extract; W_d - distilled water) on growth parameters of lateral shoots of plantain, cv. 'Apantu', at harvest.

Treatment	Shoot length (cm)	Number of leaves per shoot	Shoot girth (cm)	Number of roots per shoot
UTC	25.7 ^{abc}	3.1 ^{c}	2.6 ^{a}	2.3 ^{f}
PIF	25.3 ^{bcd}	4.1 ^{ab}	1.9 ^{de}	4.3 ^{d}
CW_a	27.8 ^{a}	4.3 ^{ab}	2.1 ^{bc}	7.6 ^{a}
CW_f	24.1 ^{cd}	4.0 ^{ab}	1.7 ^{e}	5.6 ^{bc}
CW_aSW	24.9 ^{cd}	4.4 ^{a}	1.9 ^{de}	5.5 ^{bc}
BAP	25.3 ^{bcd}	4.2 ^{ab}	2.2 ^{b}	6.1 ^{b}
CWP_a	24.4 ^{cd}	4.2 ^{ab}	1.8 ^{de}	4.4 ^{d}
SW	27.4 ^{ab}	4.4 ^{a}	1.9 ^{cd}	5.3 ^{c}
W_d	23.3 ^{d}	3.9 ^{b}	1.7 ^{e}	3.1 ^{e}
LSD	2.2	0.4	0.2	0.7
P-value	***	***	***	***

Data are means (n=50). Different letters in the same column indicate significant difference at $p \leq 0.05$. *** significant at $p<0.001$.

3.5 Discussion

Several macropropagation approaches have been explored for harvesting numerous uniform plantain plantlets and for the purpose of easy adoption by smallholder farmers (Dzomeku et al., 2014; Kwa, 2003). Moreover, it was demonstrated in several experiments that the proliferation rate of plantain can be enhanced by the application of plant hormones (Kindimba and Msogoya, 2014; Langford et al., 2012; Msogoya and Mwakisitu, 2014; Osei, 2006).

Sprouting of *Musa* AAB cultivars typically commences two weeks after corm planting (Dzomeku et al., 2014; Kindimba and Msogoya, 2014) and this was also observed in the current study. However, corms treated with PIF and autoclaved coconut water had a significantly earlier shoot emergence but also a greater shoot proliferation compared to the sole PIF treatment, which is usually employed by smallholder farmers. The high number of lateral shoots per corm in both the CW_a and BAP treatment was likely due to the efficacy of CKs in promoting axillary bud growth (Shimizu-Sato et al., 2009; Agampodi and Jayawardena, 2009; Ma et al., 2008). Osei (2006) also found a significant improvement in the shoot proliferation of two plantain cultivars by injecting coconut water under field conditions. Moreover, adding coconut water to tissue culture media increased the shoot regeneration of Dwarf Cavendish explants (Mondal et al., 2012) and potato plantlet growth (Khawaj et al., 2015; Michael, 2011), indicating that commonly used synthetic plant growth regulators can be successfully substituted. Nevertheless, exploiting the potential of papain for enzyme-mediated CKs de-conjugation reactions did not lead to greater number of shoots per corm when compared to the CW_a treatment.

The increased shoot length in the CW_a treatment is in agreement with Buah and Agu-Asare (2014) and Gbadamosi and Sulaiman (2012), who observed positive effects of coconut water as supplementary in-vitro media component on shoot elongation of banana and *Irvingia gabonensis*, respectively. In agreement with the significant difference in leaf number per shoot between the UTC and the CW_a treatment, Souza et al. (2013) showed that coconut water as a constituent in tissue culture media positively affected the number of leaves per shoot in olive. The comparable effects of the CW_a and BAP treatments on shoot girth was also observed by Buah and Agu-Asare (2014) in banana in-vitro experiments.

Compared to the UTC and PIF treatment, the positively stimulated root growth by seaweed extract was also described by Sajith et al. (2014). They reported an enhanced bud regeneration and improved root development in field-grown banana by using a bio-fertilizer. The findings of Buah and Agu-Asare (2014), observing higher number of roots on Dwarf Cavendish banana grown in media with coconut water, were confirmed in the present study. These treatment effects might be due to the composition and concentration of plant hormones in seaweed extract and coconut water, respectively (Aloni et al., 2006; Yong et al., 2009). Indeed, adventitious root development of *Dracaena purplecompacta* L. was promoted by using indole-3-acetic acid (IAA) from coconut water extract (Agampodi and Jayawardena, 2009).

The macropropagated plantlets needed to be acclimated to field conditions, which was previously shown by Sajith et al.(2014) and Dayarani et al. (2013). Specifically, the robust and well-rooted plantlets from corms treated with CW_a or BAP survived prior to planting close to 100% at the acclimatization phase (data not shown). In contrast, only about 65-70% shoots with significantly fewer roots as for example those from PIF, W_d

or UTC corms could successfully adjusts to the change in its environment (data not shown). An increased mortality rate of rootless plantlets during acclimatization was earlier reported (Baiyeri and Aba, 2007).

3.6 Conclusions

In agreement with the stated hypothesis, the vacuum infiltration of plantain corms with coconut water was proven to be an effective method for macropropagation. Moreover, autoclaved rather than untreated coconut water had a greater potential for inducing multiple shoots. Consequently, smallholder plantain farmers could substitute the conventionally used plant growth regulator BAP with autoclaved coconut water for treating corms since both hormones produced a similar number of high-quality lateral shoots within the experimental period. For inducing clusters of new shoots, it is further recommended to not only destroy the apical meristem of the corm prior to planting but also that of each two-week old lateral shoot emerging from the mother corm. There is a great potential for rapid on-farm plantlet production through the application of corms with coconut water solutions, followed by treating those and subsequently emerging shoots with the PIF technique. Since coconut fruit is widely grown in the tropics and of little expense, the substitution of BAP with this plant-based hormonal solution should not lead to a greater production cost. This approach could be exploited by farmer group organizations for mass-propagation of robust planting material to boost production of plantain.

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4. Field performance of False Horn Plantain (*Musa* AAB) corms treated mechanically and with hormone solutions

4.1 Abstract

The growth and yield performance of macropropagated plantlets of the False Horn plantain cultivar ‘Apantu’ was evaluated due to the lack of reliable data on the effectiveness of that planting material under typical farming conditions in West Africa, Ghana. Corms were either mechanically treated by the *Plants Issus de Fragments de tige* (PIF) technique to destroy the apical meristem or remained intact as untreated controls. Subsequently, PIF-treated corms were vacuum infiltrated with either natural or synthetic plant hormone solutions. Emerging plantlets were harvested, acclimatized for three months and planted in a freshly prepared field. Vegetative growth characteristics of each mother (main) plant were taken at 6 and 9 months after planting and for the main and first sucker crop along with yield parameters at harvest, respectively. The results indicate that treatment induced growth differences at 6 and 9 months after planting and were no longer significant at harvest. Consequently, final growth performance was quite homogenous across all treatments for the main and sucker crop, respectively. In contrast, fruit yield parameters of the main and sucker crops were to some extent affected by treatment; however, hormone infiltration tended to have little additional effect over the PIF-treatment. Nevertheless, treating corms with hormonal solutions enhances the production of rooted plantlets at the nursery stage and ensures improved field performance.

