Effect of Thidiazuron, Benzylaminopurine and Naphthalene Acetic acid on *In vitro* propagation of Tuberose *(Polianthes tuberosa L.)* from shoot tip explants

M. J. Hutchinson , R. Onamu and S. Obukosia

Department of Crop Science, University of Nairobi, P.O. Box 30197, Nairobi, Kenya. Email: hutchjesang@yahoo.com

ABSTRACT

Tuberose (*Polianthes tuberosa L.)* is an important export crop among small-scale farmers in Kenya. One of the main challenges facing production and marketing of good quality Tuberose cut flowers is the lack of clean planting material as the resource-poor farmers multiply their own propagules. The main objective of the present study was to evaluate the potential of Thidiazuron (TDZ), a phenyl urea, benzylaminopurine (BAP), a cytokinin and naphthalene acetic acid (NAA), an auxin, on in vitro propagation of tuberose from shoot tip explants. No multiple shooting was observed in any of the treatments tested. Results from the study indicated that TDZ, at low concentrations, was more potent than BAP in increasing shoot length and quality as well as the number of leaves per shoot. However, TDZ at high concentration (5 μM), was toxic leading to death of the shoot explant. Inclusion of NAA in media either supplemented with TDZ or BAP led to formation of calli, which did not differentiate further.

1.0 INTRODUCTION

Tuberose (*Polianthes tuberosa L.)* has gained importance as an export crop among small-scale farmers in Kenya due to its simple management practices and ease of growth outdoors (Chebet, 1999; HCDA, 1997). Further expansion of production of this crop has, however, been hampered by unavailability of clean planting material (Waithaka, 1986). Currently, most farmers multiply their own planting material on farm, resulting in high levels of pest and disease infestation with poor quality produce that cannot adequately compete in the international market (Waithaka, 1986; HCDA, 1997).

The role of tissue culture in the production of disease-free planting material is clear. Most culture systems involve the use of auxins and cytokinins (Skoog and Miller, 1957). A few attempts to propagate Tuberose *in vitro* using NAA and BAP have been carried out using leaf explants (Waithaka, 1986; Sanjal *et al.* 1998) and bulb segments

(Benschop, 1993). The use of shoot tips as explants may provide cleaner planting material because of absence of vascular connections to the tip (Salisbury and Ross, 1991).

Thidiazuron, a phenyl urea, originally developed as a cotton defoliant, has been found to be a potent chemical for morphogenesis in vitro for many plant species such as peanut, tobacco, geranium (Capelle *et al.* 1983; Gill and Saxena, 1992; Hutchinson *et al.* 1996; Mok *et al.* 1982; Thomas and Katterman, 1986; Visser *et al.* 1992). Thidiazuron has often been found to be more potent or as potent as auxins and cytokinins, sometimes substituting for requirements of both, for plant regeneration (Visser *et al.* 1992). To our knowledge, there has been no report of using shoot tips as explants or TDZ as a chemical of choice for the propagation of Tuberose.

The purpose of the study was to evaluate the potential of TDZ in the micropropagation of Tuberose (*Polianthes tuberosa L.)* using shoot tips as explants.

2.0 MATERIALS AND METHODS

2.1 Stock plant care

Tuberose stock plants with shoots and bulbs were obtained from Cianda Flowers Ltd, a commercial export flower grower in Kiambu district of Kenya, which is situated at 2300 m above sea level and around 10° South of the Equator. The Tuberose plants were planted and cared for under optimal cultural conditions (Chebett, 1998; Waithaka, 1986). Bulbs were dipped in Benlate fungicide solution before planting at a depth of 5-10 cm on 1m wide raised beds. During planting, organic manure combined with DAP (125kg/ha) was incorporated into the soil. Top dressing was carried out after planting using CAN at 100kg N/ha as a split application 30 and 60 days after planting. Manual weeding was done to keep the beds weed–free. The crop was sprayed against fungal infections e.g stem rot, botrytis using Dithane M-45 and against mites using Rogor E respectively.

