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Effect of thidiazuron on *in vivo* shoot proliferation of popular banana (*Musa* spp. L) cultivars in Tanzania

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ABSTRACT

Objective: Thidiazuron (TDZ) is a diphenyl urea-based cytokinin, which is non-degradable and persistent in plant tissues. The effect of these TDZ properties on *in vivo* banana proliferation when deshealthed corms are temporarily dipped in such growth regulator is unknown. The objective of this study was to evaluate the effect of temporary treatments with TDZ of deshealthed banana corms on *in vivo* sucker multiplication.

Methodology and Results: The study was comprised of a split plot experiment in a randomized complete design with three replications each replication with 10 corms. The main plot factor was banana cultivars (*Mtwike*, *Mzuzu* and *Bukoba*) while the sub-plot factor was TDZ concentrations (0.5, 1.0, 2.0 and 3.0 mg/l). Moistened sawdust was steam-sterilized for 45 minutes and then filled for cooling in wooden propagators. Banana suckers were cleaned to remove roots and surface-sterilized for 15 seconds. The sterilized corms were deshealthed to expose axillary buds and decorticated to suppress the apical meristems. These corns were each dipped in TDZ at 0.5, 1.0, 2.0 and 3.0 mg/l for 12 hours and then planted into the sterilized sawdust media in the propagators. Results showed that the number of shoots per corm significantly ($P < 0.05$) increased as TDZ concentration increased from 0.0 to 2.0 mg/l but decreased as TDZ increased to 3.0 mg/l. The number of leaves per sucker significantly ($P < 0.05$) decreased as TDZ concentration increased from 0.0 to 3.0 mg/l. Conversely, corms treated with TDZ at 2.0 mg/l produced suckers with the largest number of leaves of 4.9 per sucker followed by corms treated with TDZ at 1.0, 3.0 and 0.5 mg/l with 4.5, 4.3 and 3.3 leaves per sucker, respectively. Banana cultivars had a significant ($P < 0.05$) effect on the number of shoots per corm where banana cv. *Bukoba* produced the largest number of shoots of 6.4 per corm while banana cv. *Mtwike* and *Mzuzu* produced 2.3 and 2.9 shoots per corm, respectively.

Conclusion and Application: The findings from this study provide evidence that *in vivo* shoot multiplication rates and sucker growth of banana cv. *Mzuzu*, *Bukoba* and *Mtwike* can be increased by dipping for 12 hours deshealthed corms in TDZ solution at 2.0 mg/l. The low *in vivo* multiplication rates of banana cv. *Mtwike* and *Mzuzu* underscore the need for further studies to determine alternative best cytokine-based growth regulators.

Key words: Thidiazuron, *in vivo* proliferation, Sucker growth, Banana.

INTRODUCTION

In vivo macropropagation is an alternative technique for mass production of banana planting materials under *in vivo* conditions (Kwa, 2003; Njukwe *et al.*, 2005). The technique involves disinfecting, deshealthing banana corms to expose axillary buds and decorticating the apical meristem to suppress