Keywords
6-benzylaminopurine, bunch, coconut water, macropropagation, seaweed extract, sucker

4.2 Introduction

Plantain (*Musa* AAB) is an important food crop that is widely grown in the humid tropics of Africa, providing food for more than 70 million people in that region (Ortiz and Vuylsteke, 1994). The majority of plantain production comes from rural families' backyard gardens as well as cultivated fields of smallholders and is an essential income source for resource poor farmers in developing countries (Nkendah and Akyeampong, 2003; Ortiz and Vuylsteke, 1994). In West Africa, Ghana produces the largest plantain crop, which is of great socio-economic importance in terms of food security and job opportunity (Dzomeku et al., 2009). The demand for the 'cooking banana' is relatively high in both urban and rural communities and has resulted in an increased market price (Lescot and Ganry, 2010; Swennen, 1990; Tomekpe et al., 2011). Consequently, many households have gradually expanded the field cultivation of plantain (Swennen, 1990).

However, access to sufficient and inexpensive planting material by smallholder plantain farmers is a major constraint in Sub-Saharan Africa (Faturoti et al., 2002; Kasyoka et al., 2010; Lefranc et al., 2010). This is as a result of several factors, including low sucker production due to the apical dominance behaviour of the main pseudostem (Langford et al., 2017; Macias, 2001; Ortiz and Vuylsteke, 1994) and poor field management by smallholder farmers (Hauser, 2000; Tenkouano et al., 2006). Farmers often rely on young suckers or old corms directly from the field for the

establishment of new plantings; however, this plant material is frequently infested with pathogens or various pests (Kasyoka et al., 2010).

Therefore, several clonal propagation techniques have been developed to overcome the inadequate sucker production in plantain (Buah et al., 2010; Dzomeku et al., 2014; Kwa, 2003; Osei, 2006). An innovative micropropagation technique for raising plantlets is tissue culture (Buah et al., 2000; Drew and Smith, 1990; Niere et al., 2014; Osei, 1996; Vuylsteke and Ortiz, 1996); however, this procedure requires high initial investment costs for specialised laboratories. In contrast, a macropropagation technique that is now commonly employed by many smallholder plantain and banana farmers in Sub-Saharan Africa is the *Plants Issus de Fragments de tige* (PIF) treatment (Dzomeku et al., 2014; Kindimba and Msogoya, 2014; Kwa, 2003) that destroys the apical meristem with a crosswise incision and leads to robust and healthy plantlets at the nursery stage (Lefranc et al., 2010). This is especially the case, when pest (e.g. banana weevil and nematodes) infested suckers and corms are treated with hot water at 52˚C for 20 min prior to planting (Hauser et al., 2010; Hauser, 2000). Moreover, the PIF technique in combination with synthetic or natural plant growth regulators has increased the number of shoots per corm (Kindimba and Msogoya, 2014).

Despite several studies having demonstrated that large numbers of clean and healthy plantlets can be produced by macropropagation, there are only few data available on the performance of that planting material under typical farming conditions (Norgrove and Hauser, 2014). Therefore, the objective of this study was to assess the growth and yield performance of macropropagated plantlets. It is hypothesized, that suckers from corms manipulated with PIF and infiltrated with either natural or synthetic plant

hormones had a better growth and yield performance than those from only PIF-treated corms.

4.3 Materials and Methods

4.3.1 Plant material and treatments.

Macropropagation of plantain plantlets was carried out at the Crops Research Institute (CRI) in Kumasi, Ghana, in 2016. Sword and ratoon sucker corms of the False Horn plantain cultivar ‘Apantu’ were harvested from an experimental plantation at the CRI. The corms were pared by removing roots before all leaf sheaths around the collar were carefully cut-off with a sharp knife that was frequently sterilized in 70% ethanol to reduce microbial contamination of the corms. The corms were then washed under running tap water to remove any foreign material. Air-dried corms were either mechanically manipulated by employing the PIF-technique or remained unchanged as an untreated control. PIF-treated corms were vacuum infiltrated with various aqueous solutions (Table 4.1) and planted in sterilized sawdust within a germination bed. Emerging plantlets were harvested and potted in 3-liter polyethylene bags, using soil that was sterilized with hot water steam for 3 hours. Potted plantlets were then acclimatized for three months under a shade structure constructed from palm fronds.

Table 4.1. Aqueous solutions used for vacuum infiltration of corms that were mechanically manipulated with the PIF-technique.

Treatment	Complete description of treatment
1. CW_f	fresh coconut water
2. CW_a	autoclaved coconut water at 121^oC for 15 min
3. CWP_a	coconut water with 0.1% (w/v) pulverized papain, incubated at 40^oC for 40 min and stirred every 10 min for 1 min and then autoclaved at 121^oC for 15 min
4. CW_aSW	autoclaved coconut water with 0.5% (v/v) of sea weed extract (Tecamin Raiz, AgriTecno, Spain)
5. SW	0.5% (v/v) sea weed extract dissolved in distilled water
6. BAP	2.25 mg of 6-Benzylaminopurine (Carl Roth, GmbH, Germany), dissolved in 1N NaOH and diluted in 1 L of distilled water
7. W_d	distilled water

4.3.2 Experimental site.

A fallow that was not cultivated for about 10 years was selected for the field experiment at the CRI. Land preparation included clearing the sandy-loam (Arenosol) site from woody vegetation and mulching with the cuttings to improve soil fertility and water holding capacity (Swennen, 1990; Tswanya et al., 2017). This type of land preparation is often recommended instead of the slash and burn approach (Giardina et al., 2000; Smil, 1999). Acclimatized plants, each about 40 cm tall with 3-5 leaves, were selected from the nursery and planted in holes of 30 cm depth and 30 cm diameter at a spacing of 3 x 2m in the middle of the rainy season in July 2016 (Swennen, 1990; Dzomeku et al., 2012). Each hole received 250 g well-decomposed poultry manure one week prior

to planting. The experimental site was fertilized in three split applications at 3, 6 and 9 months after planting with 170 kg urea (46% N), 192 kg potassium chloride (approx. 50% K) and 42 kg triple super phosphate (46% P_2O_5) per hectare, respectively. Moreover, each plant received approximately 2 kg of cow dung prior to flowering. Weed control was carried out regularly either manually or by herbicide applications to ensure good plant growth and development. De-suckering of the main crop was performed at harvest to maintain two sword suckers and one ratoon sucker per plant mat.

4.3.3 Experimental design and data collection.

The experimental set-up consisted of nine treatments each with 5-plant-plots assigned randomly to three replicated blocks, respectively. Vegetative growth parameters were taken for each mother (main) plant at 6 and 9 months after planting, respectively, and at harvest and of the first sucker crop (ratoon) at harvest: pseudostem girth at 50 cm above the soil line, pseudostem height from girth measuring point to the most recent unfolded upper leaf, and number of unfolded green leaves. Dead yellow-brownish leaves and leaves with more than 50% black sigatoka disease symptoms at the leaf surface were excluded. In addition, number of suckers were recorded for each plant mat at harvest of the main and sucker crop, respectively.

Crop phenology of both main and first sucker plants was evaluated by recording the number of months to flowering after planting and also days to fruit maturity from flowering. Moreover, yield performance was assessed by taking records of bunch weight, number of hands and fingers per bunch for each plant. Fruit maturity for the main plant started in September 2017 and that of the sucker plants in October 2018.

4.3.4 Statistical analysis.

Analysis of variance (ANOVA) was used to evaluate the effect of mechanically manipulated and with varying aqueous solutions vacuum-infiltrated False Horn corms on pseudostem characteristics and yield performance. Data were analysed using Genstat (18th Edition Rothamsted, United Kingdom) and displayed graphically with Microsoft Excel.

4.4 Results

4.4.1 Crop phenology.

The timing of flowering after planting was not affected by the treatments and occurred between 13 and 15 months for the main crop and about 24 months for the sucker crop. Fruit maturity was reached for both crops between 90 and 105 days after flowering, irrespective of treatment.