2.2 Explant preparation and surface sterilization

Tuberose bulbs, obtained from 90 day old crop were cleaned with a detergent (dilute Econo) to remove excess soil particles, and rinsed in running tap H_2O for 15 min. Excised tips (1-2 cm long) were placed in sterile water in a beaker. The tips were surface sterilized by immersing in 70% ethanol for 5 min and then immersing in 1.5% sodium hypochlorite solution containing Tween 20 (2 drops/100ml solution) for 5 mins and then

rinsed three times with sterile distilled water. Shoot tips consisting of an apical dome and one or two leaf primodia and measuring between 0.5–1.0mm long were excised under a dissecting microscope and used as explants.

2.3 Culture of shoot tip explants

One excised shoot tip was cultured aseptically in a universal bottle containing 10 mL of media. The culture medium consisted of MS (Murashige and Skoog, 1962) salts, B5 (Gamborg *et al.* 1968) vitamins, 30 g/L sucrose and was supplemented with various plant growth regulators in various concentrations and/or combinations as follows:

- TDZ (0.1, 0.4, 1 and 5 μM)
- BAP (0.05, 0.1 and 1 mg/L)
- NAA (0.01 and 0.1 mg/L)
- BAP +NAA: (0.1mg/L BAP+0.01 mg/L NAA; 1mg/L BAP+0.01 mg/L NAA; 1mg/L BAP+0.1 mg/L NAA
- TDZ +NAA: (0.1μm TDZ+0.01mg/LNAA, 1μm TDZ +0.01 mg/L NAA, 1μm TDZ + 0.1 mg/L NAA).

Basal medium devoid of any plant growth regulators (MSO) acted as a control in all experiments. The pH of the media were adjusted to 5.7± 0.1 before autoclaving at 121^0C for 20min. The cultures were placed on growth shelves set at 25 ± 2^0 C and illuminated (16 h photoperiod 70 – 78 μmol/m^2/s) by cool white fluorescent tubes.

The explants cultured in media supplemented with TDZ were maintained on the media for 10 days and then transferred to MSO. All other explants were maintained on the medium for 4 weeks and sub-cultured to fresh similar medium after every 4 weeks. After 12 weeks in culture, 9 shoots/universal bottle were transferred to rooting media for further growth and development.

Shoot length, numbers of leaves/stem and the shoot quality rating were assessed every week for a period of 3 months (12 weeks). Shoot quality rating involved a visual rating of 0-4: 0- poor growth low chlorophyll content and browning of tissues while 4- best growth, good/ sufficient chlorophyll accumulation).

2.4 Experimental design and statistical analysis.

All experiments were laid out in a Completely Randomized Design and each treatment was replicated 3 times and all experiments were repeated at least twice.

Data on % rooting of plantlets were subjected to arcsine prior to statistical analysis. Data were analyzed using the analysis of variance (GENSTAT) stastical package (Lane and Payne, 1996) and the means were compared using Tukey's procedure at 5% level of significance.

3.0 RESULTS

A single shoot was formed during in vitro propagation of Tuberose using shoot tips as explants (Fig. 1) with no evidence of multiple shoot formation in any of the treatments tested. The addition of various plant growth regulators to the induction medium significantly influenced length and quality rating of the shoot formed as well as number of leaves per shoot. Placing Tuberose explants in a TDZ-supplemented induction medium for 10 days, and subsequently transferring them to a basal medium devoid of plant growth regulators, significantly increased the shoot length (Fig. 2). The length of shoot increased with increasing concentration up to 1.0 μM TDZ concentration (Fig. 2). Placing Tuberose shoot tips in 1 μM TDZ gave the longest shoots averaging about 2.3 cm after 8 weeks of culture while high concentrations (5 μM) had no effect after 5 weeks in culture. On the other hand, addition of low concentrations of BAP (0.05-0.1 mg/L) into the culture medium had no significant effect on shoot length up to 5 weeks in culture but showing a slight improvement in shoot length after 8 weeks of culture. Benzylaminopurine at a concentration of 1 mg/L however slightly increased the shoot length. A combined NAA + BAP or TDZ + NAA resulted in callus formation from shoot tip explants after 4 weeks of culture, the callus showing slow growth over 8 weeks of culture (data not shown).

