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THE EFFECTS OF INDOLE-3-BUTYRIC ACID AND 1-NAPHTHALENEACETIC ACID ON THE INDUCTION OF ROOTS FROM *Clitoria ternatea* L.

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Abstract: *Clitoria ternatea* L., or commonly known as butterfly pea, from the family of Fabaceae, is a perennial medicinal plant. Its flower which is deep blue or white in colour is commonly used as a natural food colorant. The plant originates from tropical Asia and known to possess essential bioactive compounds where the extracts from its roots, leaves and seeds are used in the phytochemical, pharmacological and clinical investigations for therapeutic drug development. The root extracts have been reported to exhibit analgesic, anti-pyretic, anxiolytic, anti-depressant, anti-convulsant, anti-stress, anti-diarrheal, anti-asthmatic and most importantly, in the treatment of central nervous system disorders. The root extracts have shown memory enhancing properties in neonatal rats. The aim of the study was to induce roots from the seedling explants of *Clitoria ternatea* L. for the establishment of a root suspension culture system that could function as efficient alternative to the *ex-vitro* plants from the soil for harvesting of medicinal secondary metabolites. The sterilized seeds of *Clitoria ternatea* L. were germinated on half-strength Murashige and Skoog (MS) medium, and the cotyledon and hypocotyl from the 7 - 8 days old seedlings were placed in the media supplemented with indole-3-butyric acid (IBA) and 1-naphthaleneacetic acid (NAA) at different concentrations. It was evident that NAA was the potential growth hormone for root induction in *Clitoria ternatea* L. The cotyledon explants cultured on MS medium supplemented with 2 mg/L NAA produced the highest percentage of root induction (70%) while 1 mg/L NAA produced the highest average number of roots from cotyledon explants. The study provided an efficient protocol for the induction of roots and callus from *Clitoria ternatea* L.

Keywords: *Clitoria ternatea* L., seedling explants, root induction, auxin.

Introduction

Clitoria ternatea L. or the butterfly pea or locally known as "Bunga telang", is a perennial, deep rooted climbing legume plant originating from tropical Asia and is known for its valuable medicinal properties (Gomez & Kalamani, 2003). The flower is deep blue or white in colour and is commonly used as a natural food colorant in many Asian sweet desserts and savoury rice. It is considered as an important source of bioactive compounds, medicines, dyes and also as an ornamental plant (Mohamed & Taha, 2011). The extracts from the roots, leaves and seeds of *C. ternatea* have been used traditionally for various treatments and also in modern phytochemical, pharmacological and clinical investigations for developing therapeutically effective compounds or drugs (Alok *et al*., 2015).

The roots of *C. ternatea* have bitter taste with purgative, laxative and diuretic properties commonly used in the treatment of indigestion, constipation, fever, arthritis and eye ailments (Mukherjee *et al*., 2008). The root extracts have been reported to exhibit analgesic, anti-pyretic, anxiolytic, anti-depressant, anti-convulsant, anti-stress, anti-diarrheal and anti-asthmatic properties (Devi *et al*., 2003; Jain *et al*., 2003; Taur & Patil, 2011). *C. ternatea* is also used as a brain tonic in the Indian traditional medicine and is believed to promote better memory and intelligence (Gomez & Kalamani, 2003). The root extracts of *C. ternatea* have reportedly been utilized in the development of new phytoseuticals for the treatment of central nervous system (CNS) disorders attributable to its ability as a memory enhancer in neonatal rats (Rai *et al*.,

2001; Mukherjee *et al*., 2008). The methanolic extracts from the roots increase the acetylcholine content and the acetylcholinesterase activity in rats which indirectly promote and enhance their memory (Rai *et al.*, 2001).

