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Full Length Research Paper

Morphological abnormality among regenerated shoots of banana and plantain (*Musa* spp.) after *in vitro* multiplication with TDZ and BAP from excised shoot-tips

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Five cultivars of *Musa* spp (banana and plantain); 'Berangan Intan', 'Berangan' (AAA), 'Rastali', 'Nangka' (AAB) and 'Baka Baling' (ABB) were chosen to compare the effect of benzylaminopurine (BAP) and thidiazuron (TDZ) on multiplication efficiency in relation with frequency of abnormal shoot regeneration. Shoot tips of *Musa* spp. were cultured on MS medium supplemented with various concentrations (0.0, 11.1, 22.2, 33.3 and 44.4 µM) of BAP and (0.0, 0.5, 2, 5 and 7.5 µM) of TDZ. Increasing BAP above 22.2 µM and 33.3 µM increased the number of shoots in 'Berangan Intan', 'Berangan', 'Rastali', 'Nangka' and 'Baka Baling' respectively, but above 33.3 µM significantly caused higher gross of abnormal shoot regeneration. TDZ in the media up to 2 µM for 'Baka Baling', 'Nangka' and 'Rastali' and 5 µM for 'Berangan Intan' and 'Berangan' increased the number of shoots per explant, however TDZ at 5 µM resulted in high number of abnormal shoots. In conclusion BAP at 22.2 µM and TDZ at 2 µM were assumed to be the most suitable for commercial micropropagation system with low frequency of abnormal shoot production for both banana and plantain.

Key words: Abnormality index, cytokinin, micro-propagation, *Musa* spp., shoot tip.

INTRODUCTION

Banana and plantain (*Musa* spp.) are the most important fruit as a staple food source for about 400 million people in developing countries, with a annual world production of around 70.6 million tons in 2004 (Kotecha and Desai, 1995; Ray, 2002; Pua, 2007). *In vitro* propagation is the best choice because of rapid multiplication, uniformity and disease control (Kulkarni et al., 2007). *In vitro* regeneration in banana can be achieved through shoot tip culture as a direct organogenesis (Swamy et al., 1982; Vuylsteke, 1998; Kulkarni et al., 2007). The shoot apices are used as the source materials for the establishment of *in vitro* shoot tip culture (Kulkarni et al., 2007). Different kinds of cytokinins have been used for micropropagation of banana cultivars (Arinaitwe et al., 2000; Roels et al., 2005) and shoot proliferation rate is significantly affected by cytokinin types, their concentrations and type of banana cultivars (Arinaitwe et al., 2000; Gubbuk and Pekmezci, 2004; Roels et al., 2005). Benzylaminopurine (BAP) has been mentioned to be the most commonly preferred cytokinin (Cronauer and Krikorian, 1984; Vuylsteke, 1998; Gubbuk and Pekmezci, 2004). However, Huetteman and Preece (1993) stated that thidiazuron (TDZ) may inhibit shoot elongation. Lee (2001) tested the effect of TDZ in the multiplication of adventitious buds in the banana cultivars by adding TDZ at 0.01 to 9.1 µM. It was concluded that TDZ at 0.91 µM induced the largest number of shoots, but at higher concentration of TDZ (9.1 µM), elongation of shoots was inhibited and clumps of small globular buds appeared at the base of shoots. Gubbuk and Pekmezci (2004) used banana cultivars to study the effects of different cytokinins on shoot

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Table 1. Analysis of variance of shoot regeneration in banana and plantain cultivars.

Source of variation	Df	MS	F-values
Cultivar(C)	4	53.898	101.69[s]
Cytokinin(K)	1	9.375	17.69[s]
Cytokinin rate(H)	4	673.782	1271.29[s]
C*K	4	142.058	268.03[s]
C*H	16	12.234	23.08[s]
K*H	4	5.158	9.37[s]
C*K*H	16	34.498	65.09[s]
Error	100	0.53	

[s] Significant at p = 0.001.
Coefficient of Variation = 8.94.