Figure 1. **In vitro propagation of Tuberose (Polianthes tuberosa L.) from shoot tip cultures after 8 weeks in culture**

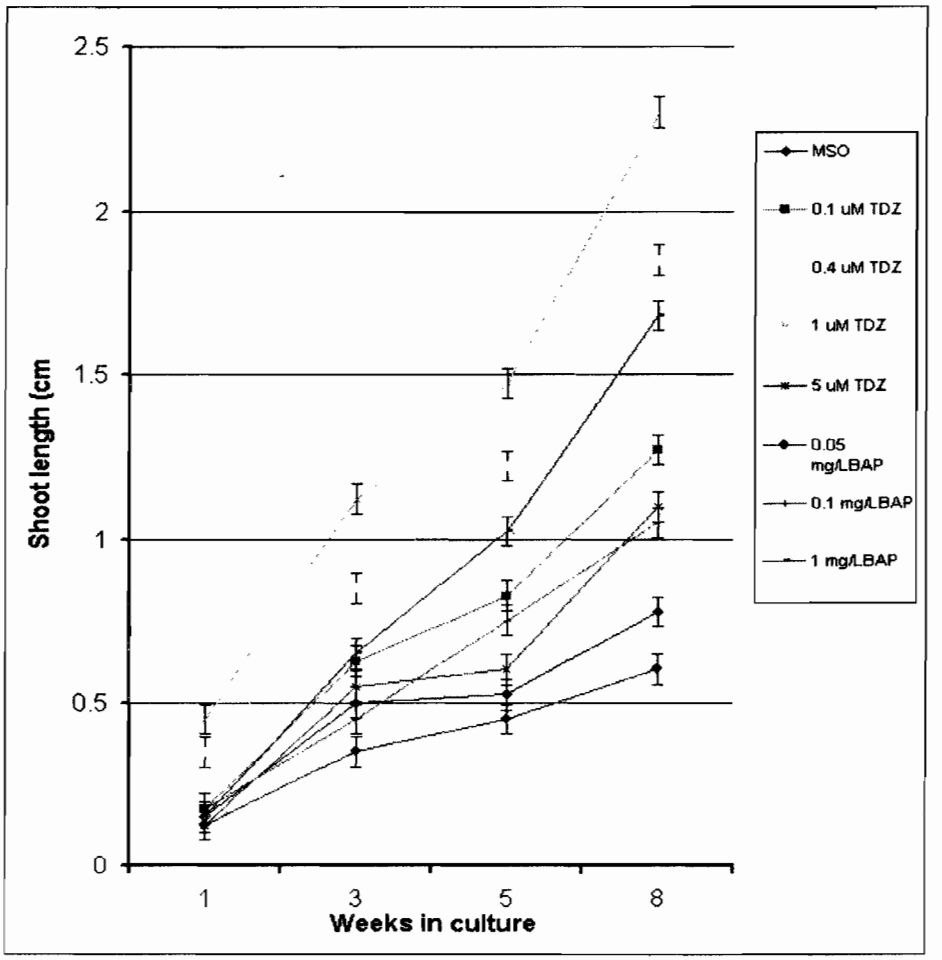

Figure 2. Effect of Thidiazuron (TDZ) and BAP on shoot length (cm) from in vitro shoot tip cultures of Tuberose (*Polianthes tuberosa* L.)

Mean separation using Fishers' LSD. Vertical bars represent standard error bars

Addition of various concentrations of TDZ or BAP improved quality of shoots within 8 week of culture above those held in basal medium (Fig. 3). Thidiazuron at 5μM concentration was especially effective in improving shoot quality. The presence of NAA in TDZ or BAP supplemented media caused a significant drop in the quality of shoots formed from Tuberose shoot tip cultures after 4 weeks in culture (Data not shown). The shoots actually turned to cream to greenish compact calli (data not shown).

