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Full Length Research Paper

Carry-over effect of Thidiazuron on banana *in vitro* proliferation at different culture cycles and light incubation conditions

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Thidiazuron (TDZ) is an active cytokinin that was shown to induce increased shoot proliferation and habituation in black walnut, *Phaseolus lunatus* and evergreen azalea, which are tree species but has not been widely investigated in bananas. Unlike other cytokines commonly in use that are adenine-based, TDZ is a urea based cytokinin and therefore is non-degradable by cytokinin-oxidase enzymes in plant tissues. This quality causes TDZ to be persistent in tissues hence transforming them from cytokinin dependence to cytokinin autonomy. This therefore makes use of TDZ cost effective but there is lack of information on this quality in banana micropropagation. A study was therefore conducted to investigate the carry over effect of varying concentrations of TDZ and 22.2 µM benzylaminopurine (BAP) as control on proliferation of five banana cultivars on a hormone free medium under various incubation conditions. The results showed that TDZ had a carry-over effect that enabled shoots to continue proliferating on a hormone free medium as the culture cycles increased and that this effect was significantly ($P<0.05$) higher than that of BAP. Accumulation of TDZ to high levels resulted in suppression of shoot proliferation but on exposing such tissues to a cytokinin-free medium in subsequent subcultures would result in increased shoot proliferation and elongation. The results further showed dark conditions enhanced higher proliferation rates than light conditions in some cultivars suggesting that banana *in vitro* proliferation is a photomorphogenically responsive process that is enhanced under dark conditions.

Key words: Thidiazuron, benzylaminopurine, micropropagation, proliferation rates, recalcitrant, cultivars.

INTRODUCTION

Bananas and plantains are a major starchy staple food in the equatorial belt of Africa stretching from East to West (Hallam, 1995). They are a staple food for nearly 400 million people in the tropics (Schoofs, 1997). The edible portion provides is a rich source of easily digestible carbohydrates, minerals: potassium, magnesium, phosphorus, calcium and iron; vitamin A (in plantains), B_6 and C (in bananas) (Stover and Simmonds, 1987; Jeger their staple food crop. It thus provides both livelihood and income to its producers and traders (MAAIF, 2001). Despite the important of bananas in Uganda, yields have declined from 8.4 tons/ha in 1970 to 5.9 tons/ha in 2000 (MAAIF, 2001). The lack of clean planting material is a serious production constraint responsible for rapid decline of bananas and plantains in Uganda (Rubaihayo and Gold, 1993). Conventional clonal propagation of bananas by suckers, the most practiced method, in addition to pest and pathogen dissemination within the propagation units (suckers) is seriously limited by low multiplication rates (5 - 10 suckers yr-1) and non-uniformity of the crop stand (Vuylsteke et al., 1990). *In vitro* propagation is a thus powerful tool for extending the potential of addressing the limitations of conventional methods of propagation by particularly overcoming the problems low multiplication rates and pathogen dissemination (Vuylsteke, 1998).

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Shoot proliferation *in vitro* largely depends on the concentration of cytokinin in the medium (Razdan, 1993; Trijilo and Garcia, 1996). However, although different micropropagation protocols using different cytokinins have been used for several *Musa* species (Vuylsteke, 1989), those employed are mainly adenine based ones like benzylaminopurine (BAP), 2-isopentyladenine (2ip) and zeatin (Talengera et al., 1994; Crouch et al., 1998). Diphenyl urea derivatives such as TDZ have not been widely used in *Musa* species. TDZ is the most potent of the urea-based compounds and the one that was first evaluated for use in plant tissue culture by Mok et al. (1982). TDZ has been shown to induce habituation in some species (Huetteman and Preece, 1993). Mok et al. (1982) showed that *Phaseolus lunatus* callus became cytokinin autonomous when cultured on media containing various concentrations of TDZ. Similar observations were also made by Neuman et al. (1993) in tree species culture when TDZ was present in the primary medium for eastern black walnut cotyledon explants. This quality makes the use of TDZ in micropropagation cost effective. However, there is lack of information on this quality of TDZ in East African Highland bananas and plantains.