The roots of *C. ternatea* contain a wide range of chemical constituents such as ternatins, alkaloids, saponins, tannins, carbohydrates, proteins, resins, starch as well as secondary metabolites such as flavonol glycoside3,5,4'-trihydroxy-7-methoxyflavonol-3-O-β-D-xylopyranosyl-(1,3)-O-β-D-galactopyranosyl (1,6) -O-β-D-glucopyranoside (Yadava & Verma, 2003), taraxerol and taraxerone (Uma *et al.*, 2009). Taraxerol and taraxerone are pentacyclic triterpenoids in which taraxerol is the fundamental compound that exhibits pharmacological values (Banerjee & Chakravarti, 1963; 1964). Taraxerol covers a wide range of pharmacological properties such as antimicrobial, antioxidant, anti-aging, anti-pyretic, anti-inflammatory, analgesic, sedative, insecticidal, anti-tumor and anti-cancer properties (Parimaldevi *et al.*, 2003; Mukherjee *et al.*, 2008). Taraxerol in *C. ternatea* can be detected using High Performance Thin Layer Chromatography (HPTLC) analysis (Kumar *et al.*, 2008), and is found to exhibit dose-dependent elevation of brain acetylcholinesterase activity related to improved learning and memory performance in rats **(**Kumar *et al.*, 2007). These studies suggest the potential of oral treatment of the root extracts for the improvement of retention and spatial learning performances on rats alongside the long term potential to increase the functional growth of the amygdala. The aqueous root extract is also effective as a potential memory enhancer for the treatment of neuronal degenerative disorders (Mukherjee *et al*., 2008).

Clitoria ternatea L. is listed as a rare species by the International Union for Conservation of Nature and Natural Resources (Kumar & Thomas, 2012). It becomes imperative for further investigation on the sustainable use of this plant and on the effects of the root extracts to the studies related to human mind, memory and wellness. Plant tissue culture functions as a tool to multiply *in vitro* plantlets for studies involving plant propagation within a short period of time to meet the pharmaceutical needs and also as an effective conservation method. Successful *in vitro* regeneration in *C. ternatea* has been reported through callus initiation (Mohamed & Taha, 2011; Shahzad *et al*., 2007), shoot regeneration (Mukhtar *et al*., 2010), somatic embryogenesis (Kumar & Thomas, 2012), from cotyledonary node (Barik *et al*., 2007; Mukhtar *et al*., 2010) and leaf explant (Mohamed & Taha, 2011) obtained from *in vitro* grown seedlings. The root tissue of *C. ternatea* is the main targeted explant for alcoholic extraction in further biochemical studies. However, harvesting the plant for root extraction will result in plant death when the roots are removed and excised from the soil.

The current study aimed to investigate the induction of *in vitro* roots using two different types of auxin, namely 1-naphthaleneacetic acid (NAA) and indole-3-butyric acid (IBA) for future establishment of root suspension culture as an alternative route to produce *C. ternatea* roots within a shorter period of time.

Materials and Methods

Plant Material

The dried matured seed pods of *C. ternatea* were collected from the herb garden at Universiti Sains Malaysia and seeds were removed from the pod and kept dry at room temperature prior to *in vitro* germination.

Seed Germination and Explant Inoculation

The seeds of *C. ternatea* were surface sterilized by rinsing under running tap water for 15 min, followed by soaking in 60% bleach solution (Clorox) with 2 drops of Tween-20 for 10 min. The seeds were gently swirled in 70% ethanol for 45 seconds, rinsed with sterile distilled water, blotted dry, inoculated on half-strength Murashige and Skoog (MS) medium (Murashige & Skoog, 1962), placed in the culture room and maintained at a constant temperature of 24 ± 2 ºC, under a 16/8h photoperiod in cool, white

fluorescent light. The germinated seedlings were excised at the cotyledons and hypocotyls and inoculated on the ½ MS medium, separately supplemented with IBA (0, 0.1, 0.2, 0.3, 0.4 and 0.5 mg/L), and NAA (0, 0.5, 1, 1.5 and 2 mg/L). Each treatment consists of 10 explant replicates of cotyledons and hypocotyls. Explants were placed in a culture room maintained at 16/8 h photoperiod.

Data Collection and Analysis

The number of explants producing callus, roots and the number of roots induced per explant were recorded after 8 weeks of culture. The percentage of explants producing callus, roots and the average number of roots induced were calculated and analysed using one-way analysis of variance (ANOVA), followed by Duncan Multiple Range Test ($P \leq 0.05$).

Results and Discussion

IBA Treatment

Figure 1 and 2 suggest that all the concentrations of IBA showed no significant difference on the induction of roots and callus as well as the number of roots induced from cotyledon and hypocotyl explants after 8 weeks of culture. There was no root formation from cotyledon explants while hypocotyl explants produced very low number of roots at 0.1 mg/L IBA (0.20±0.20) and 0.5 mg/L IBA (0.30±0.30) (Figures 1a and 1c). Callus formation was observed from both cotyledon and hypocotyl explants (Figure 1b) but the formation (0-20%) was insignificant. Despite the low root induction rate in the present study, Lakshmanan & Dhanalakshmi (1990) have proven that 0.5mg/L IBA is effective for the induction of roots in shootlets regenerated from the seedling of *C. ternatea*. The use of half-strength MS medium supplemented with 5 µm IBA could induce roots prior to acclimatization of shoot explants in the field (Shahzad *et al.*, 2007). A research on *Stevia rebaudiana,* a medicinal herb, has shown that 86% of root formation could be obtained from *in vitro* regenerated shoots treated with ½ N_6 medium supplemented with 1 mg/L IBA from cotyledon and hypocotyl explants of *C. ternatea* after 8 weeks of culture (Anbazhagan *et al*., 2010).