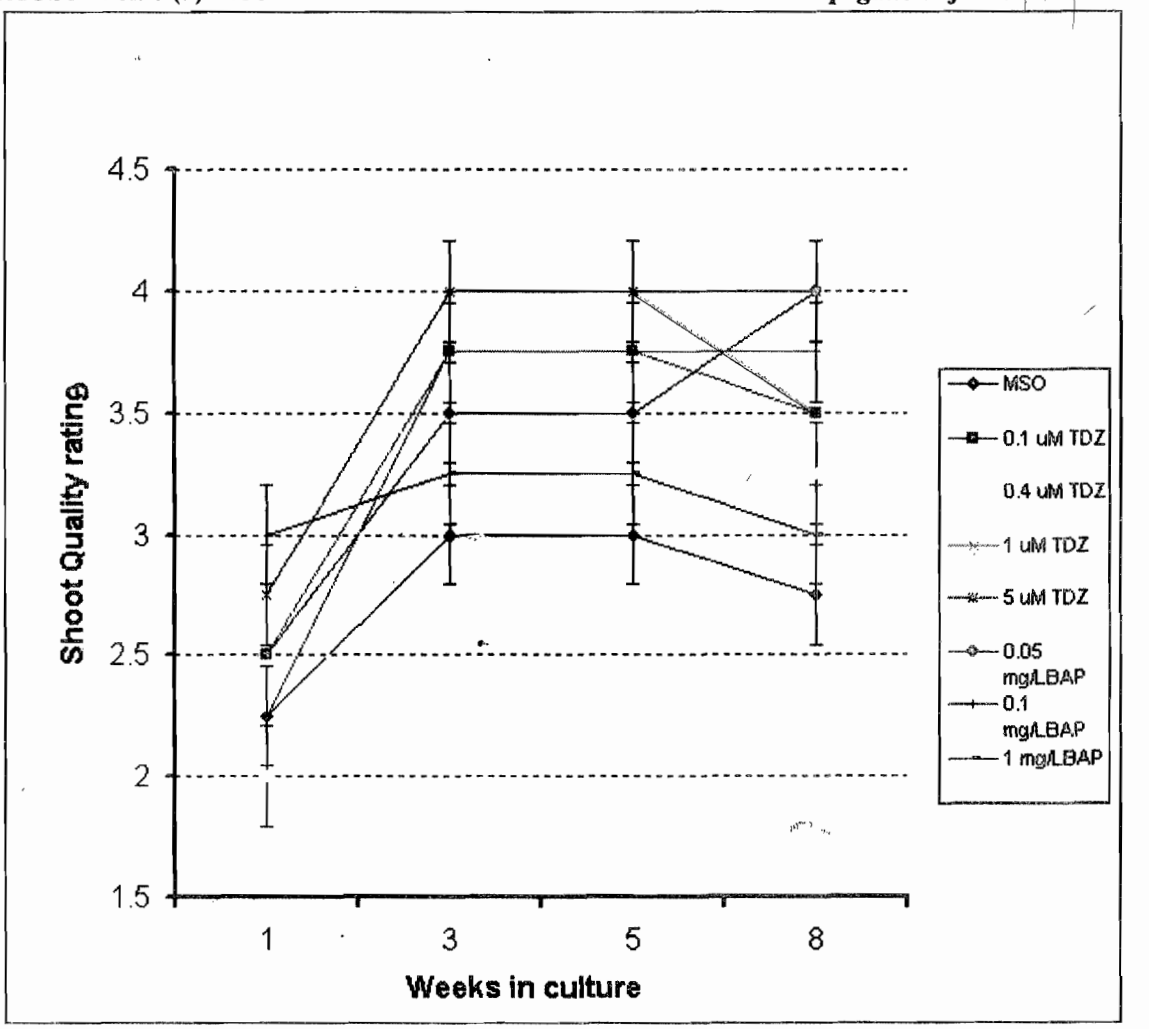

Figure 3. Effect of Thidazuron (TDZ) and BAP on quality shoot rating of shoots from in vitro tip cultures of Tuberose (*Polithes tuberosa* L.)

Mean separation using Fishers' LSD. Vertical bars represent standard error bars

The addition of various plant growth regulators to the culture medium had a significant influence on the number of leaves per shoot of Tuberose shoot tips cultured in vitro (Fig. 4). Stems of shoot tips cultured in basal medium devoid of any plant growth regulators had an average of 1-1.5 leaves while those held in low concentrations of TDZ (0.1-1.0 μM) had an average of 2.5-3.5 leaves per shoot. Low concentrations of BAP (0.05-0.1 mg/L) and high concentrations of TDZ (5 μM) had only a slight improvement on the number of leaves per shoot.