multiplication. They reported that shoot proliferation and elongation were greater with TDZ than BAP, but BAP above 20 µM and TDZ over 2 µM decreased shoot elongation. The main undesirable side effect of TDZ is abnormal shoot production (Huetteman and Preece, 1993). Bairu et al. (2008) reported that BAP at higher concentration was an inhibitor based on the abnormality index recorded in banana. Farahani et al. (2008) reported that with high concentrations of TDZ the number of normal shoots were reduced and abnormal shoots were observed. In the case of shoot multiplication, Strosse et al. (2008) stated that in contrast to BAP being the most effective at high concentrations, TDZ should be applied at lower concentrations. The objective of the present investigation was to establish the profusely proliferating shoot tips with low frequency of abnormal shoot regeneration in local bananas and plantain through determining suitable concentration of both cytokinins (BAP and TDZ). Consequently this efficient proliferating system can be used for large scale and commercial micropropagation of banana and plantain from excised shoot-tips.

MATERIALS AND METHODS

Micropropagated cultures of banana; 'Berangan Intan' (AAA), 'Berangan' (AAA), 'Rastali' (AAB) and two plantain cultivars; 'Baka Baling' (ABB) and 'Nangka' (AAB) were used as the source of materials for the establishment of *in vitro* shoot tip cultures. The shoot apices were trimmed to a size of approximately 5 to 7 mm by removing several sheathing leaves and excision with minimum basal corm tissues. The shoot apices were transferred to 100 ml capacity conical flasks containing 30 ml of multiplication medium. The multiplication MS medium (Murashige and Skoog, 1962) contained sucrose (30 g/L), gelrite (2.8 g/L) supplemented with different concentrations of benzylaminopurine (BAP) at (0.0, 11.1, 22.2, 33.3 and 44.4 µM). When designing micropropagation experiments it is often necessary to have a lower concentration range for TDZ than the other cytokinins (Huetteman and Preece, 1993), therefore, the concentrations of TDZ were prepared at (0.0, 0.5, 2, 5 and 7.5 µM). The pH of the medium was adjusted to 5.7 prior autoclaving. Cultures were incubated at 26°C ± 2°C under a 16 h photoperiod. Each treatment was replicated three times with each replicate having three explants. After two months of culture growth responses and multiplication efficiency was observed in relation to abnormality index. Parameters include the number of shoots produced per explant (determined by counting all shoots/explant) and average shoot length (determined by measuring three randomly selected shoots) were recorded. Abnormality index was calculated according to Bairu et al. (2008) by taking the ratio of abnormal to normal shoots. Low abnormality index values resulted in higher multiplication efficiency of the treatments. Regenerated shoots were categorized as normal or abnormal based on morphological appearance such as deformation, hyperhydricity and the presence of undifferentiated tissues as described by Bairu et al. (2008). The experiments were arranged in a completely randomized design with three replicates and the data collected were analysed using SAS and MSTATC computer program and comparison of means were tested for significance, using LSD test, at 0.05 level of probability.

RESULTS

The results of analysis of variance of the shoot regeneration responses among banana and plantain cultivars were significantly influenced by the cytokinin type, cytokinin concentrations, cultivar and their interactions (Table 1). The results indicated that the number of shoots significantly increased with increasing concentration of BAP up to 33.3 µM for 'Baka Baling' and 'Nangka' and up to 22.2 µM for 'Berangan Intan', 'Berangan' and 'Rastali' (Figure 1). Results indicated that BAP at 33.3 µM significantly caused higher abnormality index than 22.2 µM in 'Berangan Intan', 'Berangan', 'Rastali' and 'Baka Baling', although in 'Nangka' there was no significant difference in abnormality index between 22.2 and 33.3 µM BAP (Figure 3), with increasing in concentration of BAP up to 44.4 µM shoot regeneration was decreased and the highest gross of abnormality was observed (Figures 1 and 3). Therefore 22.2 µM concentration of BAP was optimum concentration with high normal shoot induction for all cultivars (Figures 1 and 3). Treatments with TDZ in the media up to 2 µM for 'Baka Baling' (ABB), 'Nangka' (AAB) and 'Rastali' (AAB) and 5 µM for 'Berangan Intan' (AAA) increased the number of shoots produced per explant (Figure 2). 'Berangan' (AAA) showed the most shoot proliferation with both concentrations of TDZ (2 µM and 5 µM). Although, TDZ at 5 µM was the best concentration for 'Berangan' and 'Berangan Intan' (AAA), but regarding the high abnormality index caused by TDZ at 5 µM, therefore TDZ at 2 µM was the optimum concentration for commercial micropropagation of banana and plantain (Figures 2 and 4). Figure 5 shows the intensity of abnormal proliferated shoots caused by different concentration of TDZ. With increasing concentration of TDZ above 2 µM, abnormality in regenerated shoots was increased in most cultivars (Figure 4) and also phenolic compounds exudation was observed (Figures 5d and 5e). 'Baka Baling' (ABB), 'Nangka' (AAB) and 'Rastali' (AAB) in free MS medium (without cytokinin) showed higher response of shoot regeneration ability with average of 2.33, 1.33 and 1.67 shoots per explant respectively, than 'Berangan Intan' (AAA) and 'Berangan' (AAA) with average of 0.83 and 0.67 shoots per explants, respectively (Figures 1 and 2). Therefore, it may be concluded that B