4.4.2 Growth characteristics of the main crop.

There was a significant treatment effect on pseudostem height at 6 and 9 months of plant growth in the field. Pseudostem height at 6 months after planting was on average 111 cm for CW_f treated corms, followed by corms treated with BAP, which were 21% and 12.5% taller than those of PIF-treated corms (Table 4.2). At 9 months after planting, the highest pseudostems with about 158 cm were found by corms treated with SW and CW_f, which were about 11% longer than those of corms treated with BAP

and the PIF-technique (Table 4.2). In contrast, there were no effects found at fruit harvest and average pseudostem height was at 2.9 m across all treatments.

There were no significant treatment effects on pseudostem girth at both growth stages. However, pseudostem girth tended to be with 29 cm in the CW_f and with 43 cm in the SW treatment greater than in other treatments at 6 and 9 months after planting, respectively. At harvest, all treatments had a pseudostem girth of about 50.6 cm.

There were on average 8 and 10 green leaves per pseudostem at 6 and 9 months after planting with only small differences among the treatments (Table 4.2). CW_aSW produced with about 12 leaves per pseudostem significantly more leaves than any other treatment at 9 months of growth. At harvest, treatments had averagely 2 leaves per plant. The number of young suckers growing at harvest was not significantly affected by the treatment (Figure 4.1) and ranged between 3 and 4 shoots within the mat of the main crop.

Table 4.2. Effects of treatment (UTC - untreated control; PIF - Plant Issus de Fragments de tige; CW_a - autoclaved coconut water; CW_f - fresh coconut water; CW_aSW - autoclaved coconut water with seaweed extract; BAP – 6-benzylaminopurine; CWP_a - coconut water with pulverized papain; SW - seaweed extract; W_d - distilled water) on vegetative growth characteristics of main plant pseudostem of False Horn plantain.

Treatment	6 month		9 month	
	Length (cm)	Leaf number	Length (cm)	Leaf number
UTC	84.5 **a**	8.1 **abc**	127.1 **ab**	9.9 **ab**
PIF	87.5 **a**	8.2 **abc**	137.7 **abc**	10.5 **abc**
CW_a	87.3 **a**	7.3 **a**	119.6 **a**	9.5 **a**
CW_f	110.6 **b**	8.8 **c**	156.8 **c**	10.9 **bcd**
CW_aSW	99.2 **ab**	8.3 **bc**	152.3 **bc**	11.9 **d**
BAP	100.0 **ab**	7.5 **ab**	142.1 **abc**	9.9 **ab**
CWP_a	89.5 **a**	7.2 **a**	130.1 **ab**	9.6 **ab**
SW	96.0 **ab**	8.3 **bc**	159.0 **c**	11.7 **cd**
W_d	88.3 **a**	7.9 **abc**	141.7 **abc**	9.4 **a**
Statistics				
P-value	*	*	*	**
$LSD_{0,05}$	16.1	1.1	25.6	1.4

Data are means (n=15) with different letters in the same column indicating significant difference at $p \leq 0.05$. * and ** are significant at $p \leq 0.05$ and $p<0.001$, respectively.

4.4.3 Growth characteristics of the sucker crop.

The pseudostem height and girth of the first sucker crop at harvest were not significantly affected by treatment. Plant height ranged between 3.2 m and 3.4 m across all treatments with an average girth of 63 cm. There were 1-2 green leaves per plant at harvest. The number of young suckers grown within the mat of the first sucker crop at harvest was significantly affected by the treatment. BAP and SW had with averagely 3.3 suckers the highest number of all treatments (Figure 4.1).

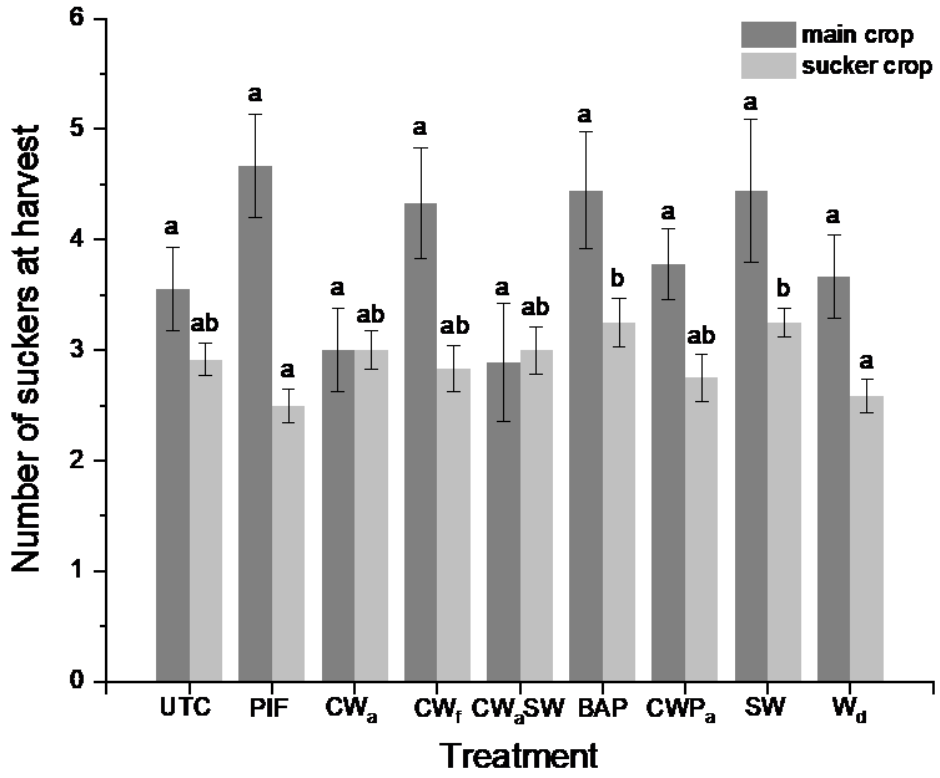

Figure 4.1. Effects of treatment (UTC - untreated control; PIF - Plant Issus de Fragments de tige; CWa - autoclaved coconut water; CWf - fresh coconut water; CWaSW - autoclaved coconut water with seaweed extract; BAP – 6-benzylaminopurine; CWPa - coconut water with pulverized papain; SW - seaweed

extract; Wd - distilled water) on sucker production of the main and sucker crop, respectively, at harvest of False Horn plantain. Vertical bars indicate standard error of the means (main crop, n= 9 and sucker crop, n=12)

4.4.4 Yield performance of main and sucker crop.

Bunch weight of the main crop was significantly affected by treatment with the heaviest bunches of about 11 kg harvested of corms treated with BAP and SW (Table 4.3). Control plants (W_d) produced the smallest bunches with 9.5 kg. In contrast, bunch weight of the first sucker crop was similar across the treatments, yielding about 11 kg (Table 4.3).

There were no treatment effects on yield parameters of the main crop with averagely 6.4 hands per bunch and 29.6 fingers per bunch (Table 4.3). However, these yield parameters were significantly affected by treatment for the first sucker crop. Plants from corms treated with SW produced with 6.9 hands per bunch and 30.9 fingers per bunch the highest numbers, but in both cases was not significantly different from the PIF, BAP and CW_f treatments, respectively (Table 4.3).

Table 4.3. Effects of treatments (UTC - untreated control; PIF - Plant Issus de Fragments de tige; CW_a - autoclaved coconut water; CW_f - fresh coconut water; CW_aSW - autoclaved coconut water with seaweed extract; BAP – 6-benzylaminopurine; CWP_a - coconut water with pulverized papain; SW - seaweed extract; W_d - distilled water) on yield characteristics of main and sucker crops, respectively, of False Horn plantain.