The activity of any growth regulator when used in *in vitro* is influenced by environmental conditions (Razdan, 1993). Light intensities of 1500 - 3000 lux are used for *in vitro* plantlet incubation though higher or less ones may be required for some plant species (Vuylsteke, 1989). The 16 h daily cycle of light at an intensity of 1173 ± 42 lux was used for routine micropropagation of East African highland bananas by Talengera et al. (1994) but there has been no research on the effect of dark conditions on the rates of proliferation in banana micro propagation. The main objective of this study was, therefore, to investigate the carry-over effect of TDZ supplemented medium on the proliferation of the selected banana cultivars in a hormone free medium under varying light incubation including dark conditions.

MATERIALS AND METHODS

The study was carried out in Plant Tissue Culture Laboratory at Makerere University Agricultural Research Institute Kabanyolo (MUARIK). The sword suckers and peepers of five cultivars from which the explants were excised were obtained from the field gene bank of East African High Bananas at MUARIK. The methods of explant excision, disinfection and inoculation were those of Talengera et al. (1994). After nine weeks of culture inoculation on modified Murashige and Skoog (1962) banana multiplication medium (Talengera et al., 1994), multiple axillary shoots that had formed on each explant were separated and re-inoculated onto MS media supplemented with 5.14, 7.14, 9.14, 11.14 and 13.14 µM TDZ with BAP at 22.2 µM (Crouch et al., 1998) used as control. The shoot cultures were incubated for a basal cycle of six weeks under16 h light of intensity 1773 ± 42 lux and temperature of 26 ± 2°C after which the proliferated shoots were separated and inoculated on hormone-free MS medium. The shoots were incubated at varying daily light incubation conditions of dark, 8 and 16 h in the growth room at the same temperature. Subculturing on hormone free-MS medium was done for three culture cycles to establish the carry-over effect of the TDZ concentrations and BAP used in the basal cycle in subsequent culture cycles. At the end of each of the three culture cycles, shoots per inoculated shoot were recorded and the data collected analysed.

RESULTS

Generally, the results indicated that mean shoot proliferation rates were significantly higher after the basal cycle with various TDZ concentrations than with 22.2 µM BAP in all the cultivars and light incubation conditions (Figures 1 - 5). The results of proliferation rates of *Kibuzi* on hormone free MS medium at different subculture cycles and light incubation conditions after a 6-week basal cycle exposure to various TDZ concentrations and 22.2 µM BAP are presented in Figure 1. Mean proliferation rates after the basal cycle of 22.2 µM BAP were significantly ($P<0.05$) lower than those where the basal cycle medium was supplemented with TDZ irrespective of the light incubation conditions and subculture cycles. This suggested that BAP had a poor carry over effect on shoot proliferation. Shoot proliferation was highest on 7.14 µM TDZ, suggesting that this was the optimum concentration for this cultivar. The results also showed a trend of increase in proliferation rates with increase in the number of culture cycles resulting from the residual effect of TDZ treatment in the basal cycle. The results proliferation rates of cultivar *Sukalindizi* are presented in Figure 2. As in cultivar *Kibuzi*, shoot proliferation of *Sukalindizi* was lower on 22.2 µM BAP than on TDZ irrespective of the light incubation conditions and subculture cycles. Shoot proliferation in cultivar *Sukalindizi* was generally highest on hormone free medium after the basal cycle with 7.14 µM TDZ except at 16 h light when it peaked at 9.14 in the third culture cycle (Figure 2C). The results of proliferation rates of cultivar *Gros Michel* on hormone free MS medium at different subculture cycles an light incubation conditions after a 6-week exposure to various TDZ concentrations and 22.2 µM BAP are presented in Figure 3. Shoot proliferation rates at 22.2 µM TDZ were significantly ($P < 0.05$) lower than those at various TDZ concentrations. For the different TDZ concentrations used in the basal cycle, the resultant proliferation rates on the hormone free medium were highest at 7.14 µM TDZ. The results proliferation rates of cultivar *Bwara* are presented in Figure 4. Unlike in cultivars *Kibuzi*, *Sukalindizi* and *Gros Michel*, the results indicated that *Bwara* proliferated best at 5.14 µM TDZ after which its proliferation rates gradually declined irrespective of the light incubation conditions. The proliferation rates on 22.2 µM were significantly ($P < 0.05$) lower than TDZ, but higher than those in the other cultivars (>2.0) suggesting that the carried over BAP was enough to induce proliferation in this highly prolific cultivar. As in the rest of the cultivars, there was a general increase in proliferation rates with increase in culture cycles on various TDZ concentrations used in the basal cycle. The results of proliferation rates