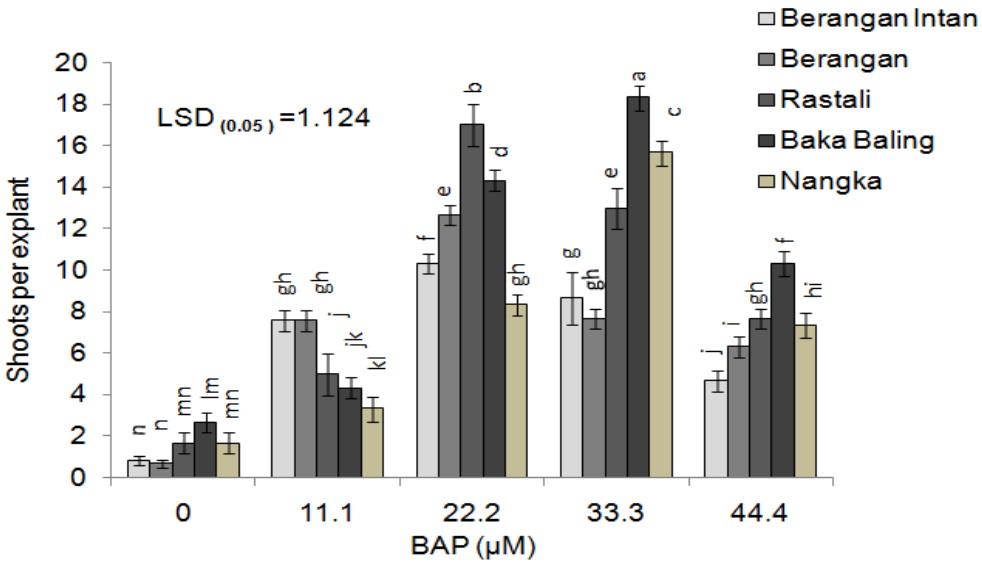

Figure 1. Shoot regeneration response to different concentration of BAP. Values with the same letter are not significantly different at the 0.05 Probability level according the LSD test.

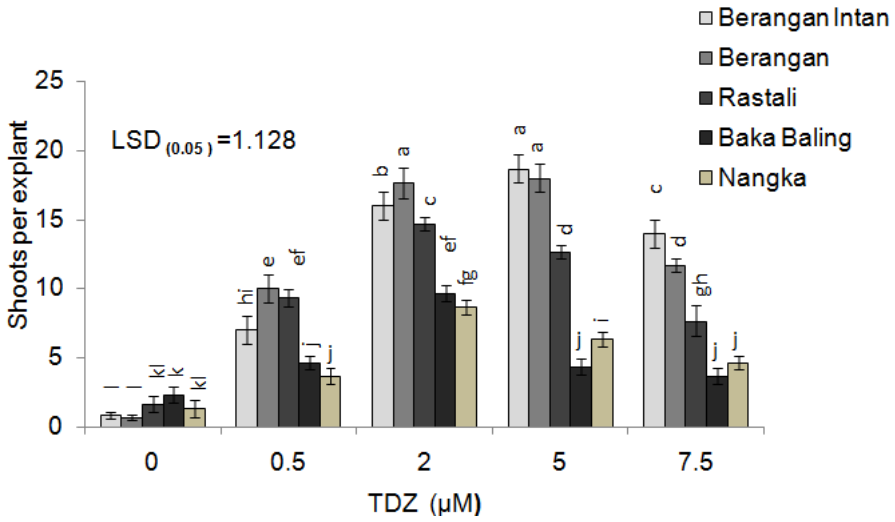

Figure 2. Shoot regeneration response to different concentration of TDZ. Values with the same letter are not significantly different at the 0.05 Probability level according the LSD test.

