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Effects of Cytokinin and Auxin on *In Vitro* Organ Development and Plumbagin Content of *Drosera peltata* Thunb.

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ABSTRACT

A rapid propagation and plumbagin production of *Drosera peltata* was developed and investigated. The research aims to study the effects of cytokinins and auxins on organ development and plumbagin production from shoot tip cultures. *In vitro* generated shoot tips were cultured on the semi-solid 1/2 MS medium containing 3.0 % sucrose, 2.0 % gelrite, and 0.1, 0.5, 1.0, and 2.0 mg L^{-1} cytokinins (BA, Kn, TDZ) and auxins (IAA, IBA, NAA and 2,4-D) for 12 weeks. The highest number of shoots (12.0 ± 1.2) was formed on the medium containing 1.0 mg L^{-1} TDZ, which was four-fold higher than in the control. Meanwhile, the highest number of roots per explant (9.4 ± 1.3) and rhizomes per explant (8.1 ± 0.8) were formed on the medium containing 2.0 mg L^{-1} NAA. The best callus induction (100%) was found on the 0.5–2.0 mg L^{-1} 2,4-D-containing medium. Moreover, the highest plumbagin content (12.04 mg g^{-1} DW) was detected from shoots regenerated on the 0.1 mg L^{-1} BA-containing medium, which was approximately two-fold higher than that in the control. The study is efficient for organs induction and enhances plumbagin content from shoot tip explants of *D. peltata*.

INTRODUCTION

The genus *Drosera*, which includes carnivorous plants is widely distributed around the world and mostly used as natural folk medicine (Länger, Pein, & Kopp, 1995). Phytochemical investigation in *Drosera* species revealed that plumbagin, 7-methyljuglone, and naphthoquinone derivatives are the main secondary compounds (Babula, Adam, Havel, & Kizek, 2009; Egan & van der Kooy, 2013; Zenk et al., 1969). Plumbagin, a natural bioactive compound, has antimicrobial (Didry, Dubreuil, Trotin, & Pinkas, 1998), anti-inflammatory and spasmolytic (Melzig, Pertz, & Krenn, 2001), anticonvulsant (Hema, Bhupendra, Mohamed Saleem, & Gauthaman, 2009), anticancer (Xu & Lu, 2010), and antifungal activities (Grevenstuk et al., 2012). Moreover, it is a candidate compound as a natural herbicide (Choi et al., 2012) and seed germination inhibitor (Marion Meyer, Van der Kooy, & Joubert, 2007). In Thailand, three species of *Drosera*, *D. indica*, *D. burmanii*, and *D. peltata* were distributed in specific habitats in the northern, western, and northeastern regions (Larsen, 1987). However, both *D. indica* and *D. burmanii* have been recorded and utilized as Thai traditional medicine to treat diseases such as lymphadenitis, dysentery, malaria and eczematous dermatitis (Department of Pharmacognosy, Mahidol University Foundation, 2000). Although chemical constituents of *D. peltata* had been subsequently detected and elucidated (He, He, He, & Wan, 2012; Tian et al., 2014), only a few studies using *D. peltata* as a medicinal plant have been reported. Because of the overharvesting

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from natural habitats for medicinal purposes, most of the *Drosera* species, particularly *D. peltata*, might become exposed to the risk of extinction from the wild; therefore, it was listed as a vulnerable, threatened, or endangered species (Jennings & Rohr, 2011).

Tissue culture is an efficient method for rapid clonal propagation and secondary metabolite production of medicinal plants (Debnath, Malik, & Bisen, 2006; Ramachandra Rao & Ravishankar, 2002). It has been achieved for both *in vitro* conservation and biomass production for secondary metabolite bioprospection in various species of *Drosera* (Crouch, Finnie, & van Staden, 1990; Grevenstuk, Coelho, Gonçalves, & Romano, 2010; Jayaram & Prasad, 2007; Kawiak, Królicka, & Łojkowska, 2003; Marczak, Kawiak, Łojkowska, & Stobiecki, 2005; Wawrosch, Benda, & Kopp, 2009). However, only few studies on *in vitro* propagation of *D. peltata* were published (Kim & Jang, 2004). Further, to the best of the knowledge *in vitro* production of plumbagin by *D. peltata* has not yet been reported. Therefore, efficient techniques for in vitro propagation and plumbagin production in *D. peltata* need to be studied and improved.

