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Original article

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

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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

Significance of this study

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.

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.

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.

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 means (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 (Plant Issus de Fragments de tige) technique is also frequently applied to destroy the apical meristem with a crosswise incision. In addition, harvested corms are frequently subjected to hot water treatments prior to paring to

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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 induced 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 compare the effectiveness of vacuum infiltration and soaking of banana corms with varying BAP concentrations on shoot regeneration under greenhouse conditions.

Materials and methods

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 were destroyed with a crosswise incision by employing the PIF technique while the others were left with intact meristems.

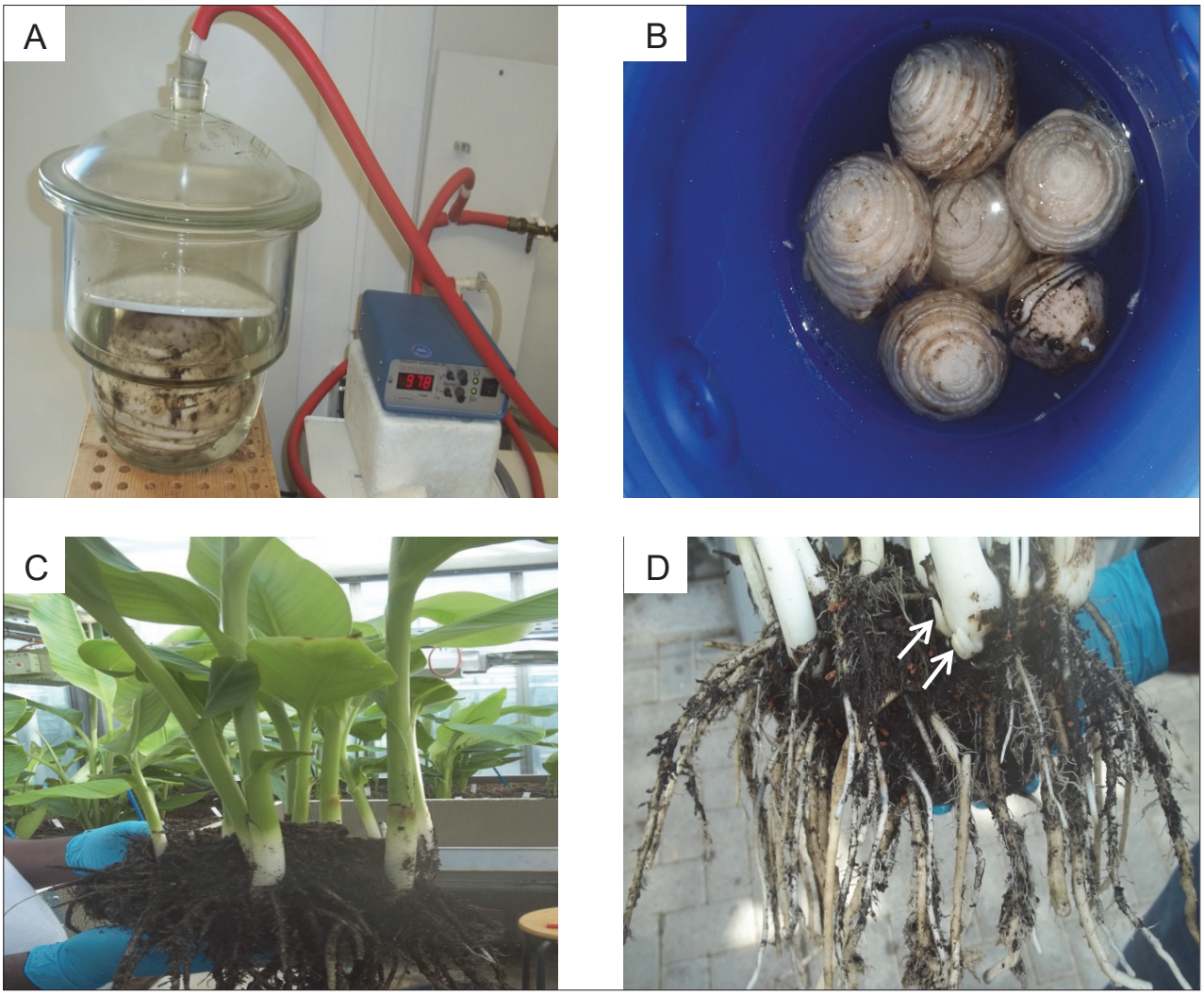

Figure 1. Corm under (A) vacuum infiltration; (B) corm under soaking condition; (C) sprouted corm with multiple shoots and roots in the greenhouse; and (D) arrows indicate shoot formation at the base of a mature shoot grown on 225.25 mg L^{-1} 6-benzylaminopurine.

Hormonal treatments

Three concentrations (0, 2.25 and 225.25 mg L^{-1}) of the synthetic plant hormone BAP (Carl Roth, GmbH, Germany) were prepared, using 1N NaOH as solvent and water as diluent. Corms with destroyed or intact apical meristem were 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 application 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 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 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.