genome has high regeneration potential rather than A genome. The obtained results showed that cultivars with AAB and ABB genotype ('Rastali', 'Nangka' and 'Baka Baling') were more sensitive than cultivars with AAA group ('Berangan Intan' and 'Berangan') to higher concentrations of BAP (44.4 µM) and TDZ (5 and 7.5 µM) as seen by a higher abnormality index (Figures 3 and 4). Elongation of shoots regenerated from explants differed from one cultivar to another at different level of TDZ and BAP (Figures 6 and 7), but at lower concentrations of BAP (11.1 and 22.2 µM) the length of shoots increased compared to control (Figure 6), as increasing BAP above 11.1 µM reduced shoot elongation in 'Berangan Intan', 'Berangan' and 'Rastali' and 22.2 µM of BAP increased length of shoots in 'Nangka' and 'Baka Baling'. Also with increasing in concentration of TDZ from 0 to 7.5 µM shoot length was reduced for most cultivars (Figure 7). Results indicated that BAP at 22.2 µM produced the optimum number of shoots (10.33, 12.67 and 17) per explants in 'Berangan Intan', 'Berangan' and 'Rastali', respectively, with low abnormality index as compared to other BAP and TDZ treatments.

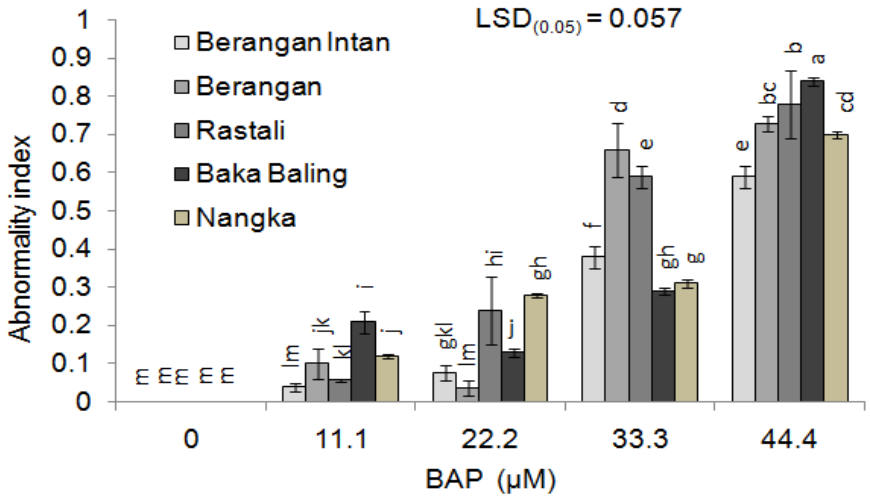

Figure 3. Abnormality index caused by different concentration of BAP. Values with the same letter are not significantly different at the 0.05 Probability level according the LSD test.