NAA Treatment

Figure 3a shows that the elevated concentrations tested at 0.5, 1.5 and 2 mg/L NAA had significant effects on the formation of roots from cotyledon explants, as compared to the control medium, with 60, 60 and 70% formation, respectively. The cotyledon and hypocotyl explants also produced the highest callus formation (100%) at 1.5 mg/L NAA and 0.5 mg/L NAA, respectively (Figure 3b). The average number of roots from cotyledon explants at 1 mg/L NAA (3.90±2.07) was significantly different from the control (Figure 3c). The morphology of roots formed from cotyledon explants treated with NAA was light brown, short and thick (Figure 4). The results from the current study was in contradiction to the earlier report that *Echinacea purpurea* hypocotyl explants produce roots effectively in the media supplemented with IBA, instead of NAA (Choffe *et al.*, 2000).

Root formation in shootlets regenerated from leaf and stem of *C. ternatea* has been reported using 1-3 mg/L NAA (Kumar *et al.*, 1993). The evidence on the highest root formation (3.17±0.92%) from the leaf explants of *C. ternatea* is obtained from DKW medium supplemented with 2.0mg/L NAA (Mohamed & Taha, 2011), suggesting that lower NAA concentration produces lower root formation. The significant effect of 2 mg/L NAA in MS medium on the formation of roots (78.3%) has been further proven in *Dahlia* but with a justification that the rate of formation of roots decreases as the concentrations of NAA increase (Wadankar & Malode, 2012). A combination of auxin and cytokinin in the production of organogenic calli is reported in MS medium containing 2,4-Dichlorophenoxyacetic acid (2,4-D) and Benzylaminopurine (BA) at the concentrations of 10 or 20 µm and 5 µm, respectively (Shahzad *et al.*, 2007).