apical dominance (Kwa, 2003; Njukwe *et al.*, 2005; Kindimba and Msogoya, 2014). Compared to *in vitro* propagation, this technique provides in a short period pest-free plantlets (Njukwe *et al.*, 2005; Kwa, 2003) and has the potential of producing 50 - 60 shoots per corm in 4 - 5 months (Baiyeri and Aba, 2004). In this technique, banana corms are planted in a sterile sawdust initiation media either without or with treatment of benzlyamimopurine (Baiyeri and Aba, 2004). This technique has shown better results when corms from flowered banana plants and maiden suckers are used (Baiyeri and Ajayi, 2000; Kwa, 2003). The inability of axillary buds from small banana suckers to sprout under *in vivo* macro-propagation is associated with the high apical dominance caused by high auxin levels (Arinaitwe *et al.*, 2000). Auxins work antagonistically with cytokinins, and thus an application of the later suppresses apical dominance and consequently promotes proliferation of axillary buds (Naseem, and Dandekar, 2012). *In vivo* macropropagation combined with an application of BAP in cavities of decorticated corms induced more sprouting of axillary buds in banana (Singh *et al*., 2011). Moreover, a temporary dipping of deshealthed and decorticated corms in BAP at 1.5 mg/l resulted to the highest *in vivo* proliferation of 17.11 suckers per corm in plantain cv." Itoke Sege" (Kindimba and Msogoya, 2014). Thidiazuron (TDZ) is a diphenyl urea based cytokinin growth regulator, which is non-degradable by cytokinin-oxidase enzymes (Makara *et al*., 2010). Furthermore, TDZ has long residual effects, increases the biosynthesis of endogenous adenine-based cytokinins and enhances nutrient uptake and assimilation in plants (Guo, *et al.* 2011). This growth regulator induces shoot proliferation in plant species that respond poorly to 6-benzylaminopurine supplemented growth medium (Huetteman and Preece, 1993). *In vitro* shoot proliferation rates of both recalcitrant and responsive banana genotypes increased with TDZ concentration from 0.01 to 2.0 mg/l (Arinaitwe *et al.,* 2000) while TDZ concentrations above 2.0 mg/l or below 0.01 mg/l reduced *in vitro* shoot proliferation and suppressed shoot elongation in banana (Crouch *et al*., 1998). It is hypothesized that the high residual effect of TDZ would increase *in vivo* proliferation rates when deshealthed and decorticated banana corms are temporarily dipped in a solution of such growth regulator. Unfortunately, studies on the effect of temporary TDZ treatments on banana *in vivo* macropropagation rates are scanty. The objective of this study was to determine the effect of temporary TDZ treatments on *in vivo* proliferation rates and shoot growth of popular banana cultivars in Tanzania.

MATERIALS AND METHODS

Materials: Three banana cultivars were used in this study namely "Mtwike" (Cavendish cv. Grande naine), "Mzuzu" (French plantain) and Bukoba (East African highland banana). Banana suckers of about 100 cm tall and 15 cm collar diameter were collected from farmers. The suckers were headed back 30 cm and sterilized by dipping them into hot water at 100 ^{0}c for 15 seconds. The sterilized corms were deshealthed to expose the axillary buds and the exposed axillary buds were wounded by cutting them transversely to stimulate multiple sprouting. The apical meristem of each corm was destroyed by decortication to overcome the apical dominance. Sawdust sourced locally was used as an initiation media. The sawdust was steam-sterilized at 100 ^{0}c for 45 minutes and then was poured in propagators for cooling. A propagator with a dimension of 3.0 m x 1.0 m x 0.5 m was constructed by using soft timbers.

Experimental design: The experiment was set as a split plot arrangement in a randomized complete block design with three replications. A replication consisted of 10 corms of each cultivar. Banana cultivars (*Mtwike*, *Mzuzu* and *Bukoba*) were the main plot factor while TDZ concentrations (0.5, 1.0, 2.0 and 3.0 g/l) were the sub-plot factor. The deshealthed and decorticated banana corms were soaked for 12 hours into each TDZ concentration. The TDZ treated corms were planted into moistened sawdust filled in the propagators. The propagators were placed under a plastic tunnel with temperature of 25 - 30 °C. The remaining TDZ solution for each concentration was poured onto the sawdust at 6 l/m^3 of the propagator. Irrigation was carried out twice per week while poly-feed starter fertilizer (N-P-P at 19 - 19 - 19) at a concentration of 0.5 g/l of water was applied after two months at 6 litres of solution per m^2.

Data collection and analysis: Data collection included the number of days to first shoot emergence, number of sprouted buds per corm, number of shoots per bud, number of shoots per corm and shoot size based on height, collar diameter and number of leaves per shoot. The collected data were subjected to analysis of variance using GenStat Statistical Programme 12th Edition (Rayne *et al*., 2009). Treatment mean separation was carried out based on Student-Newman-Keuls at P < 5 %.

