Journal of Applied Biosciences 74:6086– 6090

ISSN 1997–5902

Effect of benzylaminopurine on *in vivo* multiplication of French plantain (*Musa* spp. AAB) cv. 'Itoke sege'

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Original submitted in on 25th October 2013 Published online at www.m.elewa.org on 28th February 2014. http://dx.doi.org/10.4314/jab.v74i1.1

ABSTRACT
Objective: In vivo macropropagation is an alternative simple and cheap technique for banana multiplication. However, the response of cv. "Itoke Sege" to *in vivo* macropropagation combined with different benzylaminopurine (BAP) concentrations is not known. This study was conducted to determine the appropriate concentration of BAP for enhancing *in vivo* macropropagation of French plantain cv. 'Itoke Sege'.
Methodology and results: Sword suckers of about 70 - 80 cm tall and 14 -16 cm collar diameter were obtained from farmers' fields in Rungwe district in Mbeya, Tanzania. Moistened sawdust was steam-sterilized for 45 minutes and then filled for cooling in wooden propagators of 1.5 m x 2.20 m x 0.3 m dimension. Suckers were partially peeled, washed to remove roots and surface-sterilized for 15 seconds by dipping them in hot boiling water. The sterilized corms were desheathed to expose axillary buds and decorticated to suppress the apical meristems. Fifteen corms in three replications were each dipped in BAP at 0.0, 1.5, 3.0 and 6.0 mg/l for 12 hours and then planted into sawdust media. Irrigation was done immediately but subsequent watering was carried out when necessary. *In vivo* multiplication response was evaluated based on number of days to first shoot emergence, number of shoots per corm, number of roots per shoot and shoot size. Results showed that BAP concentration at 1.5 mg L^{-1} significantly ($P < 0.05$) reduced the number of days to first shoot emergence of 15.78 days followed by BAP at 3.0, 6.0 and 0.0 mg L^{-1} with 25.18, 28.39 and 36.43 days, respectively. Similarly, BAP concentration at 1.5 mg L^{-1} significantly ($P < 0.05$) increased sucker productivity with 17.11 suckers per corm followed by BAP at 0.0, 3.0 and 6.0 mg L^{-1} with 15.23, 13.08 and 12.96 suckers per corm, respectively. Corms treated with BAP at 1.5, 3.0, 6.0 mg L^{-1} significantly ($P \leq 0.05$) produced taller shoots with length of 27.0, 27.3 and 26.7 cm followed by corms treated with BAP at 0.0 mg L^{-1} with shoot length of 22.7 cm. Conversely, corms treated with BAP at 0.0 and 6.0 mg L^{-1} produced suckers with larger collar diameter of 3.4 and 2.4 cm followed by suckers from corms treated with BAP at 3.0 and 1.5 mg L^{-1} with collar diameters of 2.2 and 2.0 cm, respectively. Suckers from corms treated with BAP at 0.0 and 3.0 mg L^{-1} had larger number of leaves of 4.8 and 4.6 per sucker followed by suckers from corms treated with BAP at 1.5 and 6.0 mg L^{-1} with 4.0 and 3.8 leaves per sucker, respectively.
Conclusion and application: Based on these findings, it is concluded that *in vivo* macropropagation combined with BAP at 1.5 mg L^{-1} is a suitable technique for improving multiplication and sucker growth of

French plantain cv. 'Itoke Sege'. The findings of this study provide an opportunity for the use of *in vivo* macropropagation coupled with BAP at 1.5 mg L^{-1} as an alternative simple and cheap technology for rapid and mass production of planting materials for recalcitrant plantain varieties. Further study is recommended to evaluate the response of cv. "Itoke Sege" to *in vivo* macropropagation combined with other cytokine-based growth regulators. Research is also required to test the responses of other recalcitrant plantain cultivars to *in vivo* macropropagation in combination with different BAP concentrations.
Key words: Benzlyamimopurine, *in vivo* macropropagation, French Plantain, 'Itoke Sege.

INTRODUCTION

French plantain cv. 'Itoke sege' is the most important variety popularly grown in Mbeya region, Tanzania. According to Joab (2004) the variety is known for its biggest bunches, delayed fruit production and highest apical dominance (Figure 1). Like all plantain varieties, mature fruits of "Itoke Sege" are popularly used as fried and roasted meal.