Plant growth regulator is one of the important factors affecting shoot multiplication and secondary metabolite production. The medium components, particularly cytokinins and auxins, are used to control morphogenesis and regulate the production of bioactive compounds in several medicinal herbs (Ramachandra Rao & Ravishankar, 2002). Several plants multiplied and produced secondary metabolites on the media supplemented with cytokinins and/or auxins. However, *in vitro* conservation and plumbagin production from D. peltata as effects of plant growth regulators has not been investigated till present. Accordingly, the purpose of the present investigation was to develop an efficient in vitro culture system for conservation and plumbagin production by *D. peltata* stimulated by different concentrations of cytokinins and auxins on semi-solid MS medium.

MATERIALS AND METHODS

Effects of cytokinin and auxin on *in vitro* organ development and plumbagin content of *D. peltata* Thunb. was performed in Plant Tissue Culture Research Unit, Department of Biology, Faculty of Science, Naresuan University, Thailand during January-November 2015. Two main experiments conduct in this study consist of regeneration efficiency as affected by auxin and cytokinin and plumbagin production from shoot tips of *D. peltata*.

Plant Materials

The seeds of *D. peltata*, collected from a naturally growing plant in Loei province, Thailand, were stored at 4 °C for four weeks before use. The seed surfaces were first sterilized for 10 min with 15 % (v/v) Clorox® solution mixed with 0.01% (v/v) Tween 20. Thereafter, the seeds were immersed in 70 % (v/v) ethanol for 1 min and finally washed in sterilized distilled water three times. The sterilized seeds were sown on solidified [0.2 % (w/v) gelrite] medium containing half MS (Murashige & Skoog, 1962) salts and 3 % (w/v) sucrose. The seeds were cultured under cool white fluorescent light (20 µmol m^{-2} s^{-1}) under a 12-h photoperiod at 25±2 °C. The forty-five-days-old seedlings were first sub-cultured in a new medium, and consecutively sub-cultured every four weeks. At the third subculture, the shoot tips were used for further experiments.

In vitro Shoot Tip Cultures

Shoot tips approximately 15 mm in length were cut from the stock cultures and transplanted individually to the new culture media, containing half-strength MS (Murashige & Skoog, 1962) salts, 3 % (w/v) sucrose, 0.1 % (w/v) myo-inositol, 0.2% (w/v) gelrite, and 0.1, 0.5, 1.0, and 2.0 mg L^{-1} of cytokinins, 6-Benzyladenine (BA), Kinetin (Kn), Thidiazuron (TDZ) or auxins, Indole-3-acetic acid (IAA), Indole-3-butyric acid (IBA), Naphthaleneacetic acid (NAA), and 2,4-dichlorophenoxyacetic acid (2,4-D). The same medium without any hormones was used as the control. All media were adjusted to pH 5.7 and subjected to autoclaving (20 min at 121 °C). All the shoot tips were cultured under a 12-h light regime (20 µmol m^{-2} s^{-1} from cool white fluorescent lamps) at 25 ± 2 °C for 12 weeks. At the end of the treatment, explant growth and plumbagin accumulation were recorded, and all the data were statistically analyzed.

Plumbagin Analysis by HPLC

Plumbagin was extracted and analyzed according to the protocol of Marczak, Kawiak, Łojkowska, & Stobiecki (2005) with some modifications. Whole organogenic tissues (5.0 g) were dried at 50 °C for two days. The dried sample was ground and mixed with methanol (10 ml), and the mixture was then sonicated at 25 °C

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for 15 min. The suspension was subsequently centrifuged (10,000 rpm, 15 min) before collecting the supernatant. This supernatant was filtered by a 0.45-µm membrane filter (VertiClen™) and the content of plumbagin was analyzed by HPLC. The HPLC (Perkin Elmer, series 200) system was set up using a C18 column (Restek, 4.6 mm ID × 50 mm length, 3-µm particle size). The injection volume of each sample was 10 µl. The sample was eluted by a mixture of methanol and 0.4% acetic acid in water (70:30 v/v) with a flow rate of 1.0 ml min^{-1}. The presence of plumbagin was detected by a UV detector at 270 nm. The content of plumbagin was calculated by comparing with the standard curve of genuine plumbagin (Sigma-Aldrich, USA) using Total Chrom™ workstation (version 6.2.) software (Perkin-Elmer Instrument HPLC).