Treatment	Yield (kg/bunch)		Hands per bunch		Fingers per bunch	
	Main Crop	Sucker Crop	Main Crop	Sucker Crop	Main Crop	Sucker Crop
UTC	10.7 ^{bc}	10.9 ^{a}	6.3 ^{a}	6.3 ^{a}	29.3 ^{a}	28.9 ^{ab}
PIF	10.4 ^{bc}	11.0 ^{a}	6.4 ^{a}	6.4 ^{abc}	28.1 ^{a}	29.7 ^{abc}
CW_a	10.7 ^{bc}	11.0 ^{a}	6.6 ^{a}	6.4 ^{abc}	29.4 ^{a}	28.9 ^{ab}
CW_f	10.7 ^{bc}	11.1 ^{a}	6.6 ^{a}	6.7 ^{abc}	32.1 ^{a}	30.5 ^{c}
CW_aSW	10.0 ^{ab}	10.8 ^{a}	6.3 ^{a}	6.3 ^{ab}	29.6 ^{a}	28.8 ^{ab}
BAP	11.3 ^{c}	11.0 ^{a}	6.6 ^{a}	6.8 ^{bc}	28.1 ^{a}	30.0 ^{bc}
CWP_a	9.8 ^{ab}	10.9 ^{a}	6.4 ^{a}	6.2 ^{a}	30.2 ^{a}	28.2 ^{a}
SW	11.0 ^{c}	11.4 ^{a}	6.1 ^{a}	6.9 ^{c}	29.6 ^{a}	30.9 ^{c}
W_d	9.4 ^{a}	10.8 ^{a}	6.4 ^{a}	6.2 ^{a}	29.1 ^{a}	28.3 ^{a}
Statistics						
P-value	*	ns	ns	*	ns	*
$LSD_{0.05}$	0.9	0.4	0.6	0.5	3.1	1.5

Data are means (main crop n=9; sucker crop n=12) with different letters in the same column indicating significant difference at $p \leq 0.05$. * is significant at $p \leq 0.05$; ns: not significant.

4.5 Discussion

Growth and yield performance of plantain plants derived from *Plants Issus de Fragments de tige*, which is currently and widely employed by smallholder plantain farmers in West and Central Africa, has not been intensively studied. In contrast, this technique in combination with hormone applications, has been thoroughly evaluated for its potential on the rate of shoot proliferation. However, smallholder farmers are aiming to achieve high yields for securing their livelihood through product sales and thus income generation. Consequently, this study investigated the effect of plantain corms treated with PIF and hormone solutions on the growth and yield performance of both the main crop and the first sucker crop.

The lack of treatment effect on the number of suckers of the main crop might be due to all plantlets having developed sufficient roots during the nursery and acclimatization phase (Coyne et al., 2010). It has been suggested by Smith et al. (2001) that vigorous growth and high suckering ability is the results of a strong root system rather than carry-over effect from corms treated with growth hormones. Moreover, the main crop from corms treated with the PIF-technique and hormone solutions, particularly CW_f, had longer psuedostems than those that were only mechanically manipulated. This has also been described for in-vitro plantain plants, which were significantly taller when treated with growth hormones (Vuylsteke and Ortiz, 1996). The generally stronger growth of the first sucker crop compared to the main crop was also reported by (Blomme et al., 2002) and might be attributable to the application of manure (Eghball et al., 2004) in combination with the regular fertilizer.

The plantain cultivar used in the current trial is a landrace, which is susceptible to black Sigatoka, a fungal disease caused by *Mycosphaerella fijiensis* (Dzomeku et al., 2006).

Ferris et al. (1999) reported that all landrace plantain cultivars are susceptible to the black Sigatoka disease with detrimental effects on leaf production (Vuylsteke and Ortiz, 1996). In the current study little to none leaf pruning was employed to control the disease.

The time of flowering and fruit maturity of the cultivar ‘Apantu’ could not be altered with hormone and fertilizer applications. Nevertheless, the slightly longer time to flowering after planting of the main crop from PIF-treated corms was reported earlier by Mekoa and Hauser (2010). The average bunch weights are comparable to those reported in other studies with the same cultivar (Dzomeku et al., 2016; Shaibu et al., 2012). However, the sucker crop generally recorded heavier bunch weights than the main crop. Similar findings were reported by Obiefuna (1986), demonstrating bigger bunches in the ratoon crops than their respective mother crops. In summary, it should be emphasized that good management practices of plantain fields are imperative for attaining high yield (Shaibu et al., 2012).

4.6 Conclusion

The study demonstrated that both main and sucker plantain crops from hormonally treated corms did not significantly perform better than those from only PIF-treated corms in most of the growth and yield parameters. However, yield performance also depends on good agronomic practices in the field. In contrast, treating corms with growth hormones is highly relevant at the propagation stage to raise robust plantlets with high numbers of roots for improving their field performance. Moreover, the PIF-

technique is a reliable low cost option for smallholder farmers to enhance the proliferation rate of the source material in the nursery.

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5. General discussion

5.1 Improved macropropagation technique in banana and plantain

Lack of adequate and healthy planting material by smallholder farmers is a major constraint to the expansion of banana and plantain production (Tomekpe et al., 2011). Cultivated bananas and plantains are triploid and do not produce viable seeds (Ortiz and Vuylsteke, 1994). Therefore, propagation is mainly by vegetative means. Farmers often rely on growing suckers from the mat as well as old corms, which are mostly infested with pest and diseases for the establishment of new fields and also for the expansion of existing plantations (Njukwe et al., 2013). The resulting outcome of using such unhealthy planting material is poor plant stand and thus low yields. The mother plant has a low sucker regeneration ability producing between 5-10 suckers within a year after planting (Rahman et al., 2004; Vora and Jasrai, 2012). This is mainly due to apical dominance which is mediated by plant growth hormone (Ortiz and Vuylsteke, 1994; Singh et al., 2011), essentially, auxin, which suppresses the outgrowth of axillary buds (Blomme et al., 2002; Müller and Leyser, 2011). The typical mother plant of banana and plantain corm holds many potential buds which could grow into healthy suckers (Swennen and Ortiz, 1997), in the absence of apical dominance and provision of appropriate growth conditions (Dzomeku et al., 2014).

The technique carried out in the present study employed a mechanical approach to release latent buds which were under the influence of apical dominance. There were satisfactorily many latent buds sprouting into shoots even without the application of growth hormones due to the destruction of the apical meristem using the crosswise incisions. This is in line with work carried out by Dzomeku et al. (2014) where different

plantain cultivars were subjected to meristem destruction to release latent buds from apical dominance. The present study demonstrates that mechanical manipulation of corms particularly the meristem hastens the sprouting of latent buds into lateral shoots which is very important for rapid field multiplication of banana and plantain. Sprouting of most *Musa* AAB cultivars, which typically commences after two weeks of planting the corms in sawdust, was confirmed in the present study and in agreement with findings by Dzomeku et al. (2014) and Kindimba and Msogoya (2014)**.** The application of the mechanical manipulation of corms stemmed from earlier research efforts by the African Research Centre on Banana and Plantain (CARBAP) and the International Institute of Tropical Agriculture (IITA). This was in search for a reliable and inexpensive macropropagation techniques for the multiplication of banana and plantain for smallholder banana and plantain growers particularly in West and Central Africa (Tomekpe et al., 2011). More importantly, the approach since its inception has additionally received some research attempts to enhance the proliferation potential through the introduction of plant growth hormones (Kindimba and Msogoya, 2014; Langford et al., 2017).