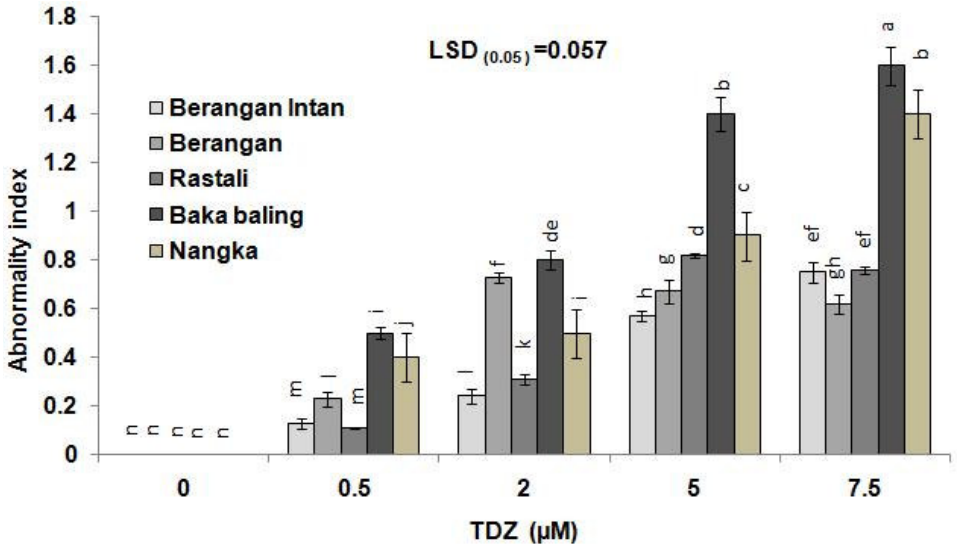

Figure 4. Abnormality index caused by different concentration of TDZ. Values with the same letter are not significantly different at the 0.05 Probability level according the LSD test.

DISCUSSION

The results showed that the types of cytokinins and their concentrations affected multiplication responses among banana and plantain cultivars. Also Arinaitwe et al. (2000) and Gubbuk and Pekmezci (2004) revealed a significant effect of both types and concentrations of cytokinins on the multiplication rate. Arinaitwe et al. (2000) also studied the effect of BAP on banana cultivars. They reported no significant increase in shoot proliferation in 'Kibusi' (AAA) and 'Ndiziwemiti' (ABB) from 16.8 to 28.8 µM, but the cultivar 'Bwara' (AAA) revealed significant increase in number of shoots from 16.8 to 28.8 µM. In our experiment a higher number of regenerated shoots were recorded at 22.2 µM of BAP. Although 'Baka Baling' (ABB) and 'Nangka' (AAB) showed the highest number of shoots (18.33 and 15.67, respectively) at 33.3 µM, the high abnormality index (0.29 and 0.31) recorded at 33.3 µM of BAP gave this treatment unusable. Previous reports (Kalimuthu et al., 2007; Venkatachalam et al., 2007; Bairu et al., 2008) indicated that 22.2 µM of BAP is the optimum cytokinin concentration for most banana tissue cultures and thus support our results. Arinaitwe et al. (2000) were the first to study of TDZ in *Musa* spp. Lee