Experimental Design and Statistical Analysis

The experiment was repeated twice with 20 biological replicates per treatment. A Complete Randomized Design (CRD) was used for statistical analysis. The data was subjected to mean comparisons using analysis of variance (ANOVA) and Duncan's multiple range test (DMRT) at the $p < 0.05$ level of significance.

RESULTS AND DISCUSSION

Plant growth regulators affected organ development, shoot proliferation, and plumbagin production of *D. peltata* after culturing for 12 weeks. The results revealed that the cytokinins tended to stimulate multiple shoots with a succulent stem, and an undeveloped lamina on petiole (Fig. 1a), whereas auxins tended to promote shoot with rhizome and roots (Fig. 1c). The number of developed shoots in plants growing on the medium containing cytokinins (BA, Kn, and TDZ) was higher than that in the plants cultured on the medium containing auxins (IAA, IBA and NAA) or the control (Fig. 1b). The highest number of shoots per explant (12.0 ± 1.2) derived via direct organogenesis was found on the medium augmented with 1.0 mg L^{-1} TDZ (Table 1, Fig. 1d). This might be due to the properties of TDZ, which is known to induce shoot multiplication rate in many plant species (DiCosmo & Misawa, 1995; Rout et al., 2000; Smetanska, 2008) such as *Dionaea muscipula* (Banasiuk, Kawiak, & Krölicka, 2012), including *Drosophyllum lusitanicum* (Gonçalves & Romano, 2005), *Drosera peltata* (Kim & Jang, 2004) and *Drosera gigantea* (Taraszkiewicz, Jafra, Skrzypczak, Kaminski, & Krolicka, 2012). The TDZ-supplemented medium potentially promoted the highest shoot number and shoot buds, which was approximately four-fold as compared to the control plants. Subsequently, these could be regenerated to new shoots. It is possible that TDZ directly promotes growth in a manner similar to that of N^6-substituted cytokinins or affects the synthesis and/or accumulation of some endogenous plant hormones (Capelle, Mok, Kirchner, & Mok, 1983; Mok & Mok, 1985). The other reason is based on the effects of the high ability of TDZ to induce cytokinin-dependent shoot regeneration and modulation of endogenous levels of cytokinins (Mok & Mok, 1985). However, multiple shoots showed different levels of unusual characteristics in all the media containing cytokinins. In this occurrence, explants regenerated swollen shoots with small thick leaves. Some stunted shoots produced thick leaves with short petiole and malformed figure. Further, shoot malformation, as induced by cytokinins, was observed in *D. indica* (Jayaram & Prasad, 2007) and *D. burmanii* (Jayaram & Prasad, 2008), whereas regenerated shoots of *D. peltata* derived from the control and the auxins-supplemented media exhibited normal growing. Abnormalities of in vitro shoot organogenesis induced by cytokinins may be associated with an increase in the expression of a knotted1-type homeobox gene (*TobH1*) isolated from normal shoot-forming cultures. This gene is generally known to control different developmental programs, particularly those involved in the formation of shoot meristem during morphogenesis. An overexpression of *TobH1* gene may induce abnormal shoot morphology (Ramage & Williams, 2004).

Furthermore, shoot tips of *D. peltata* cultured on the medium augmented with BA and Kn could produce a number of inflorescences, but there were no inflorescence formation was induced on the medium supplemented with TDZ and auxins. The flowering percentage tended to increase with the raising of BA and Kn concentration. The highest flowering percentage (21.8 %) was in plants cultured on the medium supplemented with 1.0 mg L^{-1} Kn. However, flowering induction in *D. indica* was earlier reported in cultures growing on cytokinin-free medium (Jayaram & Prasad, 2007), whereas a low concentration of Kn and BAP (0.1–0.5 mg L^{-1}) could stimulate higher flowering in *D. burmanii* (Jayaram & Prasad, 2008). Adenine-type cytokinins, such as BA and Kn, can stimulate cell division in the central

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zone of the meristem and subsequently cause flowering (Bernier, 1988). Moreover, cytokinins play an important role in physiological signals in flowering (Bernier, Havelange, Houssa, Petitjean, & Lejeune, 1993) and activate *SaMADS* A, a gene apparently involved in regulation of the floral transition in some plants (Bonhomme, Kurz, Melzer, Bernier, & Jacqmard, 2000). Unlike TDZ, a synthetic phenyl urea cytokinin-like compound stimulates only shoot bud and shoot formation. These results suggested that it could be a factor that affected *in vitro* flowering.