5.2 Impact of growth hormones on the proliferation potential of banana and plantain corms

Plant growth hormones play significant role in shoot and root proliferation in plants including banana and plantains. Synthetic plant hormone specifically 6-benzylaminopurine in various concentrations has been widely used to induce shoot growth in banana and plantain (Kindimba and Msogoya, 2014; Langford et al., 2017; Osei, 2006). The combination of BAP with bio-fertilizers as additives and its effect on

shoot proliferation in banana has also been researched on (Sajith et al., 2014). Furthermore, there has been exploitation of various plant biostimulants on plant growth and development (Calvo et al., 2014). The application of cytokinins to plants externally for effective uptake and metabolism is quite complex (Kamínek, 1992).

The synthesis of cytokinin is mainly in the root and is transported mainly through the xylem into the aerial plants parts which may be involved in the shoot and root development (Aloni et al., 2005; Haberer and Kieber, 2002; Müller and Leyser, 2011).

The current study employed solutions of natural and synthetic plant hormones, seaweed and papain in treating mechanically manipulated banana and plantain corm to induce multiple shoots. Moreover, the study investigated the impact of solution uptake on corms from various hormonal application methods, thus submergence and infiltration. The submergence or soaking has been a common practice carried out by most smallholder farmers for hormonal treatment application on banana and plantain corms (Dayarani et al., 2013; Msogoya and Mwakisitu, 2014). In spite of its wide usage, there are some obvious challenges to the approach as it practically makes use of large volumes of hormonal solution in order to ensure complete submergence of corms. Moreover, the practice also requires long soaking duration to enhance the uptake of the hormonal solution. Seed priming technique similar to the soaking method has also been employed to enhance sprouting of seeds and plant growth in many crops through the addition of nutrients and other growth hormones (Ajouri et al., 2004; Sedghi et al., 2010).

The vacuum infiltration method employed in the present study revealed a higher solution uptake when compared with the method of soaking. It was observed further in the present trial that infiltration could go as higher as 33% more solution uptake than

the method of soaking. Furthermore, the infiltration process requires less treatment time (10-12 min), unlike the soaking method which usually requires 12 hr long of submergence for effective solution uptake (Kindimba and Msogoya, 2014; Langford et al., 2017; Msogoya and Mwakisitu, 2014). Infiltration as a hormonal application method, additionally, contributed to the amount of shoot regeneration from the banana corms. Corms subjected to vacuum infiltration resulted in 16% more shoot than the soaking method. Despite a positive effect of infiltration on shoot proliferation, the combined effect of infiltration and the various concentrations of 6-benzylaminopurine employed in shoot regeneration was not significantly observed.

In spite of a higher solution uptake with higher shoot numbers under infiltration, rapid corm decay was observed. This could be attributable to the vacuum pressure employed having adverse effect on the tissue of the corms. However, the general higher shoot numbers on vacuum infiltrated corms could have further contributed to the accelerated corm decay.

Ntamwira et al. (2017) reported of higher number of plantlets growth on corm, resulting in the consumption of the limited corms reserves and a further accelerated corm decay.

BAP is commonly used in the multiplication of banana and plantain to induce proliferation of shoots (Singh et al., 2011). However, BAP which is a synthetic plant hormone is quite expensive for the acquisition by smallholder farmers in developing countries (Buah and Agu-Asare, 2014)**.** Therefore, under the current study an attempt was made to assess the effectiveness of two main concentrations of benzylaminopurine, a high (225.25 mg L^{-1}) and a low concentration (2.25 mg L^{-1}) compared to a water control**.** The findings demonstrated that shoot production was independent of the various concentrations of BAP employed in the present study.

Nonetheless, these two concentrations significantly resulted in an increased in multiple shoot in banana corms compared to the water control treatment. Similar findings in terms of concentration effect has also been reported by some authors demonstrating that, higher concentrations did not enhance higher shoot and root proliferation (Kindimba and Msogoya, 2014). Moreover, there has been previous report by Gübbük and Pekmezci (2004) demonstrating the adverse effect of higher cytokinin on shoot growth in banana. Higher abnormality in shoot regeneration of banana and plantain with TDZ and BAP has also been reported by Shirani et al. (2009). Tissue culture media supplemented with various concentration of BAP showed that a higher concentration (33 µM) resulted in an increased abnormal shoot formation in banana (Najmeh et al., 2011). In a sharp contrast to these findings, a higher shoot proliferation with higher concentration of cytokinin has been reported by Thiemele et al. (2015) in the multiplication of various cultivars of plantains employing macropropagation technique. The rate of shoot proliferation in banana and plantain cultivars in response to BAP has been attributed to the genetic variability as well as the amount of auxins and cytokinins content in the plant tissue (Arinaitwe et al., 2000). Sucker production tends to increase with a corresponding decrease in ploidy levels and in particular with increase in *M. balbisiana* genomic group within the genomic constitution of the particular clone (Bhende and Kurien, 2015).

The application of the two major concentrations of BAP in the current study triggered more root formation than the control treatment. However, the impact of BAP on root formation between the two BAP concentrations were also not observed. It was presumed that the high application of BAP concentration may have resulted in high cytokinin content in the banana corms to such an extent of creating a low auxin to cytokinin ratio prerequisite for root formation (Kamínek, 1992). In culture medium, a

high auxin to cytokinin ratio triggers the formation of root (Haberer and Kieber, 2002; Kamínek, 1992). Importantly, the ratio of these two hormones is very critical in the formation of shoots and roots rather than their absolute amount (Haberer and Kieber, 2002). In the proliferation of plantain, media supplemented with both auxin (NAA) and cytokinin (BAP) at optimum concentrations resulted in high bud formation (Feyisola et al., 2015). This has further been demonstrated in the proliferation of banana and plantain applying various concentrations of cytokinin and auxin with optimum amounts to trigger shoot and root formation in in-vitro culture (Buah et al., 2010; Najmeh et al., 2011; Ngomuo et al., 2013).

Exploiting plant growth hormones derived directly from plant based in the propagation of plants has also been widely investigated (Agampodi and Jayawardena, 2009; Khawaj et al., 2015). Prominent among these plants which have been identified to contain various plant hormones relevant for in-vitro propagation is coconut (*Cocos nucifera*) water. Various approaches have been employed from a number of researchers to identify and quantify the various growth hormones and their respective amounts in coconut water (Ge et al., 2004; Ma et al., 2008; Tan et al., 2014). Apart from phytohormones in coconut water, there are other growth promoting substances in coconut water such as vitamins, organic acids, nitrogenous compounds, amino acids etc., (Yong et al., 2009), which are requisite elements especially in tissue culture media. The current study demonstrated the effectiveness of various coconut water treatments with regard to days of shoot emergence, quantity of shoots and roots proliferation.

Early shoot emergence in banana and plantain is very important as this facilitate rapid shoot multiplication on-farm for smallholder farmers. Therefore, an approach to induce

rapid shoot multiplication in banana and plantain is very imperative. Under the influence of autoclaved coconut water in the present study, an earlier shoot emergence in treated plantain corms was found compared to the other hormonal treatments. Mondal et al. (2012) found a similar trend with higher frequency of shoot regeneration of Dwarf Cavendish explants in growing media supplemented with coconut water.