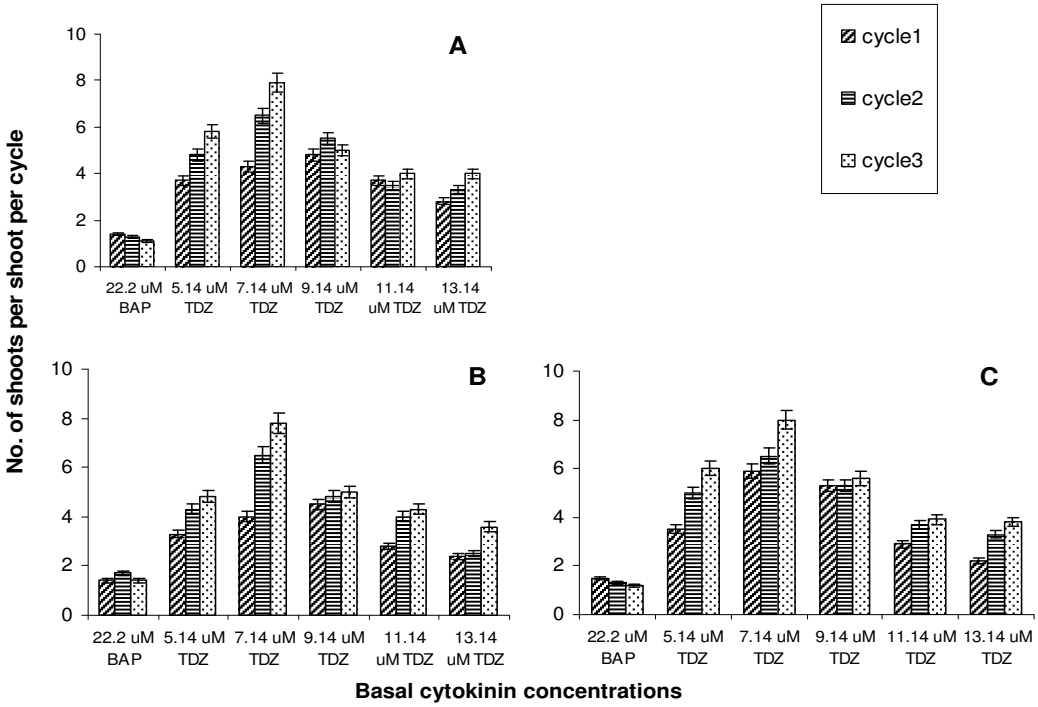

Figure 1. Proliferation rates of *Kibuzi* on Hormone free MS medium at different culture cycles and light incubation conditions (A-dark, B- 8 and C-16 hours light/d) after a basal cycle with various TDZ concentrations and 22.2 µM BAP (control).