RESULTS

Effect of TDZ concentration on in vivo multiplication and sucker growth: The number of sprouted buds per corm, number of shoots per bud and number of shoots per corm significantly (P < 0.05) increased as TDZ concentration increased from 0.0 to 2.0 mg/l but decreased as TDZ increased to 3.0 mg/l (Table 1). Thidiazuron at 2.0 mg/l resulted in the largest number of sprouted buds of 2.9 buds per corm followed TDZ at 1.0, 3.0 and 0.5 mg/l with 2.6, 2.5 and 1.7 sprouted buds per corm, respectively. Similarly, TDZ at 2.0 g/l resulted in the largest number of shoots of 2.0 per bud followed by TDZ at 3.0, 1.0 and 0.5 mg/l with 1.8, 1.5 and 1.2 shoots per bud, respectively. Overall, TDZ at 2.0 mg/l resulted in the largest number of shoots per corm of 6.3 shoots followed by TDZ at 3.0, 1.0 and 0.5 mg/l with 4.6, 4.0 and 1.9 shoots per corm, respectively.

Table 1: Effect of TDZ concentration on *in vivo* multiplication of different banana cultivars

TDZ conc. (mg/l)	Days to 1st shoot emergence	No. of sprouted buds/corm	No. of shoots per bud	No. of shoots per corm
0.5	29.4	1.7[a]	1.2[a]	1.9[a]
1.0	25.0	2.6[b]	1.5[ab]	4.0[b]
2.0	28.8	2.9[b]	2.0[b]	6.3[c]
3.0	28.5	2.5[ab]	1.8[ab]	4.6[bc]
LSD	3.7	0.6	0.6	1.5
F-test	ns	s	s	s
S.E	3.8	0.6	0.58	1.5
CV (%)	13.9	20.4	24.9	25.5

Means followed by the same letters(s) within the column are not significant different at P < 5 % based on Student-Newman-Keuls. ns = not significant and s = significant at P < 5 %.

Sucker collar diameter and number of leaves per sucker significantly (P < 0.05) decreased as TDZ concentration increased from 0.0 to 3.0 mg/l (Table. 2). Corms treated with TDZ at 0.5 mg/l produced suckers with the biggest collar diameter of 2.2 cm followed by corms treated with TDZ at 1.0, 2.0 and 3.0 mg/l with shoot collar diameter of 1.7, 1.6 and 1.6 cm, respectively. However, corms treated with TDZ at 2.0 mg/l produced suckers with the largest number of leaves of 4.9 per sucker followed by corms treated with TDZ at 1.0, 0.5 and 3.0 mg/l with 4.5, 3.3 and 4.3 leaves per sucker, respectively.

Table 2: Effect of TDZ concentration on growth of *in vivo* derived banana suckers

TDZ conc. (mg/l)	Sucker height (cm)	Sucker diameter (cm)	No. of leaves per sucker
0.5	15.2	2.2[b]	3.3[a]
1.0	15.5	1.7[ab]	4.5[b]
2.0	15.1	1.6[a]	4.9[b]
3.0	15.5	1.6[a]	4.3[ab]
LSD	2.9	0.4	0.84
F-test	ns	s	s
SE	3	0.4	0.86
CV (%)	19.8	22.8	20.2

Means followed by the same letters(s) within the column are not significant different at 5 % level based on Student-Newman-Keuls. ns = not significant and s = significant at P < 5 %.

Effect of banana cultivars on *in vivo* multiplication and sucker growth: Banana cultivar had a significant ($P < 0.05$) effect on the number of sprouted buds per corm, number of shoots per bud and number of shoots per corm (Table 3). In banana cv. Bukoba, 3.2 buds sprouted per corm while 1.7 and 2.4 buds sprouted per corm in banana cv. Mtwike and Mzuzu, respectively. Banana cv. Bukoba also produced 2.0 shoots per bud while cv. Mtwike and cv. Mzuzu produced 1.4 and 1.6 shoots, respectively. Moreover, cv. Bukoba produced the largest number of shoots of 6.4 per corm compared with banana cv. Mtwike and Mzuzu with 2.3 and 2.9 shoots per corm, respectively. Banana cultivar had no significant ($P < 0.05$) effect on shoot height, collar diameter and number of leaves per sucker (Table 4).