Coconut water subjected to autoclaving proved to be very effective in shoot proliferation when plantain corms were treated with it. Similar finding has been demonstrated by Osei (2006) with a higher production of young plantain suckers in the field. Mintah et al. (2018) revealed that treating plantain with trans-zeatin riboside (T-ZR) and indole-3-acetic acid (IAA) present in coconut water has a high potential in the initiation of axillary bud, growth and development of plantain plantlets. The shoot multiplication potential of autoclaved coconut water in comparison to the synthetic growth hormone, 6-benzylaminopurine under the current study did not show significant treatment effect. In an in-vitro study to proliferate banana shoots Buah and Agu-Asare (2014) observed almost equal performance between media supplemented with coconut water and BAP respectively. This therefore demonstrated the effectiveness of coconut water as a suitable low cost alternative for the treatment and proliferation of suckers in plantain and banana. Despite the significance of the growth promoting properties of coconut water in various studies, there has also been report of negative effect of coconut water on plant growth. Baque et al. (2011) observed a higher concentration of coconut water resulted in a decreased in growth and other morphological features with further abnormal growth characteristics of *Calanthe* hybrids.

In macropropagation of plantains and bananas, morphological attributes such as leaf number, shoot girth and more importantly the amount of roots are very critical for the survival of the plants under the acclimatization stage (Baiyeri, 2005). Young harvested shoots with robust stems and roots have a higher survival rate under favourable acclimatization conditions (Baiyeri and Aba, 2007). Rootless plantlets which usually occur during macropropagation often results to high mortality rate during acclimatization (Baiyeri and Aba, 2007). Higher root amounts were characterized by shoots derived from plantain corms treated with autoclaved coconut water. Phytohormones mainly auxin and cytokinin found in coconut water (Mintah et al., 2018; Yong et al., 2009) might have contributed to the greater amounts of roots in the current study. These roots amounts ensured better survival at the acclimatization phase. Elsewhere, higher amount of roots has been reported in in-vitro propagation of banana and other crops with media supplemented with coconut water compared to synthetic growth hormone, BA (Buah and Agu-Asare, 2014; Gbadamosi and Sulaiman, 2012).

It has been recommended that cultural and other relevant practices at both the propagation and the nursery stages that enhance root growth and development should also be pursued (Baiyeri and Aba, 2007). This in a way could ensure plantlets survival at the nursery stage and further improve survival ability of newly plantlets when planted out in the field.

Coconut water treatment prior to its application is very relevant. The use of coconut water for propagation from various studies showed that coconut water was initially subjected to some form of heat treatment prior to its application. This was either achieved through boiling for some minutes (Mintah et al., 2018; Osei, 2006) or added as a media supplement and autoclaved together (Buah and Agu-Asare, 2014; Mondal

et al., 2012). This in a way might have resulted in either a more active form of the phytohormones or the released of hormonal conjugates especially those of cytokinin in endogenous coconut water. The influence of the untreated coconut water on plantain corms at the multiplication stage was not realized, thus, shoot proliferation was not as high as compared to the treated (autoclaved) coconut water. This outcome was presumably due to proteins and fats in the coconut water which were not precipitated prior to its application. Aside the use of natural plant hormones derived from coconut water, there has also been considerable exploration of other sources of plant based hormones and biostimulants with their exploitation in the propagation of elite and economic plants including banana and plantain (Khan et al., 2009; Sajith et al., 2014; Stirk and Van Staden, 1997). Seaweed extract is one of those and has extensively being employed in manipulating plants to achieve desirable characteristics (Arthur et al., 2003; Crouch and Van Staden, 1992; Sajith et al., 2014). The use of seaweed extract and its impact on the current trial, specifically at the propagation phase did not exhibit significance treatment response of the corms in terms of shoot proliferation. Nonetheless, the application of seaweed extract resulted in more root growth than the untreated control and PIF treatments respectively. Sajith et al. (2014) observed enhanced bud regeneration and improved root development in banana through bio-fertilizer applications on corm. The effect of the addition of papain to coconut water as a proteolytic enzyme to stimulate denaturing of proteins (Amri and Mamboya, 2012) in coconut water to a large extent was also not achieved under the present study. Again, it was anticipated to see similar effect as that of autoclaved coconut water with regard to shoot and root formation. The PIF technique which involves the mechanical preparation of the corm and further destroying the apical meristem with crosswise incisions to breakdown apical dominance resulting in axillary buds' growth into lateral

shoots showed similar results to those of seaweed extract and coconut water combined with papain in the present study. The PIF techniques has been demonstrated to induce bud proliferation under favourable conditions of temperature and moisture in the absence of growth hormone (Kwa, 2003).

5.3 Growth and yield performances of hormonally derived macropropagated plants in the field

There has been a couple of researches on appropriate techniques of improved macropropagation of banana and plantain especially in Sub-Saharan Africa (Dzomeku et al., 2014; Kwa, 2003; Osei, 2006). This to a large extent has recorded some relevant outcomes in the context of enhancing banana and plantain cultivation in Sub-Saharan Africa (Tomekpe et al., 2011). Despite the significance investment into these appropriate multiplication techniques there has been no concrete follow-up of field performance of these outcomes. The present research focus on the multiplication potential of improved macropropagation technique with subsequent assessment of growth performance of the derived plantlets in the field. Lefranc et al. (2010) reiterated that macropropagated plantlets raised through the PIF technique are very robust, however, their performance in the field is lacking. Subjecting PIF technique derived plantlets and plantlets obtained from PIF treated corms with hormones in the field revealed that treatment performances were same. Most of the vegetative growth performance for both the main and sucker crop across all treatment were all the same. Nonetheless, the present study revealed that coconut water treated corms of the PIF technique had longer pseudostems than those that were only mechanically treated. Similar results has been described for in-vitro plantain plants which were significantly

taller when treated with growth hormones (Vuylsteke and Ortiz, 1996). Large numbers of sucker production are very important for smallholder growers as they rely on these new suckers for further multiplication (Bhende and Kurien, 2015). In the present study, there were attempts to evaluate carry-over effect of applied hormones on shoots derived from the treated corms in terms of sucker proliferation at fruit harvest in the field. However, there was a lack of treatment effect on sucker production particularly with the main crop in the field. This might possible be due to sufficient root development of all plantlets from the various treatments during the nursery and the acclimatization phase (Coyne et al., 2010). It has been suggested that vigorous growth and high suckering ability is the results of a strong root system rather than carry-over effect from corms treated with growth hormones (Smith et al., 2001). Moreover, external factors such as planting season, planting depth and spacing as well as good management practices especially proper nutrient application significantly contribute to sucker production (Bhende and Kurien, 2015). Proper nutrient and weed management practices were timely carried out in the present study during plants growth in the field which obviously contributed to good growth performances across all the treatments. It appears that for higher sucker production particularly with the cultivar employed in the present study, a particular physiological stage of growth is very critical for external response to growth hormones to induce more suckers as demonstrated by Osei (2006) and Mintah et al. (2018). That notwithstanding, the first sucker crop at harvest exhibited higher sucker production at harvest particularly with plants derived from the synthetic plant hormone, benzylaminopurine (BAP) and seaweed extract. Vigorous growth in terms of height and corm size in ratoon plants compared to main crop has earlier been reported by Blomme et al. (2002).

The time of flowering and fruit maturity of the False Horn cultivar ‘Apantu’ could not be altered with plant growth hormones and fertilizer application in the present study. However, the slightly longer time to flowering after planting of the main crop from PIF-treated corms was reported earlier by Mekoa and Hauser (2010). Nonetheless, there was a short time interval among the treatments with respect to flowering, thus, contributing to uniform harvesting. This in a way demonstrates the significance of uniformity of seedling generated at the nursery stage which is very important to most farmers when it comes to large scale production of the crop.