Figure 1: Plant and fruit appearance of French plantain cv. "Itoke Sege"

The suckering ability of 'Itoke Sege' is very low with an average of about 3 suckers per year per stool depending on agro-climatic conditions and managerial practices (Joab, 2004). The low suckering ability is associated with the strong apical dominance (Swennen and De Langhe, 1985). The high apical dominance in banana is linked to high endogenous auxin levels (Arinaitwe *et al.*, 2000). Cytokinin and auxin work antagonistically and thus an application of cytokinins decreases the apical dominance while an application of auxin increases the apical dominance. Benzylaminopurine is an adenine-based cytokinin popularly used for *in vitro* induction of axillary and adventitious shoots in banana (Osei, 2006; Kalimutha *et al*., 2007). An application of BAP at 3.0 and 6.0 mg/l has been recommended for enhancing *in vitro* shoot proliferation in plantains and bananas, respectively (Talengera *et al*., 1994; Maerere *et al*., 2003). *In vivo* macropropagation is an alternative technique that involves disinfecting, deshealthing banana corms to expose axillary buds and decorticating the apical meristem to suppress the apical dominance (Kwa, 2003) (Figure 2).

Figure 2: Steps for *in vivo* macropropagation: Left - Disinfected, deshealthed and decorticated banana corms, Middle - banana corms planted in sawdust and Right - Banana shoots sprouting from the planted corms

In vivo macropropagation technique is relatively simple (Njukwe *et al*., 2005) and provides in a short period pest-free plantlets (Kwa, 2003). However, according to Kwa (2003) *in vivo* macropropagation enhances sucker productivity in plantain cultivars than Cavendish banana cultivars. The deshealthed and decorticated corms are planted in sawdust in propagators either without or with treatment of BAP (Baiyeri and Aba, 2004). *In vivo* macropropagation combined with an application of BAP at concentration of 0.16 mg/l induces sprouting of axillary buds in Cavendish banana (Singh *et al*., 2011). The response of French plantain cv. 'Itoke Sege' to *in vivo* macropropagation technique in combination with BAP at different concentrations is hardly known. The objective of this study was to evaluate the effect of BAP concentration on *in vivo* proliferation of French plantain cv. 'Itoke Sege'.

MATERIALS AND METHODS

The study was carried out from February to October 2012 at the Horticulture Unit of Sokoine University of Agriculture (SUA) in Tanzania. An experiment was laid out in a randomized complete block design with four treatments each replicated three times. The treatments consisted of four BAP concentrations (0.0, 1.5, 3.0 and 6.0 mg L^{-1}). A careful selection of sword suckers of plantain cv. 'Itoke Sege' of about 3-4 months was done from healthy mother plants grown by small-scale farmers in Rungwe district in Mbeya region. Sword sucker were pared to remove roots and packed into plastic bags for transport to SUA in Morogoro region. Propagators each with a dimension of 200 cm long, 100 cm width and 75 cm high were made from soft timbers. Water moistened sawdust was sterilized by heating it for one hour and thirty minutes. The propagators were filled to three quarter-full with the sterilized sawdust and left for 24 hours to cool before planting the prepared corms. The suckers were also sterilized by dipping them in boiling water for 30 seconds. Using sterilized sharp knife, the outer sheaths were removed one by one to exposed axillary buds. The exposed apical meristem and axillary buds were wounded by making crosscuts to suppress the apical dominance and induce sprouting, respectively. The corms were soaked in each BAP concentration for 12 hours. Thereafter the corms were removed from BAP solution and planted into the sawdust at spacing of 15 x 15 cm. Watering was done immediately after planting but subsequent irrigation was conducted twice per week. Shoots from sprouted corms were treated with booster (poly- feed starter (N-P-K)) at dose of 0.5 g per litre per month to enhance their growth. Data were collected on number of days to first shoot emergence, number of shoots emerged per corm, number of roots per corm, shoots height, shoot collar diameter and number of leaves per shoot. The collected data were analysed using COSTAT6.4 (Cohort Software, Minneapolis, USA, 2006). The Bartlett's test for normality was performed prior to analysis of variance (ANOVA) and mean separation was carried out based on the least significant difference (LSD) at a probability of $P \leq 0.05$.

RESULTS

Results indicated that BAP concentration had a very high significant ($P \leq 0.05$) influence on the number of days from corm sowing to first shoot emergence and number of suckers per corm (Table 1). Banana corms treated with BAP at 1.5 mg L^{-1} produced the first shoot earlier at 15.8 days followed by corms treated with BAP at 3.0, 6.0 and 0.0 mg L^{-1} with 25.2, 28.4 and 36.4 days, respectively. Moreover, banana corms dipped in BAP at 1.5 mg L^{-1} produced the largest number of shoots per corm of 17.1 followed by corms dipped in BAP at 0.0, 3.0 and 6.0 mg L^{-1} with the number of shoots per corm of 15.2, 13.1 and 13.0, respectively. Treating banana corms with BAP had a significant ($P \leq 0.05$) effect on shoot length, diameter and number leaves per shoot (Table 2). Corms treated with BAP at 1.5 and 3.0 mg L^{-1} produced the tallest shoots with length of 22.4 and 21.6 cm respectively, followed by corms treated with BAP at 6.0 and 0.0 mg L^{-1} with shoot length of 19.8 and 17.7 cm.