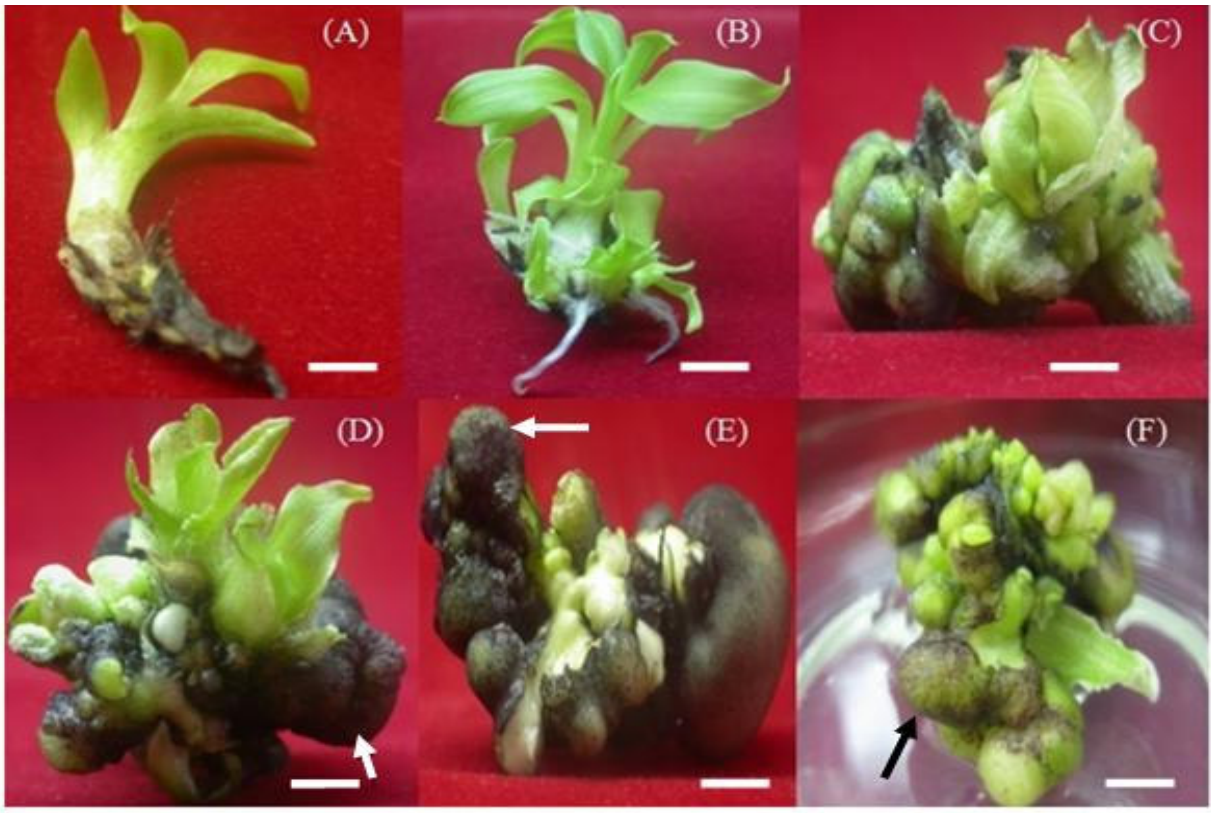

Figure 5. Abnormality index caused by TDZ in *Musa accuminata* cv. 'Berangan Intan' (AAA). A, B, C, D and E, show shoot regenerated on TDZ at 0.0 µM, 0.5 µM, 2 µM, 5 µM and 7.5 µM, respectively; F shows the abnormality in 'Baka Baling' (ABB) at 7.5 µM of TDZ. Arrows show phenolic compounds accumulation and abnormality in regenerated shoots. Bar = 8 mm.

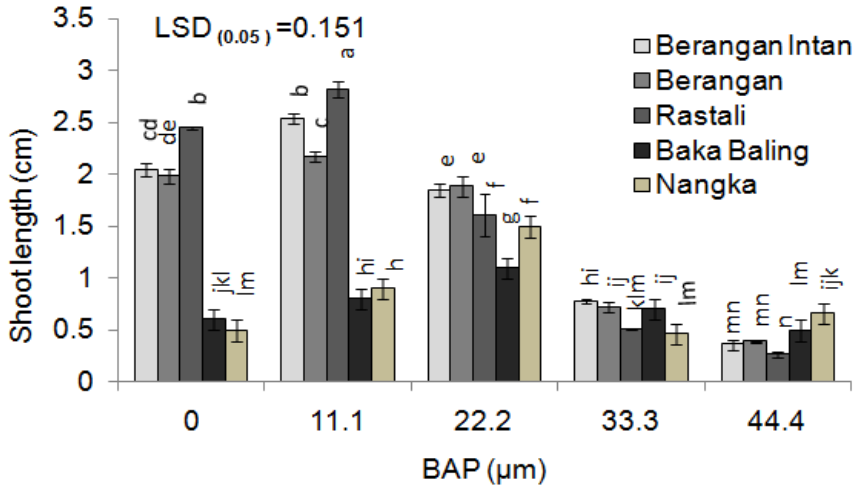

Figure 6. Shoot elongation responses to different concentration of BAP. Values with the same letter are not significantly different at the 0.05 Probability level according the LSD test.

(2001) reported that the effect of TDZ on the multiplication rate varied among banana cultivars (AAA) and (AAB). Higher concentration of both TDZ and BAP generally reduced the number of shoots per explants which is in agreement with the findings of previous reports (Gubbuk and Pekmezci, 2004; Venkatachalam et