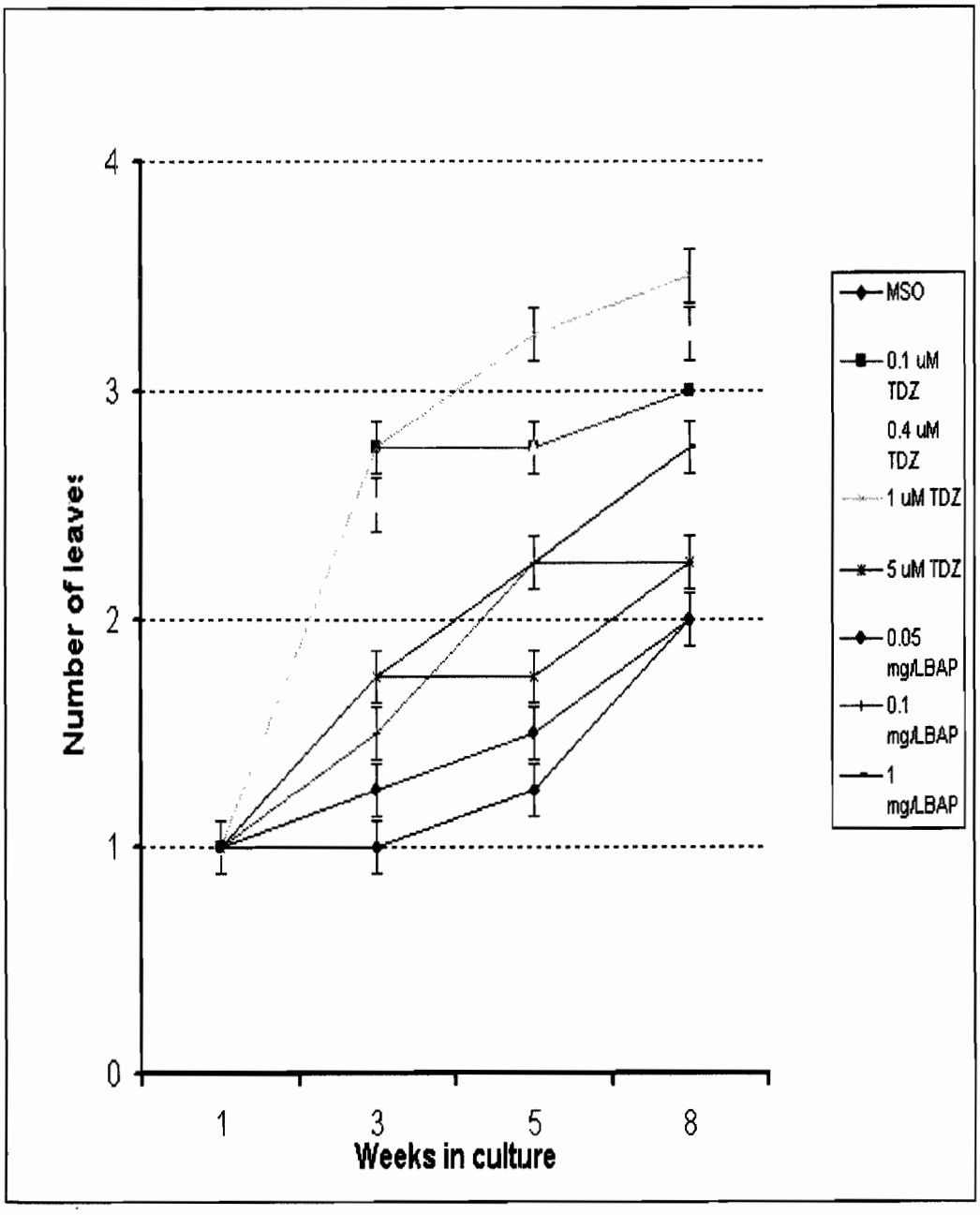

Figure 4. Effect of Thidiazuron (TDZ) and BAP on number of leaves per shoot from in vitro shoot tip cultures of Tuberose (*Polianthes tuberosa* L.)