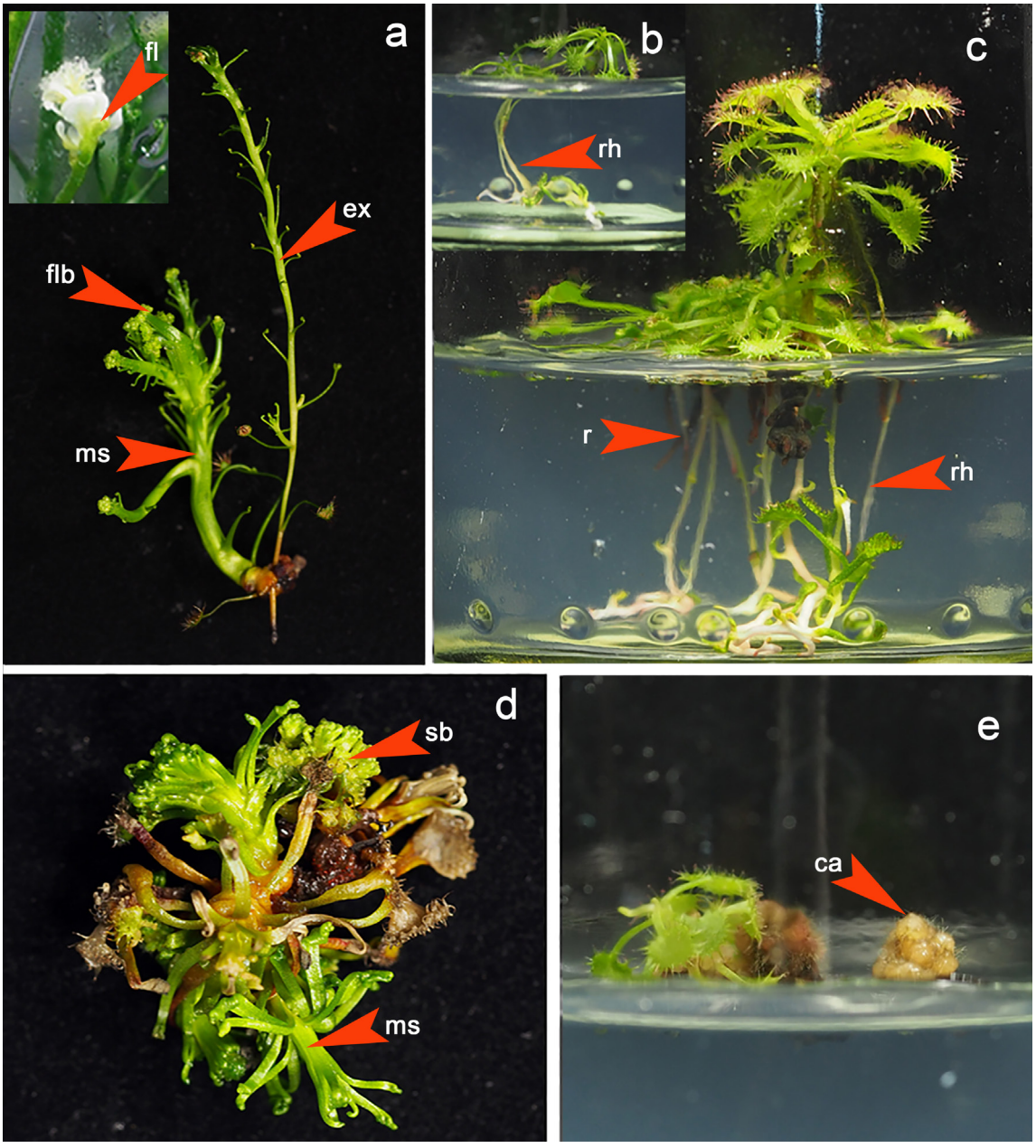

Fig. 1. Shoot regeneration from explant (ex) showed flowering malformed shoot (ms) with flower bud (flb) when cultured on ½ MS with 1.0 mg L^{-1} BA [a], rhizome (rh) and root (r) formation were observed on ½ MS with no hormone [b] and ½ MS medium added with 2.0 mg L^{-1} NAA [c], respectively. Shoot bud (sb) induction was noticed on ½ MS medium supplemented with 1.0 mgL^{-1} TDZ [d] whereas callus (ca) formation was found on ½ MS medium filled with 2.0 mgL-1 2,4-D [e] for 12 weeks of culture. (Scale bar = 0.5 cm)