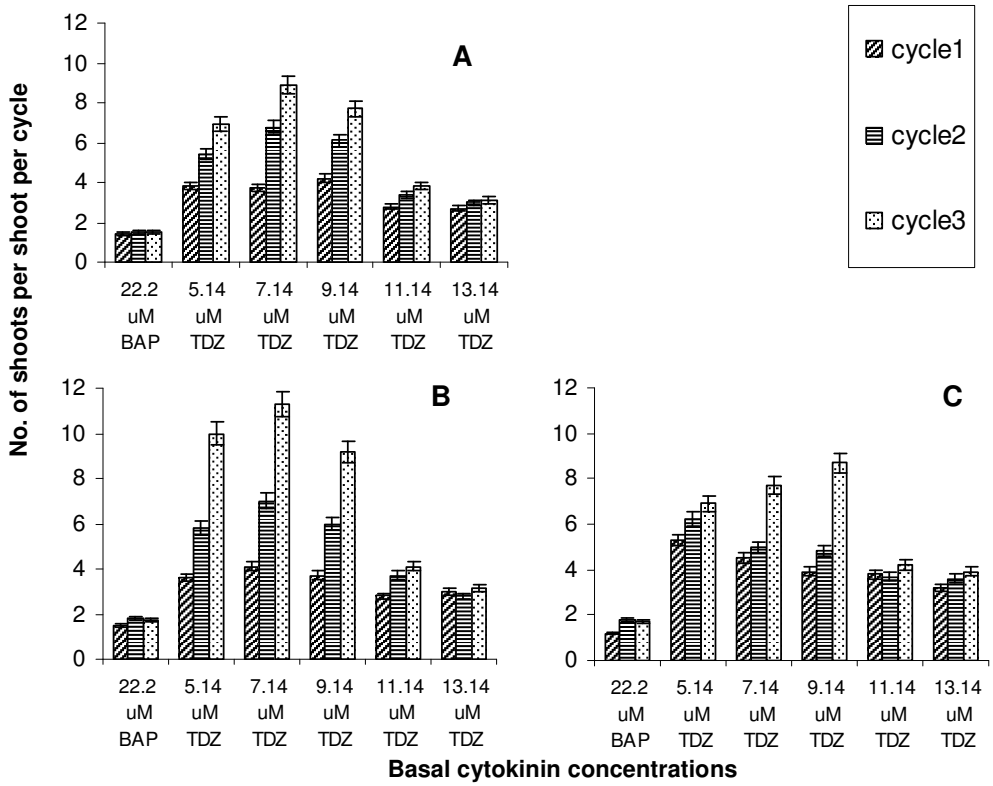

Figure 2. Proliferation rates of *Sukalindizi* on Hormone free MS medium at different culture cycles and light incubation conditions (A-dark, B- 8 and C-16 hours light/d) after a basal cycle with various TDZ concentrations and 22.2 µM BAP (control).

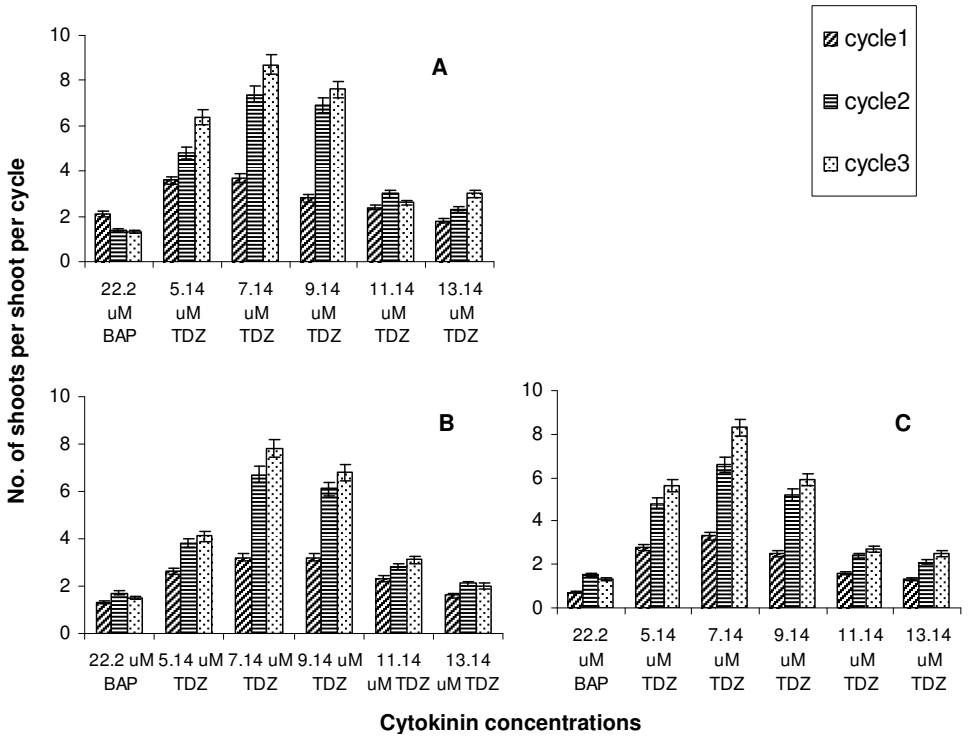

Figure 3. Proliferation rates of *Gros Michel* on Hormone free MS medium at different culture cycles and light incubation conditions (A-dark, B- 8 and C-16 hours light/d) after a basal cycle with various TDZ concentrations and 22.2 µM BAP (control).