Table 3: Effect of banana cultivar on *in vivo* shoot proliferation

Cultivar	No. of days to 1st shoot sprouting	No. of sprouted buds per corm	No. of shoots per bud	No. of shoots per corm
Mtwike	25.0	1.7[a]	1.4[a]	2.3[a]
Mzuzu	27.2	2.4[ab]	1.6[ab]	2.9[a]
Bukoba	23.2	3.2[b]	2.0[b]	6.4[b]
LSD	5.4	0.6	0.46	1.67
F-test	ns	s	s	s
S.E	2.4	0.3	0.5	0.7
CV (%)	8.6	11.5	12.2	17.5

Means followed by the same letters(s) within the column are not significant different at 5% level based on Student-Newman-Keul. ns = not significant and s= significant at P < 5 %.

Table 4: Effect of banana cultivars on growth of *in vivo* derived multiplication suckers

Cultivar	Sucker height (cm)	Sucker diameter (cm)	No. of leaves per sucker
Mtwike	14.11	1.71	4.05
Mzuzu	15.98	1.75	4.18
Bukoba	16.04	1.88	4.52
L.S.D	2.4	0.4	0.7
F-test	ns	ns	ns
S.E	3.0	0.18	0.34
CV (%)	6.9	10.3	7.9

ns = not significant at P < 0.05.

Effect of interaction of cultivar and thidiazuron on *in vivo* sucker multiplication and growth: Banana cv. Mzuzu - TDZ interaction had a significant ($P < 0.05$) effect on the number of shoots per corm (Table 5). Banana cv. Mzuzu produced the largest number of shoots of 10.4 and 5.3 per corm in TZD at 2.0 and 3.0 mg/l followed by cv. Bukoba with 5.3 and 5.2 shoots per corm in TDZ at 2.0, 3.0 mg/l, and cv. Mtwike with 3.1. and 3.2 shoots per corm in TDZ at 2.0 and 3.0 mg/l.

Table 5: Interaction effect of banana cultivar and Thidiazuron concentrations on *in vivo* sucker proliferation

Cultivar x TDZ conc. Interaction mg/l	No. of days of 1st sucker sprouting	No of buds per corm	No. of suckers per bud	No. of suckers per corm
Bukoba x 0.5	24.9	1.2	1.6	1.9[cd]
Bukoba x 1.0	25.0	2.5	1.2	3.2[cd]
Bukoba x 2.0	26.6	2.7	1.7	5.2[bc]
Bukoba x 3.0	24.4	2.8	1.8	5.3[bc]
Mtwike x 0.5	33.7	1.3	1.0	1.3[d]
Mtwike x 1.0	26.6	1.5	1.0	1.6[d]
Mtwike x 2.0	32.1	1.9	1.5	3.1[cd]

Mtwike x 3.0	32.3	2.0	2.0	3.2^{cd}
Mzuzu x 0.5	29.4	2.3	1.2	2.7^{cd}
Mzuzu x 1.0	23.6	3.6	2.1	7.2^{b}
Mzuzu x 2.0	27.1	3.9	2.9	10.5^{a}
Mzuzu x 3.0	28.1	2.5	1.9	5.3^{bc}
LSD	7.1	0.9	0.8	2.5
F-test	ns	ns	ns	s
S.E	2.4	0.3	0.3	0.8
CV (%)	14.3	23.6	21.9	25.5

Means followed by the same letters(s) within the column are not significant at different 5 % level based on Student-Newman-Keuls. ns = not significant and s = significant at p < 5 %.

The interaction of banana cultivars and TDZ concentrations had no significant (P < 0.05) effect on sucker height, sucker collar diameter and number of leaves per sucker (Table 6).

