Thidiazuron amends the organ development of endangered aquatic plant Cryptocoryne elliptica Hook. F

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Abstract. Cryptocoryne elliptica is a Malaysia endogenous aquatic plants that has been categorized as endangered species. The plant received a highly demand for aquarium trade and being collected from wild. Slow growth and propagates only through runners has obstructs the plantlets production. Micro propagation is the best option. However, study on the effects of cytokinin particularly thidiazuron (TDZ) on the proliferation rate of this plant in vitro is still limited. Thus, the objective of this study was to determine the effects of thidiazuron on multiple shoots proliferation under in vitro conditions. A complete randomized design was used. TDZ was tested at the concentrations of 0.2 – 1.0 mg/l in two media: Murashige and Skoog (MS) and Linsmaier and Skoog (LS), respectively. Single shoot explant was used for each culture vessel with thirty cultures per treatment. Results showed that the highest shoots number generated per explant was obtained in MS + 0.8 mg/l TDZ, which was 7.33 ± 3.08 after 8 weeks of cultures. The protocorm-like-bodies (PLBs) were produced in MS + 0.4 – 0.8 mg/l TDZ and LS + 0.2 – 1.0 mg/l TDZ, after 28 weeks of culture, respectively. The inflorescences were also formed on the explants cultured on TDZ containing medium. This finding suggested that TDZ had alters the proliferation and differentiation on C. elliptica cells.

1. Introduction
Cryptocoryne belongs to the family of Araceae. The plant is commercially important for aquarium trade [1]. Nonetheless, nursery production of many Cryptocoryne species is restricted due to low seed production and slow rhizome propagation [2]. Thus, most of the Cryptocoryne available in market or for aquarium trade are collected from natural environment. Cryptocoryne elliptica is an aquatic or amphibious plant that is endemic to Malaysia. This plant can be found only at three localities in Peninsular Malaysia, which are the Gunung Pongsu Forest Reserve, Pondok Tanjung Forest Reserve, and Bukit Punchor, Penang [3, 4]. This Cryptocoryne species is also facing the same problem of being collected from the wild for aquarium trade and also by other treat such as deforestation. The plants already fall under endangered categories and the need to conserve and to increase production is seriously needed [4]. Micro propagation technique is the best alternative technique to increase the plant production and high quality plantlets. Cytokinin is commonly used to induce the shoot proliferation. Thidiazuron (TDZ) a synthetic cytokinin, which is a urea-derived cytokinin has been widely used in tissue culture for proliferation of woody plant [5]. Unfortunately, report regarding the efficiency of TDZ on aquatic...
plants, especially the *Cryptocoryne* is lacking. The current study objective was to determine the effects of TDZ on *in vitro* culture of *C. elliptica*.

2. Materials and Methods

2.1. Materials

The established tissue culture plantlets of *C. elliptica* was obtained from the Biotech Laboratory, School of Fundamental Science, Universiti Malaysia Terengganu (UMT), Malaysia for used in this study.

2.2. Culture medium and treatment

Two culture media, the Murashige and Skoog, (MS) [6] and Linsmaier and Skoog, (LS) [7] basic salt were used were used in this study. Both medium were added with 20g/l sucrose, 0.1 g/l myo-inositol and solidified with 2g/l Gelrite®. The pH of the medium was adjusted to 5.6 prior to autoclaving at 121°C (1.4 kgcm⁻²) for 15 min. TDZ was added into medium before being autoclaved at concentration of 0, 0.2, 0.4, 0.6, 0.8 or 1.0 mg/l, respectively. Shoots tips, approximately 1.0 - 1.5 cm long were excised from plantlets and inoculated onto treatment medium. A complete randomized design was used in this experiment. Single shoot explant was used for each culture vessel with thirty cultures per treatment. TDZ-free medium was used as control. Subsequently, all cultures were incubated in growth room at temperature of 25 ± 2 °C, 9-h photoperiod with an irradiance of 50 µmol m⁻²s⁻¹ provided by cool white fluorescent lamps. Numbers of shoots per explant were recorded after 8 weeks of culture. Culture were kept for 28 weeks to observe the effect of TDZ on shoots after being exposed for a longer period of time. Data were statistically analysed using SPSS Ver. 21 (SPSS Inc. Chicago, USA).