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 (Figures 1c, 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. Least significant difference (LSD) was calculated to determine significant differences between means at 5% probability level using Genstat (18th edn., Rothamsted, United Kingdom) and data were displayed graphically with Origin (v. 19, Wellesley Hills, MA, USA).

Results

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). 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).

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 3). The soaked corms with an intact meristem needed significantly longer to first lateral

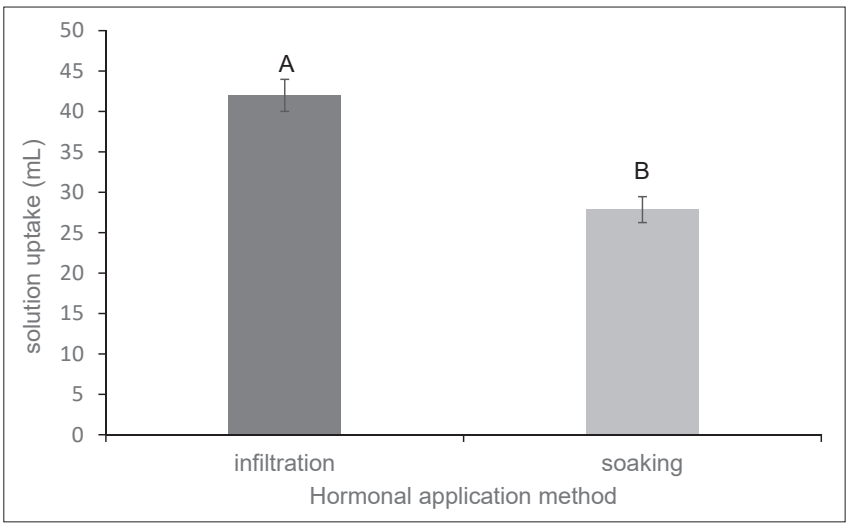

Figure 2. Solution uptake of cv. 'Khai Thong Ruang' corms by soaking and infiltration. Vertical bars indicate standard error of the means ($n = 36$) and different letters represent significant difference using LSD test at $P \leq 0.05$.

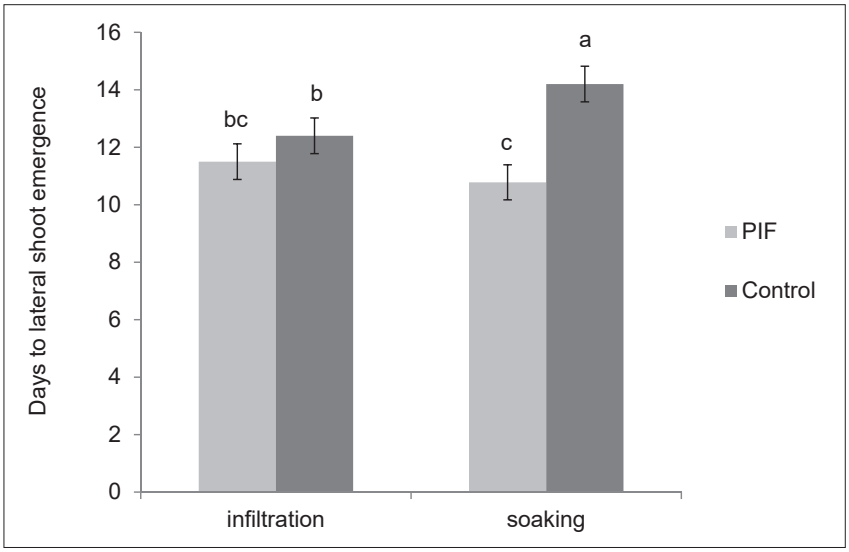

Figure 3. Effects of corm manipulation (PIF – crosswise incision and control – non-crosswise incision) and hormonal solution application (infiltration and soaking) on days to shoot emergence of the banana cv. 'Khai Thong Ruang'. Vertical bars indicate standard error of the means ($n = 18$) and different letters represent significant difference using LSD test at $P \leq 0.05$.

Table 1. Main effects and interaction of corm manipulation (PIF – crosswise incision and control – non-crosswise incision), hormonal application 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. × App. × 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. × app. × conc.	<0.001	0.013

Means with different letters within columns and statistical effects are significantly different at $P \leq 0.05$.

shoot emergence than any other treatment (Figure 3). The various concentrations of BAP applications had no effect on the number of days to lateral shoot emergence (data not shown).

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 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 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 1).

Root production

Root production of the banana cultivar was neither significantly affected by the corm manipulation technique nor the hormone application method (Table 1). However, corms treated with either of the two BAP concentrations produced 4 roots per shoot, which was about 34% more 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 1).

Shoot characteristics

There were significant main effects on the average shoot length (Table 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 technique; however, it was significantly affected by the hor-

Table 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 and statistical effects are significantly different at $P \leq 0.05$.

mone application method and BAP concentrations (Table 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.

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 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, 2007).

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.

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