The yield response in terms of average bunch weights for both the main crop and the first sucker crop with the various treatment were reasonably good and similar to those that have been reported in other studies with the same cultivar (Dzomeku et al., 2016; Shaibu et al., 2012; Vuylsteke et al., 1996). Moreover, the ratoon crops generally recorded heavier bunch weight than their respective plant crops. Similar findings were reported by Obiefuna (1986), demonstrating bigger bunches in the ratoon crops than their respective plant crops, however, this was attributed to the time of planting of the crops. Drew and Smith (1990) reported that superior performance particularly micropropagated banana is mostly attributable to seedlings which have active roots and shoot system at the time of planting and additionally free from pests and diseases. It is obvious from the current study that the propagation technique contributed to high performance among treatments which stemmed from uniformity of seedlings at the acclimatization phase with well-developed roots systems and active leaves prior to transplanting in the main field. In general, the well-developed root systems ensure that plants were able to explore water and nutrient in the soil to further inure to variable field conditions. Additionally, it must be emphasized that good management practices (fertilizer application, weed management, insect and disease control etc.) which were

observed throughout the study might have also contributed to the general growth and yield performance in the field. General emphasis has been placed on good management practices in plantain cultivation as a prerequisite to attaining higher yields (Shaibu et al., 2012).

5.4 General Recommendations

A huge potential exists for timely raising of adequate, uniform and affordable banana and plantain seedlings for the establishment of productive plantations by smallholder farmers. This could be achieved by subjecting the PIF treated corms to hormonal treatment. The use of plant based hormone solutions, naturally derived, particularly from coconut water, subjected to autoclaving could induce more uniform shoots with numerous roots on treated corms by vacuum infiltration. Lower concentration of 6-benzylaminopurine at 2.25 mg L^{-1} could be used to treat banana corms triggering reasonable amount of shoot and root proliferation instead of higher concentrations. The infiltration method of treating banana corms contributed to higher solution uptake by the corms when compared to the soaking method. Limited time is required to treat many corms with the infiltration process with less hormonal solution.

Despite the significance of the infiltration technique, it is recommended that various vacuum pressures above 40kPa should be employed to avoid adverse effect on the corms. Vacuum pressure of 40kPa enhanced solution uptake which subsequently resulted in more shoot proliferation, however, it appears to have some adverse effect on corm tissues. Treated corms did not stay in the germination bed for long compared to the control and the soaked corm treatments. We further recommend that the

infiltration technique would be more appropriate if it is applied on harvested mother corms which have more woody tissue compared to young sucker corms

Moreover, attainment of good yield for both main and sucker crops in plantain could be achieved by observing good farm management practices. This underscores earlier recommendations by Shaibu et al.(2012) that good farm management is prerequisite for achieving higher yields under plantain cultivation.

5.5 Conclusions and outlook

It is important to state that macropropagation of banana and plantain using the PIF technique still serves as an important approach to raising plantlets for smallholder farmers particularly in West and Central Africa. Nonetheless, there exist a huge opportunity to improve on the PIF technique through the use of plant hormones in particular, coconut water. Coconut (*Cocos nucifera*) grows ubiquitously in Ghana and other African countries and could easily be accessed and afforded by resource poor farmers. Subjecting coconut water to autoclaving prior to treatment of corms is prerequisite for its effectiveness in inducing multiple and uniform shoots of banana and plantain characterized with many roots and leaves. These attributes subsequently contribute to a high shoot survival at the acclimatization phase prior to field establishment. Alternatives to treating coconut water apart from autoclaving could further be explored. The research findings proved that vacuum infiltration is an effective method of hormonal solution application which ensures high solution uptake. The approach could easily be practiced by smallholder farmers with little training. Banana and plantain seedlings propagators could employ it as one of the main propagation

techniques and incorporate it into the existing propagation techniques they currently practiced. Smallholder farmers in rural communities with limited resource could pool resources together to purchase an affordable vacuum pump for the infiltration of their corms during propagation. Moreover, further research is needed particularly with a more appropriate vacuum pressure which could totally overcome tissue damage of corms. It is noteworthy to mentioned that growth hormones have a significant influence at the propagation phase of banana and plantain. However, field performances of propagated plantain plants are hugely influenced by management practices.

6. General References

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7. Summary

Despite the significant role of banana and plantain (*Musa* spp.) in the livelihood of millions of people mostly in developing countries and in particular Sub-Saharan Africa, cultivation of these important crops is impeded by numerous challenges. Against this backdrop, research attempts were made to improve shoot proliferation in banana and plantain by employing innovative macropropagation techniques. Banana and plantain suckers were harvested in the greenhouses of the University of Hohenheim, Stuttgart, Germany and research fields of the Crops Research Institute, Ghana. These were subjected to *Plants Issus de Fragments de tige technique* (PIF), which is a mechanical preparation technique. It involves paring, thus cutting off the roots of the corms with a sharp sterilized knife. Thereafter, the leaf sheaths of the corms were carefully removed, consequently exposing latent axillary buds and the apical shoot meristem. Some of the corms had the exposed meristem destroyed with crosswise incision whiles others had the meristem left intact. Various hormonal treatments with the synthetic plant hormone 6-benzylaminopurine (0, 2.25 and 225.25 mg L^{-1} BAP) and natural plant hormones derived from coconut water were used. Fresh and autoclaved coconut water and other additives such as papain and root growth biostimulant from seaweed were employed in various combination to treat banana and plantain corms by either soaking or vacuum infiltration. The treated corms were planted in germination beds filled with growth substrates inside growth chambers. Evaluation of solution uptake from the two application methods and subsequently effects on number and growth characteristics of lateral shoots from the treated corms were carried out. Field evaluation of growth and yield of acclimatized plantlets from the PIF technique and hormonally derived plantlets were also undertaken. The hormonal solution application method

demonstrated a higher solution uptake with the method of infiltration which was about 33% more compared to the method of soaking. Results revealed an earlier shoot emergence in corms which had the apical meristem destroyed with crosswise incision, demonstrating the breakdown of apical dominance. Nonetheless, this did not contribute to significantly higher shoot numbers when compared to corms with intact apical meristem. BAP treated corms had triggered greater number of strong shoots with comparatively higher number of roots than untreated controls, however, the effect was independent of the concentration applied. The study further revealed the importance of natural growth hormones particularly the application of autoclaved coconut water as an alternative to the expensive plant growth hormone, 6-benzylaminopurine. There was a marked effect of autoclaved coconut water, resulting in earlier shoot development characterized with higher root numbers compared to corms subjected to 6-benzylaminopurine and the PIF technique, respectively. Moreover, the addition of the proteolytic enzyme papain and the root growth biostimulant seaweed extract to coconut water did not influence the growth performance of the treated corms. Vegetative growth performance, specifically pseudostem length of the main crop, was significantly influenced by the treatment at 6 and 9 months of growth in the field. BAP and seaweed extract recorded the highest numbers of suckers. Uniformity of acclimatized plantlets with well-developed roots and active leaves at the nursery stage might have contributed immensely for the uniform vegetative growth. Treating the corms with BAP and seaweed significantly influenced the bunch weight of the main crop resulting in a bunch weight of about 11 kg. However, there was no significant difference among treatments regarding bunch weight of the first sucker crop with each treatment recording a bunch weight of 11 kg.