3. Results

Results showed that the highest shoots number regenerated per explant (7.33 ± 3.08) was obtained in MS + 0.8 mg/l TDZ after 8 weeks of cultures (Table 1, Figure 1A and 1B). The inflorescences were also formed on explants cultured on TDZ containing medium after 8 weeks of cultures (Figure 1C and 1D). Interestingly, the protocorm-like-bodies (PLBs) were developed on explants cultured in MS + 0.4 – 0.8 mg/l TDZ and LS + 0.2 – 1.0 mg/l TDZ, after 28 weeks of culture, respectively (Table 2, Figure 1B).

**Table 1.** Effect of TDZ on multiple shoot regeneration from shoot tips of *Cryptocoryne elliptica* in MS and LS medium after 8 weeks of culture.

| TDZ concentration (mg/l) | shoots regeneration (Mean ± SE) | Flower occurrences | Shoots regeneration (Mean ± SE) | Flower occurrences |
|--------------------------|---------------------------------|--------------------|---------------------------------|--------------------|
| 0                        | 1.71 ± 0.91 b                   | nil                | 3.44 ± 1.74 b                   | nil                |
| 0.2                      | 3.50 ± 2.43 a                   | yes                | 6.10 ± 2.38 a                   | yes                |
| 0.4                      | 6.25 ± 1.28 a                   | yes                | 6.90 ± 3.28 a                   | yes                |
| 0.6                      | 5.11 ± 2.42 a                   | yes                | 6.75± 1.98 a                    | yes                |
| 0.8                      | 7.33 ± 3.08 a                   | yes                | 5.88 ± 2.10 a                   | yes                |
| 1.0                      | 3.33 ± 0.58 a                   | yes                | 5.30 ± 1.95 a                   | yes                |

Note: Values represent means ± standard errors of two independent experiments of ten replicates. Means followed by the same superscript letter within columns did not significantly different by Tukey’s test (*p* = 0.05).
Table 2. Occurrence of protocome-like-bodies (PLBs) of Cryptocoryne elliptica in MS and LS medium after 28 weeks of culture.

| TDZ Concentration (g/ml) | TDZ Concentration (g/ml) | LS medium |
|--------------------------|--------------------------|-----------|
| 0                        | nil                      | nil       |
| 0.2                      | nil                      | PLB       |
| 0.4                      | PLB                      | PLB       |
| 0.6                      | PLB                      | PLB       |
| 0.8                      | PLB                      | PLB       |
| 1.0                      | nil                      | PLB       |

Figure 1. Effects of TDZ on organ development of Cryptocoryne elliptica cultures; (A) multiple shoots formation on cultures in MS medium + 0.8 TDZ after 8 weeks, (B) multiple shoots and PLBs on cultures LS medium + 0.6 TDZ after 28 weeks, (C) multiple shoots and flowers buds on cultures in MS medium + 0.2 TDZ after 8 weeks, (D) multiple shoots and flowers buds on cultures in LS medium + 0.8 TDZ after 8 weeks, (E) protocorm-like bodies (PLBs) and shoots under microscope, x4 magnification, (F) protocorm-like bodies PLBs under Scanning Electron Microscope (SEM), x20 magnification.

4. Discussion
To our knowledge this is the first results on the effects of TDZ on Cryptocoryne plant. Application of TDZ in the culture medium was found to increase the length of shoots, leaves width and green leaves colours. As shown in Figures 1A to 1D above, TDZ has managed to induce an explant to increase the number of shoot tips, formation of flower buds and the protocorm like bodies. Effects of TDZ on clustered short shoots with very small leaves was also reported in Ludwigia repens [8], Cercis canadensis var alba [9] and Hibiscus rosa-sinensis [10]. Cytokinins commonly stimulate shoot proliferation and inhibit their elongation, therefore; inhibition of shoot bud elongation by TDZ was consistent with its high cytokinin activity. The formation of flower in TDZ treatments were observed after 2 months of culture in growth regulator-free medium. The same observation was reported in Dendrocalamus strictus Nees [11]. The formation of PLBs on the TDZ treated explant was recorded after 28 weeks of culture in both media types. The capability of TDZ to induce the PLBs was also reported on Doritaenopsis orchid after 5 weeks culture [12] and Dendrobium nobile orchid after 8 weeks [13].
5. Conclusion
TDZ induced multiple shoots and inflorescence of explant shoot tips of *C. elliptica* when cultured for 8 weeks. TDZ also induced PLBs of *C. elliptica* when cultured for longer period of time (28 weeks). This finding suggested that TDZ had alters the proliferation and differentiation on *C. elliptica* cells.

6. References
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