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Original article

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

J. Opata[1], P.-F. Melichar[1], M. Hegele[1,†], S. Hegele[1], B.M. Dzomeku[2] and J.-N. Wünsche[1]

[1] University of Hohenheim, Institute of Crop Science, Section Crop Physiology of Specialty Crops, Emil-Wolff-Str. 25, 70599 Stuttgart, Germany

[2] CSIR-Crops Research Institute, P.O. Box 3785, Kumasi, Ghana, West Africa

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

Significance of this study

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.

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.

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 becomes necessary.

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, 2007). Other techniques, which involve the submergence of excised and mechanically treated corms with 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.

Materials and methods

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-de-

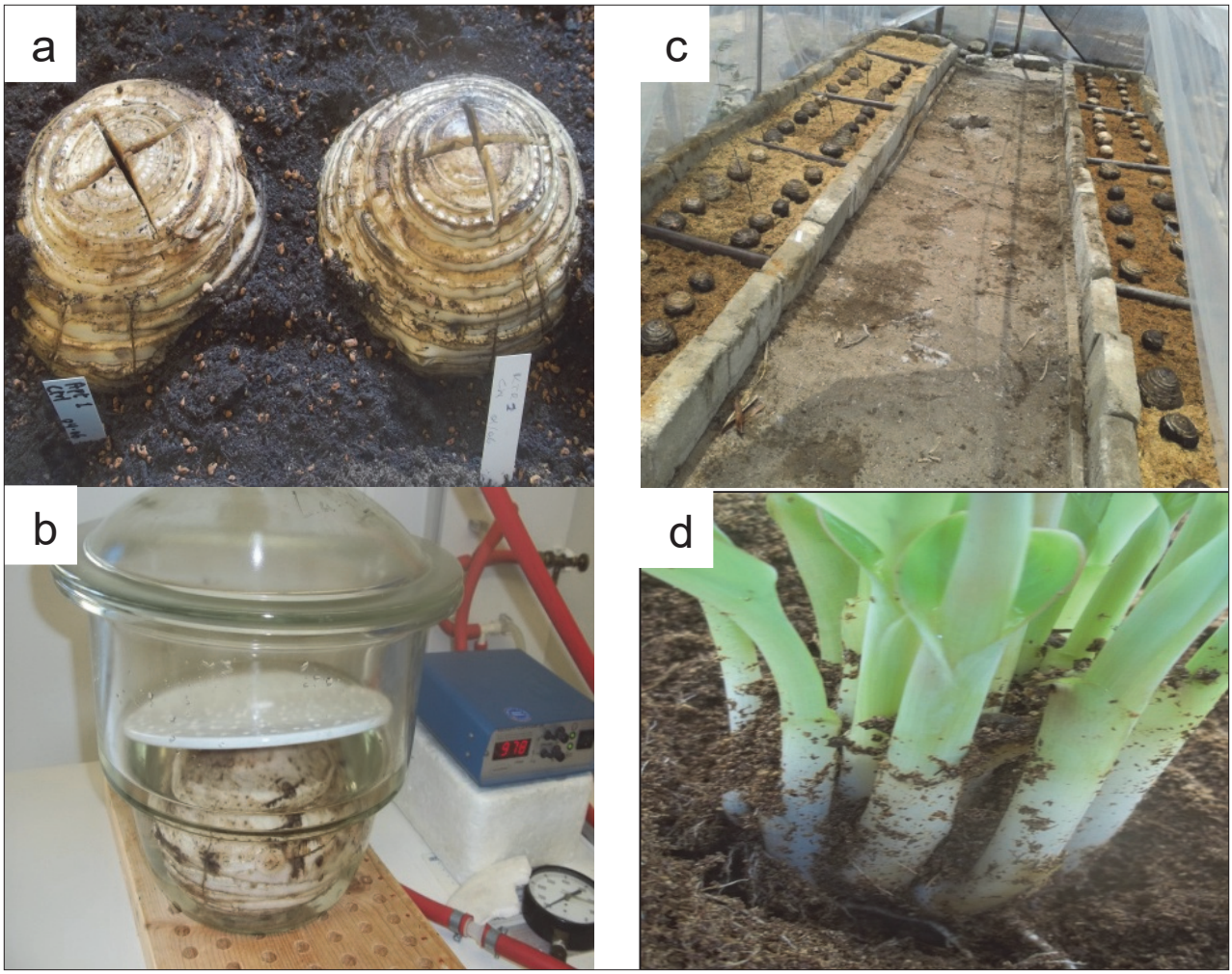

Figure 1. Corms with the crosswise incision known as *Plant Issus de Fragments de tige* (PIF) technique (a); a corm under vacuum infiltration (b); arrangement of corms inside a shade house prior to covering them with sawdust (c); and cluster of shoots after destroying the apical meristem of two-week old lateral shoot using the PIF technique (d).

ciduous 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.