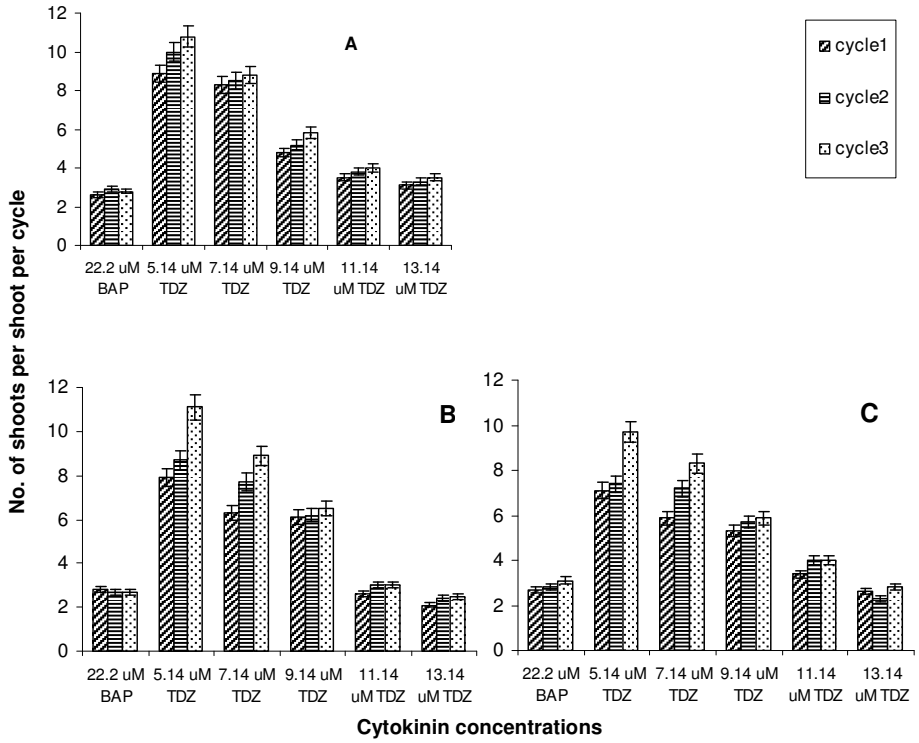

Figure 4. Proliferation rates of *Bwara* on Hormone free MS medium at different culture cycles and light incubation conditions (A-dark, B- 8 and C-16 hours light/d) after a basal cycle with various TDZ concentrations and 22.2 µM BAP (control).