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The medium containing 2.0 mg L^{-1} NAA provided the highest number of rhizomes (8.1 ± 0.8) and roots (9.4 ± 1.3) via direct organogenesis (Table 2). Similar results were obtained in *Cymbidium goeringii*, it was found that the apical flower bud of explants could produce a mass of rhizome branches, when cultured on a medium supplemented with NAA (Shimasaki & Uemoto, 1991). NAA affected not only rhizome induction, but also stimulated root formation from the shoot tip of the explants of *D. peltata*. In addition, a related result was achieved in shoot tip culture of *Drosophyllum lusitanicum* (Gonçalves & Romano, 2005). Callus induction in *D. peltata* could be observed on the medium supplemented with BA, Kn, TDZ, or 2,4-D, whereas no callogenesis was detected on the medium with IAA, IBA, or NAA, and in the control. The auxin 2,4-D is generally known to elicit rapid cell proliferation and induce callus in many plants, and the highest callogenesis (100%) was noticed on the 0.5–2.0 mg L^{-1} 2,4-D-supplemented medium. However, hairy nodular-like callus induced on 2,4-D-supplemented medium showed a yellow-brown color (Fig. 1e). The phytohormone 2,4-D performs signal transduction by stimulating ARF transcription factors, which in turn activate E2Fa, associated to the cell cycle for division (Ikeuchi, Sugimoto, & Iwase, 2013). In addition, a successful callus induction and indirect organogenesis were reported in *Drosera spathulata*, when its leaves were cultured on semi-solid MS medium consisting of 0.05 mg L^{-1} or 1.0 mg L^{-1} 2,4-D, 1.0 mg L^{-1} Kn, and 5.0% coconut milk (Bobák, Blehová, Šamaj, Ovečka, & Krištín, 1993).

The results of multiple shoot induction and plantlet production from shoot tip culture of *D. peltata*, as influenced by cytokinin and auxin, were practicable and effective for *ex situ* conservation. However, *in vitro*-produced plantlets of *D. peltata* were unsuitable for reintroduction to the natural habitat because of dormancy after transplantation to the distribution area. Therefore, the possible objective of using *in vitro*-produced plantlets of *D. peltata* would be bioprospection of plumbagin, because we can reproduce the in vitro stock plants as raw materials reproduce continuously.