8. **Zusammenfassung**

Trotz der wichtigen Rolle von Bananen und Kochbananen (*Musa* spp.) als Lebensgrundlage für Millionen von Menschen in Entwicklungsländern, insbesondere in Subsahara-Afrika, ist der Anbau dieser Kulturen von verschiedensten Problemen geprägt. Vor diesem Hintergrund wurden Forschungsanstrengungen unternommen um durch den Einsatz innovativer Makrovermehrungstechniken die Schösslingsvermehrung in Bananen und Kochbananen zu verbessern. Schösslinge von Bananen und Kochbananen wurden in den Gewächshäusern der Universität Hohenheim, Stuttgart und auf den Versuchsfeldern des Crops Research Institute, Ghana gesammelt. Die Schösslinge wurden mit der Methode *Plants Issus de Fragment de tige technique* (PIF) vorbereitet. Bei dieser mechanischen Behandlung werden zunächst die Wurzeln des Rhizoms mit einem scharfen, sterilisierten Messer abgeschnitten. Dann werden vorsichtig die Blattscheiden der Rhizome entfernt um die latenten Seitenknospen und das apikale Sprossmeristem freizulegen. Bei einem Teil der Rhizome wurde das freigelegte Meristem mit einem Kreuzschnitt zerstört. Anschließend wurden verschiedene Hormonbehandlungen mit dem synthetischen Pflanzenhormon 6-Benzylaminopurin (0, 2.25 und 225.25 mg L^{-1} BAP) und mit natürlichen Pflanzenhormonen aus Kokosnusswasser angewendet. Die Rhizome der Bananen und Kochbananen wurden durch Einlegen oder Vakuuminfiltrierung mit frischem und autoklavierten Kokosnusswasser und weiteren Additiven wie Papain und einem Wurzelstimulanz aus Seegras behandelt. Die behandelten Rhizome wurden in Klimakammern in Pflanzsubstrat eingebracht. Im Anschluss wurde die verschiedene Aufnahme der Lösungen durch die zwei Anwendungsmethoden gemessen und der Einfluss auf Anzahl und Wachstum der lateralen Sprosse der behandelten Rhizome evaluiert. Außerdem wurde das Wachstum und der Ertrag der akklimatisierten Pflanzen aus der PIF Methode

und aus der hormonbasierten Behandlung getestet. Das Infiltrieren mit der Hormonlösung erzielte eine etwa 33% höhere Aufnahme im Vergleich zum Einlegen in die Lösung. Ergebnisse zeigen außerdem, dass die apikale Dormanz bei den eingeschnittenen Rhizomen erfolgreich gebrochen wurde da bei diesen Rhizomen die Sprosse früher austrieben. Allerdings wurde die Anzahl der Sprosse im Vergleich zu den nicht-eingeschnittenen Rhizomen nicht erhöht. Rhizome, welche mit BAP behandelt wurden, brachten mehr Sprosse mit einer stärkeren Bewurzelung hervor als die unbehandelten Kontrollen; allerdings war dieser Effekt unabhängig von der Konzentration der Lösungen. Die Studie bestärkte außerdem die Rolle natürlicher Wachstumshormone; insbesondere stellte sich autoklaviertes Kokosnusswasser als Alternative zu dem teuren Wachstumshormon 6-Benzylaminopurin heraus. Rhizome, die mit autoklaviertem Kokosnusswasser behandelt wurden, zeigten ein früheres Sprosswachstum mit einer größeren Wurzelanzahl als Rhizome, die mit 6-Benzylaminopurin oder der PIF Methode behandelt wurden. Die Beigabe des proteolytischen Enzyms Papain und dem Wurzelstimulanz aus Seegras Extrakt zum Kokosnusswasser beeinflusste das Wachstumsverhalten der behandelten Rhizome nicht. Das vegetative Wachstumsverhalten, insbesondere die Länge des Scheinstammes der Hauptpflanze, wurde durch die Behandlung nach 6 und 9 Monaten im Feld signifikant beeinflusst. Die Behandlungen mit BAP und Seegras Extrakt erzielten die höchste Anzahl an Schösslingen. Die Homogenität der akklimatisierten Pflanzen mit gut entwickelten Wurzeln und Blättern bereits im Jungstadium förderte wahrscheinlich das gleichförmige vegetative Wachstum. Die Behandlungen der Rhizome mit BAP und Seegras Extrakt beeinflussten das Gewicht der Fruchtstände der Hauptpflanze signifikant und brachten Fruchtstandgewichte von 11 kg hervor. Das Fruchtstandgewicht der Folgetriebe wurde von den Behandlungen nicht signifikant beeinflusst und betrug ebenfalls 11 kg.

9. Author’s declaration

I hereby declare that this doctoral thesis is a result of my own work and that no other than the indicated aids have been used for its completion. All quotations and statements that have been used are indicated. I did not accept the assistance from commercial agency or consulting companies. Furthermore, I assure that the work has not been used, neither completely or in parts, for achieving any other academic degrees.

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John Opata

Stuttgart, ……

10. Curriculum vitae

Personal details

Date of birth	9th May 1981
Place of birth	Accra-Ghana
	Educational Background
11. 2013 till date	PhD studies, University of Hohenheim, Stuttgart, Germany.
03.2010- 03.2012	MSc. (Crops Science), Rheinische Friedrich-Wilhelms-University of Bonn. Bonn, Germany.
08.2003-05.2007	BSc. (Crops Science), University of Ghana, Legon, Accra.
	Work Experience
2014 till date	Junior Research Assistant, University of Hohenheim. Stuttgart, Germany
2014 till date	Research Participant of the Food Security Centre, University of Hohenheim
07.2007-08.2018	Research and Teaching Assistant, at the University of Ghana Forest and Horticultural Crops Research Centre-Kade.
01.2001- 07.2003	Teacher at St. Peter's Mission School, Accra, Ghana

Research Experience

2014 till date	Innovative Propagation Techniques in Banana and Plantain (University of Hohenheim, Germany and Crops Research Institute, Kumasi, Ghana).
05 -10. 2012	Seed Priming Effects on Rice- Weed Competition (Africa Rice Centre, Cotonou, Benin).
10.2016-05.2007	Effect of mulching on the growth and yield of okra (University of Ghana, Legon, Accra)
04 – 08 2008	Different Compound fertilizer regimes on the growth and yield of maize (University of Ghana Forest and Horticultural Crops Research Centre-Kade).
08.2007-08-2008	Different Legumes Management under Citrus Plantation (University of Ghana Forest and Horticultural Crops Research Centre-Kade).

Scientific conferences

04.2018	Affordable Phenotyping Workshop (Sterile Tissue Culture), CSIR Crops Research Institute (CSIR-CRI), Kumasi, Ghana.
05.2016	1st European Conference of Postgraduate Horticulture Scientist, Palermo, Italy.
11.2015	ELLS Scientific Student Conference. Social, environmental and economic dimensions. Challenges of global resource management, Prague, Czech.

09.2015 Management of land use systems for enhanced food security: conflicts, controversies and resolutions, Humboldt-Universität, Berlin, Germany

03.2015 GlobE: Global Food Security. Improving food security in Africa through increased system productivity of biomass-based value webs- Biomassweb. Postdam, Germany

Language skills

Fluent English (official). Basic German and French

Publication

2011 J.C. Norman, J. Opata, and E. Ofori (2011). Growth and yield of okra and hot pepper as affected by mulching. Ghana Journal of Horticulture. 9, 35-42.

2020 J. Opata, J. Skala, M. Hegele, B.M. Dzomeku and J.-N. Wünsche (2020). Macropropagation of banana (*Musa* AAA): Responses to hormonal and mechanical corm manipulation. Fruits, The International Journal of Tropical and Subtropical Horticulture, 75(2), 78–83.

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John Opata

Stuttgart,