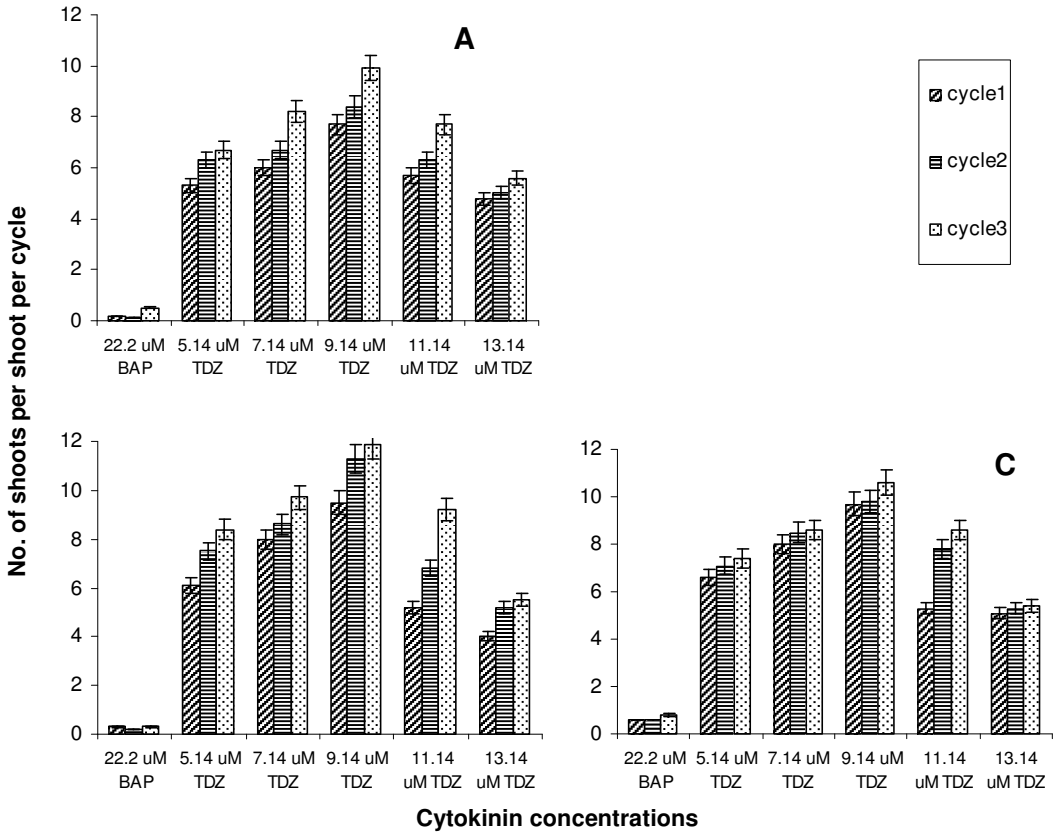

Figure 5. Proliferation rates of *Kifuba* on Hormone free MS medium at different culture cycles and light incubation conditions (A-dark, B- 8 and C-16 hours light/d) after a basal cycle with various TDZ concentrations and 22.2 µM BAP (control).

of Kifuba on hormone free MS medium at different subculture cycles and light incubation conditions after a 6-week exposure to various TDZ concentrations and 22.2 µ BAP are presented in Figure 5. In *Kifuba*, proliferation rates on 22.2 µM BAP used in the basal cycle were much lower than in the rest of the cultivars (<1) suggesting that the carried over BAP concentration was too low to induce proliferation in this highly recalcitrant cultivar (Talengera et al., 1994). There was an increase in proliferation rates from shoots initially cultured on various TDZ concentrations up to 9.14 µM but after which there was decline in proliferation rates irrespective of light incubation conditions. As in the rest of the cultivars, the highest proliferation rates were recorded in the third culture cycle with TDZ irrespective of the light incubation conditions. In cultivars *Sukalindizi*, *Gros Michel* and *Bwara*, higher proliferation rates were recorded in the dark than at 8 and 16 h of light when TDZ was used (Figures 2, 3 and 4).

DISCUSSION

The results of this study indicate that the proliferation rates of shoots originating from the basal cycle medium with various TDZ concentrations were significantly ($P < 0.05$) higher than those from 22.2 µM BAP in all the five cultivars (Figures 1 - 5) suggesting that TDZ had a high carry over effect which enabled the shoots to continue proliferating on the hormone free medium. Similar observations were made by Neuman et al. (1993) while working on eastern black walnut (*Juglans nigra*) cotyledon cultures in which they found that when cultured on a primary medium containing TDZ, the cultures continued to grow on transfer to a secondary medium lacking growth regulators. Similar findings were also reported by Gill and Oziaz-Akins (1998) in peanut callus cultures while Christena et al. 1995 demonstrated that 2-day exposure of *Geranium* cultures to 5 µM TDZ was sufficient to evoke higher embryogenic response than continuous exposure. TDZ therefore has the capacity of transforming cultured tissues from cytokinin dependence to cytokinin autonomy (Mok et al. 1987).