Plant growth regulators (such as auxins or cytokinins) play a significant role in the production of secondary metabolites in both *in vitro* and *in vivo* conditions (DiCosmo & Misawa, 1995; DiCosmo & Towers, 1984; Mantell & Smith, 1984; Rodriguez-Sahagun, Del Toro-Sánchez, Gutierrez-Lomelí, & Castellanos-Hernandez, 2012; Staba, 1980). The results revealed that cytokinins and auxins stimulated both shoot multiplication and plumbagin production in *D. peltata*. A low concentration of cytokinin (except for TDZ) and auxin tended to significantly promote plumbagin production. From those results, this research found that the highest plumbagin content was detected on media containing 0.1 mg L^{-1} BA (12.04 mg g^{-1} DW) and 0.1 mg L^{-1} 2,4-D (11.21 mg g^{-1} DW), as shown in Table 1 and Table 2. Cytokinin is one of the most effective plant growth regulators used to stimulate secondary metabolite production by gene expression in the process of macronutrient transport for secondary metabolite production (Shilpashree & Rai, 2009). Furthermore, cytokinin-mediated signaling and crosstalk with defense pathways initiates, activates, and regulates the gene expression that promotes plant immunity and plant defense against pathogens, thus indirectly activating salicylic acid (SA) response (De Vleesschauwer, Yang, Vera Cruz, & Hofte, 2010; Naseem, Kaltdorf, & Dandekar, 2015; Pantelides, Tjamos, Pappa, Kargakis, & Paplomatas, 2013). Salicylic acid could then act as an elicitor for plumbagin production in plants (Widhalm & Rhodes, 2016) or cultured plant cells (Juengwatanatrakul, Sakamoto, Tanaka, & Putalun, 2011; Putalun et al., 2010). In tissue culture, 2,4-D can replace IAA as a hormone supplement for normal cell development. However, another usage of 2,4-D is the regulation of defensive responses in plants (Zhou et al., 2009). A low dose of 2,4-D can be used as potent defense elicitors for inducing a strong defensive reaction upstream of the jasmonic acid, salicylic acid, ethylene pathways, and significantly increased trypsin proteinase inhibitor activity and volatile production (Xin et al., 2012). In addition, the beneficial effect of 2,4-D on stimulating plumbagin biosynthesis in *Drosera peltata* may be due to the induction of stress on the cultured explants, which activates signaling response and causes gene expression for specific enzymes essential for the biosynthesis of plumbagin to cope with stress (Patidar, 2012). A similar result observed in *Drosophyllum lusitanicum* revealed that low concentration of BA could stimulate higher content of plumbagin (Nahálka, Blanárik, Gemeiner, Matúšová, & Partlová, 1996). Within the cytokinin group, the TDZ-induced plumbagin content was lower than that in the other treatments supplemented with cytokinins (Table 1). Within the auxin group, 2,4-D at higher concentration (0.5–2.0 mg L^{-1}) produced

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the lowest plumbagin content as compared to the auxin-supplemented medium (Table 2). From the results, *in vitro* shoot tips of *D. peltata* surprisingly produced higher plumbagin content than that previously reported in *D. burmanii* (Putalun et al., 2010) and *D. indica* (Juengwatanatrakul, Sakamoto, Tanaka, & Putalun, 2011). These noticeable results also improved more efficient methods than what was produced by *Plumbago zeylanica* (Choosakul, 2000) and *P. indica* (Gangopadhyay, Sircar, Mitra, & Bhattacharya, 2008; Jindaprasert et al., 2001).

Table 1. Effect of cytokinins on morphological change of *D. peltata* shoot tips cultured on ½ MS medium for 12 weeks

Cytokinins	Concentration (mg L^{-1})	Number of shoot	Number of rhizome	Number of root	Flower bud formation (%)	Callus formation (%)	Plumbagin (mg g^{-1} DW)
No hormone	-	3.2 ± 0.9 e*	1.5 ± 0.3 b	0.3 ± 0.3 b	0.0	0.0	6.74 ± 0.01 d
BA	0.1	9.4 ± 1.5 abc	0 ± 0.0 c	0 ± 0.0 b	5.0	2.7	12.04 ± 0.03 a
	0.5	7.4 ± 0.8 bcd	0 ± 0.0 c	0 ± 0.0 b	13.9	15.3	4.07 ± 0.09 f
	1.0	10.8 ± 1.4 ab	0 ± 0.0 c	0 ± 0.0 b	17.8	16.7	5.96 ± 0.09 e
	2.0	8.9 ± 1.1 abc	0 ± 0.0 c	0 ± 0.0 b	10.9	4.7	5.66 ± 0.49 e
Kn	0.1	7.2 ± 0.7 cd	1.9 ± 0.2 a	4.6 ± 0.8 a	2.0	0.0	9.62 ± 0.05 b
	0.5	5.4 ± 0.6 de	0 ± 0.0 c	0.6 ± 0.4 b	7.9	0.7	9.91 ± 0.07 b
	1.0	4.7 ± 0.4 de	0 ± 0.0 c	0.2 ± 0.2 b	21.8	4.7	4.26 ± 0.29 f
	2.0	7.9 ± 0.8 bcd	0 ± 0.0 c	0.3 ± 0.3 b	20.8	3.3	9.10 ± 0.06 c
TDZ	0.1	7.6 ± 0.8 bcd	0 ± 0.0 c	0 ± 0.0 b	0.0	15.3	1.18 ± 0.04 h
	0.5	9.2 ± 0.9 abc	0 ± 0.0 c	0 ± 0.0 b	0.0	15.0	2.16 ± 0.14 g
	1.0	12.0 ± 1.2 a	0 ± 0.0 c	0 ± 0.0 b	0.0	12.7	1.69 ± 0.03 gh
	2.0	9.0 ± 1.1 abc	0 ± 0.0 c	0 ± 0.0 b	0.0	8.7	4.35 ± 0.02 f