Table 1: Effect of BAP concentrations on *in vivo* sucker sprouting and productivity of French plantain cv. 'Itoke Sege'

BAP concentration (mg/l)	Number of days to first sucker emergence	Number of suckers per corm
0.0	36.8[a]	15.2[ab]
1.5	15.9[d]	17.1[a]
3.0	25.1[c]	13.1[b]
6.0	28.4[b]	13.0[b]
LSD (0.05)	0.4	2.4
F-test (0.05)	***	**
CV (%)	1.9	19.6

Means followed by the same letter (s) within the column are not significant different at a probability of 5 % based on LSD test. ** = highly significant ($p \leq 0.01$), *** = very highly significant ($p \leq 0.001$).

Conversely, corms treated with BAP at 1.5 mg L^{-1} had largest collar diameter of 2.2 cm followed by corms treated with BAP at 3.0, 6.0 and 0.0 mg L^{-1} with sucker collar diameters of2.0, 1.8 and 1.7 cm, respectively. Suckers from corms treated with BAP at 3.0 mg L^{-1} had also largest number of leaves of 4.3 per sucker followed by corms treated with BAP at 0.0, 6.0 and 1.5 mg L^{-1} with number of leaves of 4.1, 4.0 and 3.9 per sucker, respectively.

Table 2: Effect of BAP concentrations on rooting and growth of *in vivo* derived suckers of French plantain cv. 'Itoke Sege'

BAP conc. (mg/l)	Sucker length (cm)	Sucker collar diameter (cm)	Number of roots per sucker	Number of leaves per sucker
0.0	17.7[c]	1.7[d]	11.0[a]	4.1[b]
1.5	22.4[a]	2.2[a]	11.5[a]	3.9[b]
3.0	21.6[a]	2.0[b]	11.7[a]	4.3[a]
6.0	19.8[b]	1.8[c]	10.8[a]	4.0[b]
LSD (0.05)	1.3	0.1	1.1	0.2
F-test (0.05)	***	***	Ns	***
CV (%)	8.0	7.5	11.5	5.1

Means followed by the same letter (s) within the column are not significant different at a probability of 5 % based on LSD test. ns = not significant, *** = very highly significant ($p \leq 0.001$).

DISCUSSION

The present study shows that treatment of desheathed and decorticated corms with BAP solution at 1.5 mg L^{-1} enhanced *in vivo* sucker emergence, proliferation and shoot growth of French plantain cv. 'Itoke Sege'. As cv. "Itoke Sege" shows a very apical dominance in the field, it was expected that the cultivar would respond well to BAP at relatively higher concentration. The differential responses in multiplication rates to BAP concentration levels have frequently been reported in banana and plantains, and have been associated with the genetic variability within the plantain cultivars, especially the endogenous levels of auxin and cytokinins (Arinaitwe *et al*., 2000; Kalimutha *et al*., 2007, Muhammad, 2007). For instance, *in vitro* multiplication of 7 - 8 shoots per explant of plantain cultivars has been reported when MS basal medium was supplemented with BAP at 2.0 mg L^{-1} (Kalimutha *et al*., 2007). Increasing cytokinin concentration above 3 mg L^{-1} decreased *in vitro* proliferation of plantain cultivars (Muhammad, 2007). The low *in vitro* proliferation at higher cytokinin concentration has been associated with lower endogenous levels of auxins (Arinaitwe *et al*., 2000).On the contrary, Sreeramanan *et al*. (2008) found higher *in vitro* shoot induction in plantain cv. 'Oniaba' and 'Apantu' when treated with BAP at 4.5 mg L^{-1} (Buah *et al*., 2010).. The higher shoot growth in corms treated with BAP at 1.5 mg L^{-1} corresponds well with the enhanced shoot emergence. An injection of BAP in plantain corms under field conditions enhanced bud formation as well as the

speed of shoot development (Swennen, and De Langhe, 1985). To conclude, the results from the present study clearly show that *in vivo* macropropagation combined with BAP at 1.5 mg L^{-1} enhances sucker productivity and growth of recalcitrant French plantain cv. "Itoke Sege". The findings of this research provide evidence for the use of *in vivo* macropropagation coupled with BAP at 1.5 mg L^{-1} as an alternative simple and cheap technology for rapid and mass production of plantain materials for recalcitrant plantain varieties. Further studies are required to test the responses of other recalcitrant plantain cultivars to *in vivo* macropropagation in combination with different BAP concentrations. Research is also recommended to evaluate the responses of cv. "Itoke Sege" to *in vivo* macropropagation combined with other cytokine-based growth regulators.

ACKNOWLEDGEMENT

The authors are thankful to Tanzania Commission for Science and Technology (COSTECH) for financing this study.

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