Corm preparation

Sword and ratoon suckers of the False Horn 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 ensures that soil-borne microorganisms, especially root nematodes and banana stem borers, were 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 thoroughly 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 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.

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 °C for 15 min (CW_a); coconut water with 0.1% (w/v) pulverized papain, incubated at 40 °C for 40 min and stirred every 10 min for one minute throughout the incubation time and then autoclaved at 121 °C for 15 min (CWP 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).

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 to 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 h at the CRI. The freeze-dried latex was pulverized into fine powder with a hammer mill (A11, IKA-Werke, Germany).

Vacuum infiltration

The prepared corms were weighed and completely submerged in glass desiccators which contained the respective hormonal solutions (Figure 1b). To prepare 4 L 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 hormonal solutions 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.

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 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 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.

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 se-

lected 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 edn., Rothamsted, United Kingdom) and displayed graphically with Origin (v. 19, Wellesley Hills, MA, USA).

Results

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.

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 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 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.

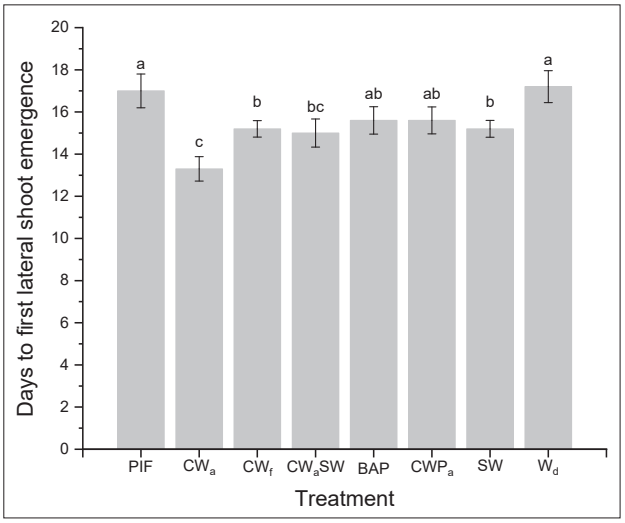

Figure 2. Effects of treatments (PIF - *Plant Issus de Fragments de tige*; CW_a - autoclaved coconut water; CW_f - fresh coconut water; CW_aSW - autoclaved coconut water with seaweed extract; BAP - 6-benzylaminopurine; CWP_a - coconut water with pulverized papain; SW - seaweed extract; W_d - distilled 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 $P \leq 0.05$. Untreated control plants had no later shoot growth.

Number of lateral shoots

Significant treatment differences were found for the number of lateral shoots after two weeks of corm sprouting (Figure 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). 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).

There were significant treatment differences in the accumulated total number of shoots per corm that were harvested every two weeks (Figure 3). The highest number of shoots were produced in the BAP and CW_a treatments with averagely 38 shoots per corm, which were significantly 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). The least number of shoots was produced by UTC corms, which was about 60% less compared to BAP and CW_a, respectively.

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 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 1). The UTC shoots had close to 20% stronger

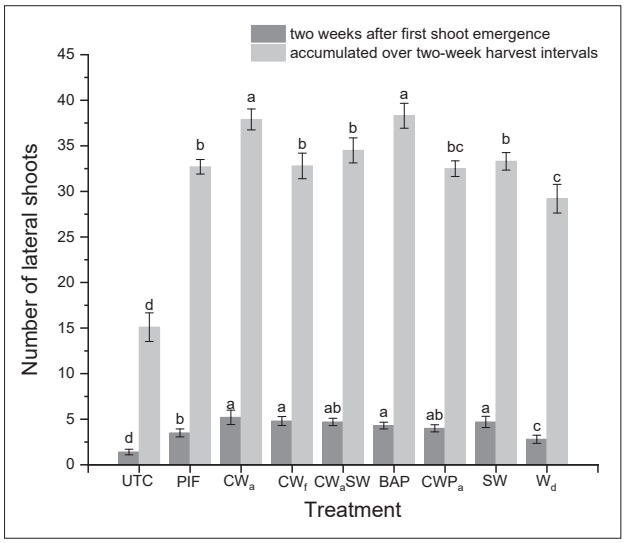

Figure 3. Effects of treatments (UTC - untreated control; PIF - *Plant Issus de Fragments de tige*; CW_a - autoclaved coconut water; CW_f - fresh coconut water; CW_aSW - autoclaved coconut water with seaweed extract; BAP - 6-benzylaminopurine; CWP_a - coconut water with pulverized papain; SW - seaweed 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 $P \leq 0.05$.

Table 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 $p < 0.001$.

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 1). Shoots from corms treated with CW_a had the highest number of roots (Table 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 1).

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, 2007).

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 (2007) 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% 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 adjust 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).

Conclusion

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 not only to 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 plantlet production through the application of corms with coconut water solutions, and subsequently treating the emerging shoots with the PIF technique. Since coconut fruit is widely grown in the tropics at little expense, the substitution of BAP with this plant-based hormonal solutions in macropropagation techniques would not lead to high 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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