Table 6: Interaction effect of banana cultivar and Thidiazuron concentrations on growth of *in vivo* multiplication derived suckers

Cultivar x TDZ conc. Interaction mg/l	Sucker height (cm)	Sucker diameter (cm)	No. of leaves per sucker
Bukoba x 0.5	16.2	2.2	3.6
Bukoba x 1.0	15.3	1.5	4.6
Bukoba x 2.0	15.1	1.8	5.0
Bukoba x 3.0	14.1	1.6	4.0
Mtwike x 0.5	11.7	2.0	3.4
Mtwike x 1.0	15.8	1.9	4.4
Mtwike x 2.0	14.0	1.3	4.6
Mtwike x 3.0	14.2	1.7	4.4
Mzuzu x 0.5	16.6	2.1	3.3
Mzuzu x 1.0	15.3	1.7	4.2
Mzuzu x 2.0	13.6	1.6	4.2
Mzuzu x 3.0	16.0	1.6	4.3
LSD	5.3	0.7	1.5
F-test	ns	ns	ns
S.E	1.8	0.2	0.5
CV (%)	22.2	23.5	22.8

ns = not significant and s = significant at P < 0.05).

DISCUSSION AND CONCLUSION

Effects of TDZ on banana *in vivo* shoot proliferation and growth: In this study, the number of shoots increased from 1.9 to 6.3 shoots per corm as TDZ concentration increased from 0.5 to 2.0 mg/l but decreased to 4.6 shoots per corm as TDZ further increased to 3.0 mg/l. This trend is in agreement with Arinaitwe *et al.* (2000) who also found that *in vitro* proliferation of banana cv. Ndiziwemiti (ABB) increased as TDZ concentration increased from 0.01 to 1.25 mg/l. Similarly, Youmbi *et al*. (2006) reported higher *in vitro* proliferation in lower TDZ concentration ranging from 0.01 to 0.02 mg/l. The *in vivo* multiplication rate reported in this study is relatively low and does not therefore demonstrate any benefits of residual effect of TDZ treatments. Comparatively, a temporary treatment of deshealthed corms of plantain cv. "Itoke Sege" in benzylaminopurine at 1.5 mg /litre produced 17.11 *in vivo* suckers per corm (Kindimba and Msogoya, 2014). In the present study TDZ concentration from 0.5 to 2.0 mg/l increased *in vivo* shoot growth based on the number of leaves per shoots though the number of leaves per sucker decreased as TDZ concentration increased to 3.0 mg/l. The trend in these results is in agreement with Gubuku *et al*. (2004) who also reported that *in vitro* shoot elongation increased as TDZ concentration increased from 0.01 mg/l to 0.22 mg/l but decreased when TDZ increased above 0.44 mg/l.

Similarly, Youmbi *et al.* (2006) reported an increasing number of leaves per *in vitro* shoot as TDZ concentration increased from 0.01 to 0.09 mg/l. In this study banana cv. Bukoba exhibited the highest *in vivo* shoot proliferation rate followed by Mzuzu and Mtwike. Using banana cv. Kibuzi (AAA East Africa highland banana) and cv. Ndiziwemiti (ABB), Arinaitwe *et al.* (2000) also reported that *in vitro* proliferation rate depended on banana genomic groups and cultivars. According to Blakesley (1991) the differential responses to TDZ concentrations among banana genotypes is associated with their difference in cytokinin uptake, translocation to the meristematic region and degradation. Specifically, Youmbi *et al.* (2006) reported *in vitro* proliferation rate of 18.7 shoots per explant of banana (AAA) cv. Gross Michel at TDZ concentration of 0.08 mg/l while banana (ABB) cv. Fougamou required TDZ concentration of 0.20 mg/l to produce the same number of suckers per explant. To conclude, the findings from this study reveal that *in vivo* shoot multiplication of banana cv. Mzuzu, Bukoba and Mtwike can be increased by dipping for 12 hours deshealthed and decorticated corms in TDZ solution at 2.0 mg/l. The response of banana to TDZ depends on cultivars where cv. Bukoba provides the highest *in vitro* multiplication rate in TDZ at 2.0 mg/l. The differential response among banana cultivars to TDZ concentrations underscores the need to determine alternative cytokine-based growth regulators for increasing *in vivo* multiplication of banana cv. Mzuzu and Mtwike.

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