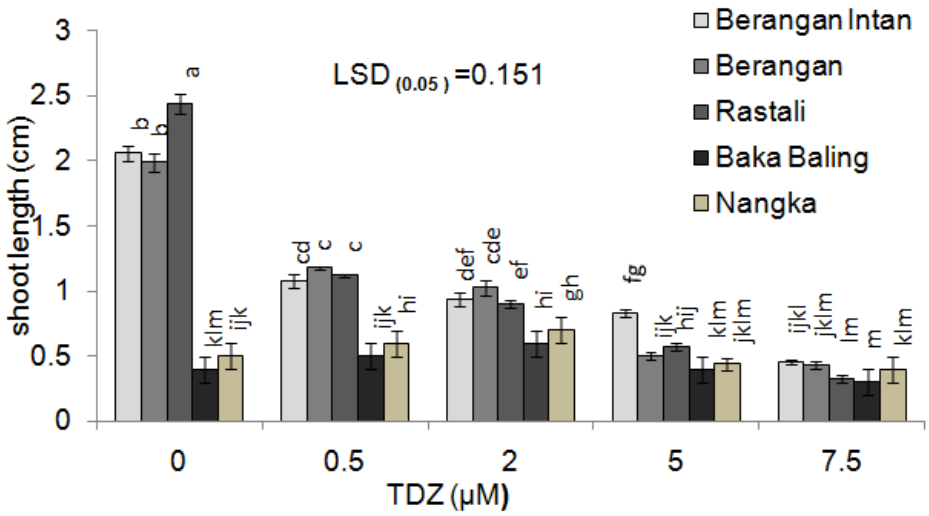

Figure 7. Shoot elongation responses to different concentration of TDZ. Values with the same letter are not significantly different at the 0.05 Probability level according the LSD test.

al., 2007; Strosse et al., 2008; Bairu et al., 2008). Bairu et al. (2008) reported different range of sensitivity to cytokinin concentrations among the genomic group of banana. In this study, TDZ increased the average number of shoots in 'Berangan Intan' and 'Berangan' (AAA) from 0.83 and 0.67 at 0 µM to 18.67 and 18 at 5 µM, respectively, then a reduction in multiplication rate was recorded at 7.5 µM for both cultivars, but in 'Rastali', 'Nangka' (AAB) and 'Baka Baling' (ABB) the most shoots were produced with 2 µM (Figure 2). Therefore it was concluded that AAB and ABB group was more sensitive to higher concentration of TDZ than AAA group. In the case of BAP the results nearly were opposite, as AAA group showed more sensitive to higher concentrations of BAP (Figure 1). These differences in sensitivity may be due to different responses of banana and plantain cultivars to TDZ and BAP. The high rate of abnormal shoot production was recorded at higher concentration of both BAP and TDZ, in a way that BAP at 44.4 µM produced a significantly larger number (0.59, 0.73, 0.78, 0.84 and 0.70) of abnormal shoots per explants for 'Berangan Intan', 'Berangan', 'Rastali', 'Baka Baling' and 'Nangka' respectively and TDZ above 5 µM caused the highest gross of abnormality index in most cultivars tested in this experiment. Roels et al. (2005) reported a lot of morphological abnormality with application of TDZ. The comparatively higher rate of abnormal shoots regenerated with TDZ rather than BAP for all cultivars in our study might be due to the highest cytokinin activity of TDZ, as Huettemen and Preece (1993) stated that after micropropagation with TDZ, the main undesirable side effect is increase of abnormal shoot production. The optimum TDZ concentration varied significantly by cultivars. In our study, shoot regeneration was decreased with increasing concentration of TDZ above 2 µM, as 2 µM was the most suitable concentration for nearly all banana and plantain cultivars tested in this experiment. Also Gubbuk and Pekmezci (2004) reported that the most shoots were produced with 2 µM of TDZ. However, we presented the abnormality index caused by TDZ in all banana and plantain cultivars which can be considered for comercial micropropagation of banana using TDZ. With higher levels of BAP (22.2-44.4 µM) and TDZ (5-7.5 µM), enhanced exudation of phenolic compounds were observed (Figures 5d and 5e), which was similar to studies by Roels et al. (2005). Shoot elongation depends on BAP and TDZ concentration as reported by Gubbuk and Pekmezci (2004) on different banana cultivars. With increasing of the cytokinins concentrations, shoot length was also reduced. Our results indicated that BAP lower than 22.2 µM improved shoot elongation even more than the control treatment but higher than 22.2 µM of BAP reduced shoot length. With increasing of TDZ concentration the shoot length was reduced in all cultivars. The previous studies showed that the types of cytokinin and their concentration significantly influenced shoot multiplication and elongation (Gubbuk and Pekmezci, 2004; Farahani et al., 2008). The study showed that, the lower abnormality index rate the more effective of the treatments, therefore, TDZ at 2 µM and BAP at 22.2 µM were assumed to be the most suitable for an efficient micropropagation system with low frequency of abnormal shoot production for both banana and plantain.

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