Remarks: *Mean ± SE within each column followed by the same letters are not significantly different ($p < 0.05$) using DMRT's test

Table 2. Effect of auxins on morphological change of *D. peltata* shoot tips cultured on ½ MS medium for 12 weeks

Cytokinins	Concentration (mg L^{-1})	Mean number of shoot	Mean number of rhizome	Mean number of root	Callus formation (%)	Plumbagin (mg g^{-1} DW)
No hormone	-	3.2 ± 0.9 a*	1.5 ± 0.3 cde	0.3 ± 0.3 c	0.0	6.74 ± 0.01 e
IAA	0.1	2.4 ± 1.0 abc	1.7 ± 0.2 cd	0.6 ± 0.3 c	0.0	8.19 ± 0.01 c
	0.5	2.7 ± 0.7 abc	2.0 ± 0.3 c	1.2 ± 0.4 c	0.0	6.70 ± 0.01 f
	1.0	2.6 ± 0.5 abc	1.3 ± 0.2 cde	0.9 ± 0.4 c	0.0	8.85 ± 0.01 b
	2.0	2.2 ± 0.3 abc	2.4 ± 0.5 c	1.3 ± 0.5 c	0.0	6.43 ± 0.00 g
IBA	0.1	2.8 ± 0.8 ab	1.4 ± 0.3 cde	1.1 ± 0.8 c	0.0	7.72 ± 0.03 d
	0.5	2.3 ± 0.3 abc	2.5 ± 0.6 c	1.1 ± 0.5 c	0.0	6.34 ± 0.01 h
	1.0	2.6 ± 0.3 abc	2.4 ± 0.4 c	0.2 ± 0.2 c	0.0	6.40 ± 0.01 g
	2.0	2.5 ± 0.5 abc	4.3 ± 0.7 b	0 ± 0.0 c	0.0	5.52 ± 0.01 j
NAA	0.1	2.5 ± 0.6 abc	2.3 ± 0.4 c	0.9 ± 0.5 c	0.0	5.59 ± 0.01 i
	0.5	2.9 ± 0.6 ab	4.7 ± 1.2 b	3.8 ± 1.2 b	0.0	4.13 ± 0.01 k
	1.0	2.8 ± 0.4 abc	8.1 ± 1.1 a	8.6 ± 1.6 a	0.0	3.31 ± 0.00 l
	2.0	2.8 ± 0.3 abc	8.1 ± 0.8 a	9.4 ± 1.3 a	0.0	3.19 ± 0.00 m
2,4-D	0.1	2.2 ± 0.4 abc	1.6 ± 0.3 cd	0.9 ± 0.4 c	14.3	11.21 ± 0.01 a
	0.5	0.9 ± 0.1 bc	0.2 ± 0.1 de	0 ± 0.0 c	100	2.65 ± 0.00 o
	1.0	1.0 ± 0.1 bc	0 ± 0.0 e	0 ± 0.0 c	100	2.70 ± 0.00 n
	2.0	0.8 ± 0.1 c	0 ± 0.0 e	0 ± 0.0 c	100	2.35 ± 0.00 p

Remarks: *Mean ± SE within each column followed by the same letters are not significantly different ($p < 0.05$) using DMRT's test

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CONCLUSION AND SUGGESTION

This study indicates that plant growth regulators affect shoot multiplication in addition to stimulating higher plumbagin production from *in vitro* produced shoot tips of *D. peltata*. Induction of multiple shoots was successfully obtained when TDZ was used as a cytokinin source, whereas NAA stimulated the highest root and rhizome number. Although shoot multiplication induced in this study was not very high, the higher plumbagin content detected from these regenerated tissues was improved and reported in *D. peltata* for the first time. A suitable concentration of BA and/or 2,4-D would promote higher plumbagin content, and this can be an alternative method of improving the regeneration system and confirming efficient methods for *ex situ* conservation as well as plumbagin production by shoot tip culture of *D. peltata*.

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