The results also suggested that the carry-over effect of TDZ (manifested in shoot proliferation rates on hormone free MS medium) on shoot proliferation rates was influenced by the concentration used in the basal cycle (Figures 1 - 5). Cultivar *Bwara* proliferated highest (7.6 - 9.9) a the lowest TDZ concentration of 5.14 µM used in

the basal cycle (Figure 4) suggesting that it is highly prolific (highly non-recalcitrant) cultivar as reported by Talengera et al. (1994) has a high content of endogenous cytokinins hence requiring low TDZ concentrations for effective proliferation. In contrast, *Kifuba* proliferated highest (8.6 - 10.6) at a relatively high TDZ concentration of 9.14 µM (Figure 5) suggesting that it is recalcitrant cultivar (Talengera et al., 1994). It therefore probably has less endogenous cytokinin content, hence requiring higher exogenous cytokinins concentrations for effective proliferation. Cultivars *Kibuzi, Sukalindizi* and *Gros Michel* proliferated maximally at 7.14 µM TDZ suggesting that they have moderate endogenous cytokinin content (Figures 1 - 3). Pierik, 1987 suggested that the inherent endogenous cytokinin levels in different cultivars account for the expression of variations in cultivars shoot proliferation responses to different exogenous cytokinin concentrations.

Very high levels of TDZ beyond 9.14 µM resulted in proliferation decline indicating that high TDZ levels suppress shoot proliferation. Unlike adenine and purine-based cytokinins like BAP, TDZ is resistant to cytokinin-degrading enzymes, and hence when used at high levels in the basal medium, the persistent concentrations in the tissues remain high thus inducing excessive suppression of lateral buds, consequently resulting in reduced proliferation rates (Hueteman and Preece, 1993). Arinaitwe et al. (2000) reported that high TDZ concentrations inhibit axillary shoot proliferation and stimulate formation of undistinguishable bulbous structures called scalps in bananas while Thomas and Katterman (1986) reported that high TDZ concentrations resulted in a higher number of extremely stunted and undifferentiated shoots in tobacco cultures.

There was a general trend of increase in proliferation rates with culture cycles on a hormone free medium after TDZ had been used in the basal cycle, the highest being in the third subculture cycle in all the cultivars (Figures 1 - 5) suggesting that subculturing on a hormone free medium had a resultant reduction in the amount of TDZ carried over at each subculture cycle hence releasing the dormant buds that result in increased shoot proliferation. Similar observations were made by Christena et al. (1995) in somatic embryogenesis of *Geranium* in which embryogenic response of callus cultures increased at every cycle of subculture on a secondary medium devoid of hormones after an eight-day exposure to 5 µM TDZ.

Cultivars *Sukalindizi, Gros Michel* and Bwara had higher proliferation rates in the dark than in 8 and 16 h light at different TDZ concentrations suggesting that *in vitro* proliferation of bananas is enhanced under dark conditions. This could be true since *in vitro* photosynthesis was found to be unnecessary by Hartmann et al. (1990). Similar observations have also been reported in other crops. For instance Pinker (2001) reported higher growth parameters of shoot proliferation rates and fresh weight in the dark than in chopper (intermittent) or continuous light in deciduous plant cultures while Rusli et al. (1998) reported similar observations when using dark and low irradiance in *in vitro* culture of *Rosalia hybrida*.

Conclusions and Recommendations

From this study, it is deduced that TDZ has a carry-over effect that transforms cultured tissues of East African Highland bananas from exogenous cytokinin dependence to cytokinin autonomy. It is therefore, recommended that for cost-effective micropropagation, prolific, intermediate and recalcitrant banana cultivars with different levels of endogenous levels of cytokinin content be respectively cultured using TDZ at 5.14, 7.14 and 9.14 µM for a single cycle after which subculturing should be done on a hormone free medium for three or more cycles since the proliferation rates were still increasing at three culture cycles on a hormone free medium. It is also noted from this study that dark conditions or low intensities can substitute for the relatively high light intensities conventionally used in the culture growth rooms. It is thus recommended that in routine banana micropropagation, cultures be incubated in the dark during multiplication and provided with light at the rooting stage to reduce the production costs per plantlet since costs of providing artificial light and controlling temperatures that accrue from this will have been eliminated.

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