Mean separation using Fishers' LSD. Vertical bars represent standard error bars

4.0 DISCUSSION AND CONCLUSION

All morphogenic responses in vitro are regulated, primarily, by an intricate balance of phytohormones especially auxins and cytokinins (Komamine *et al.* 1992; Skoog and Miller, 1957). Thidiazuron, however, has been shown to exhibit strong cytokinin-like activity in several plant systems (Capelle *et al.* 1983; Mok *et al.* 1982) acting either directly or through modulation of endogenous levels of plant hormones (Murthy *et al.* 1995; Hutchinson *et al.* 1996). Thidiazuron has been found to be more potent or as potent as combined auxins and cytokinins, sometimes substituting for requirements of both for plant regeneration (Visser *et al.* 1992).

The results from the present study indicate that Thidiazuron is more potent, at low concentrations, than BAP in increasing shoot length and quality as well as number of leaves per shoot. These results suggest that TDZ could be exhibiting stronger cytokinin activity than BAP, results that are consistent with observations in most cytokinin bioassays (Capelle *et al.* 1983; Mok *et al.* 1982). Higher concentrations of TDZ although indicating a dwarfing effect, improved the greening of shoots formed, suggesting greater chlorophyll accumulation in shoots (Kaul and Sabharwal, 1972; Kefford *et al.* 1973).

Supra-optimal levels of TDZ have been reported to inhibit morphogenic responses such as somatic embryogenesis in geranium (Hutchinson *et al.* 1996; Visser *et al.* 1992), shoot elongation of peanut (Murthy *et al.* 1995), oats, maize and radish (Devlin *et al.* 1989) and pumpkin hypocotyls (Burkhanova *et al.* 1984). The inhibition of shoot elongation by TDZ at higher concentrations may be attributed to ethylene effects. Thidiazuron has been reported to cause elevation in endogenous ethylene (Hutchinson *et al.* 1996), which in turn causes stem thickening and shortening (Salisbury and Ross, 1991).

Combined BAP and NAA or TDZ+NAA induced friable to compact, cream to green callus. Callus formation could be as a result of modulation of endogenous phytohormones in the tissues such that the auxin: cytokinin ratio attained was sufficient to induce calli (Skoog and Miller, 1957). Similarly, combined BA with NAA (0.2-0.5 mg/L) in Tuberose leaf explants (Sanjal *et al.* 1998) resulted in callus formation. TDZ alone or in combination with NAA had no significant difference in the regeneration of plantlets from carnation shoots (Watada *et al.* 1996). Although inclusion of both auxin and cytokinin in the propagation medium supported the survival of shoot tip explants of

onion (Rabinowich and Brewster, 1990), inclusion of NAA (0.01-0.1 mg/L) resulted in the death of shoot tip explants, suggesting that phytohormones could have been present in toxic concentrations.

Although TDZ has been found to promote formation of multiple shoots in several plant systems, lack of multiple shoots in the current study indicate Tuberose has a strong apical dominance, a phenomenon common to most monocot plants (Bond and Alderson, 1993). Plants possessing strong apical dominance usually contain high levels of endogenous auxins (Salisbury and Ross, 1991), which could favor elongation as opposed to multiplication of shoots, a situation that could explain formation of necrotic and dead tissues when additional auxin is added exogenously. The development of multiple shoots has been shown to require an intricate balance of auxin and cytokinin in several plant systems (Kyte, 1991) and may be influenced by the type of explant used. Multiple shoots were formed from leaf-derived calli of Tuberose over a narrow range of BAP: NAA of 0.1-0.25 (Sanjal *et al.* 1998).

After about 3 months, effect of TDZ on shoot quality rating became more evident. The improvement of shoot quality by TDZ could be due to chlorophyll synthesis or preservation from degradation as found in detached leaves of barley (You *et al.* 1992), geranium tissues (Visser *et al.* 1995), peanut cotyledons (Murthy *et al.* 1995), and other cytokinin bioassays (Mok *et al.* 1982).

In summary, Thidiazuron has been shown to be more potent than BAP with or without NAA in evoking morphogenic responses in vitro from shoot tip cultures of Tuberose (*Polianthes tuberosa* L.).

ACKNOWLEGEMENTS

Mbugwa farm of Tigoni, Limuru for providing the Tuberose plants used in the study. Professor Jasper Imungi for providing the Thidiazuron and technicians of the Biotechnology laboratory for technical assistance.

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