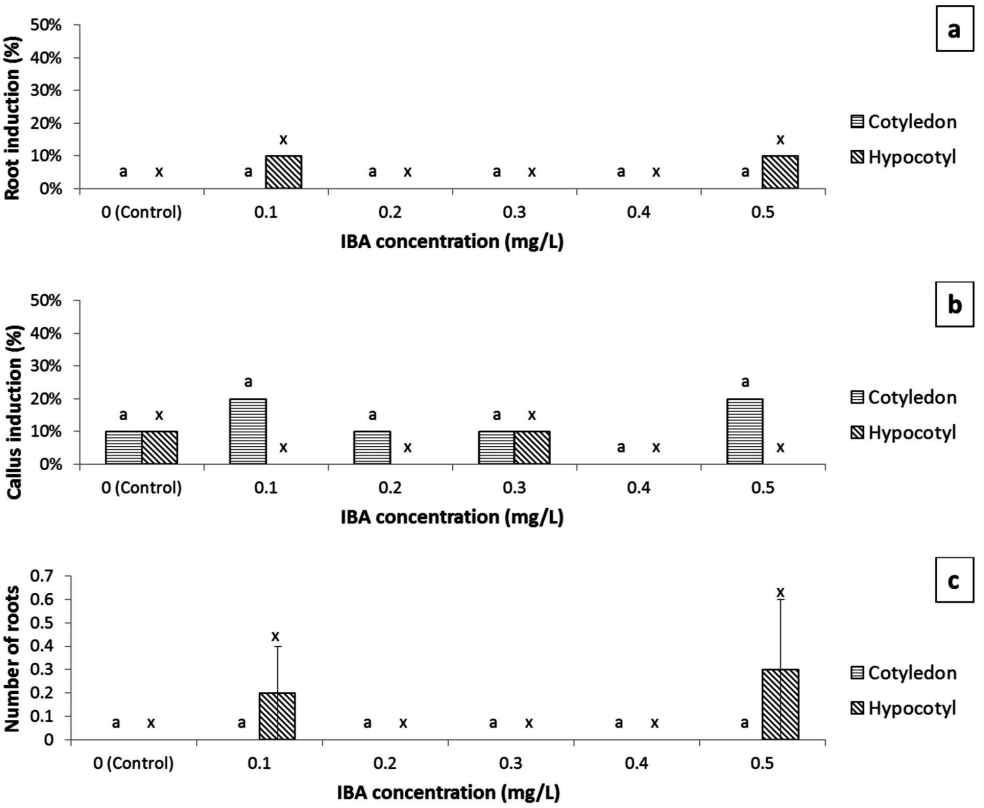

Figure 1: The effects of Indole-3-Butyric Acid (IBA) on a) root induction; b) callus induction; c) number of roots induced from cotyledon and hypocotyl explants of *C. ternatea* after 8 weeks of culture

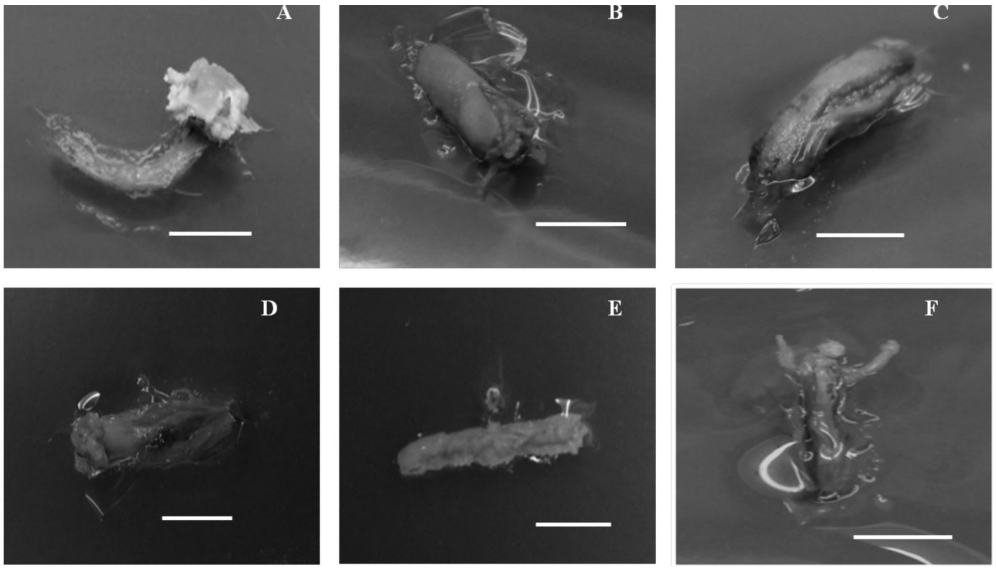

Figure 2: Root and callus induction of hypocotyl explants of *C. ternatea* inoculated in half-strength MS medium supplemented with different concentrations of Indole-3-Butyric Acid (IBA) after 8 weeks of culture. A) control; B) 0.1 mg/L; C) 0.2 mg/L; D) 0.3 mg/L; E) 0.4 mg/L and F) 0.5 mg/L. The scale bars represent 0.5 cm

The roots of various medicinal plants have been considered as the valuable parts as secondary metabolites and bioactive compounds are found specifically in the roots for pharmaceutical and nutraceutical uses. The induction of root from hypocotyl and cotyledon has been carried out for a number of medicinal plants. Auxins are found in all plants. Being natural or synthetic, auxins involve in numerous developmental processes in plants such as stem and internodes elongation, apical dominance, tropisms, abscission and rooting. Auxin is needed together with other plant growth regulators for cell division and organized cell expansion (Skoog & Miller, 1957) that have influence in various impacts on plant physiology and growth (Vanneste & Friml, 2009). The formation of callus and roots by different auxins may rely greatly on the presence of other endogenous plant hormones that indirectly impacts the development and morphology of regenerated organs. The results with IBA therefore may be improved at higher concentrations tested or in the presence of other cytokinins.

Conclusion

NAA was proven to be efficient in the root induction of *C. ternatea* at the concentrations tested. Explants inoculated on half-strength MS medium supplemented with NAA showed higher root and callus induction than the medium supplemented with IBA albeit at lower concentration range. Cotyledon explants showed better root induction (70%) at 2 mg/L NAA and effective callus induction (100%) at 1.5 mg/L NAA, while the hypocotyl explants showed excellent callus induction (100%) at

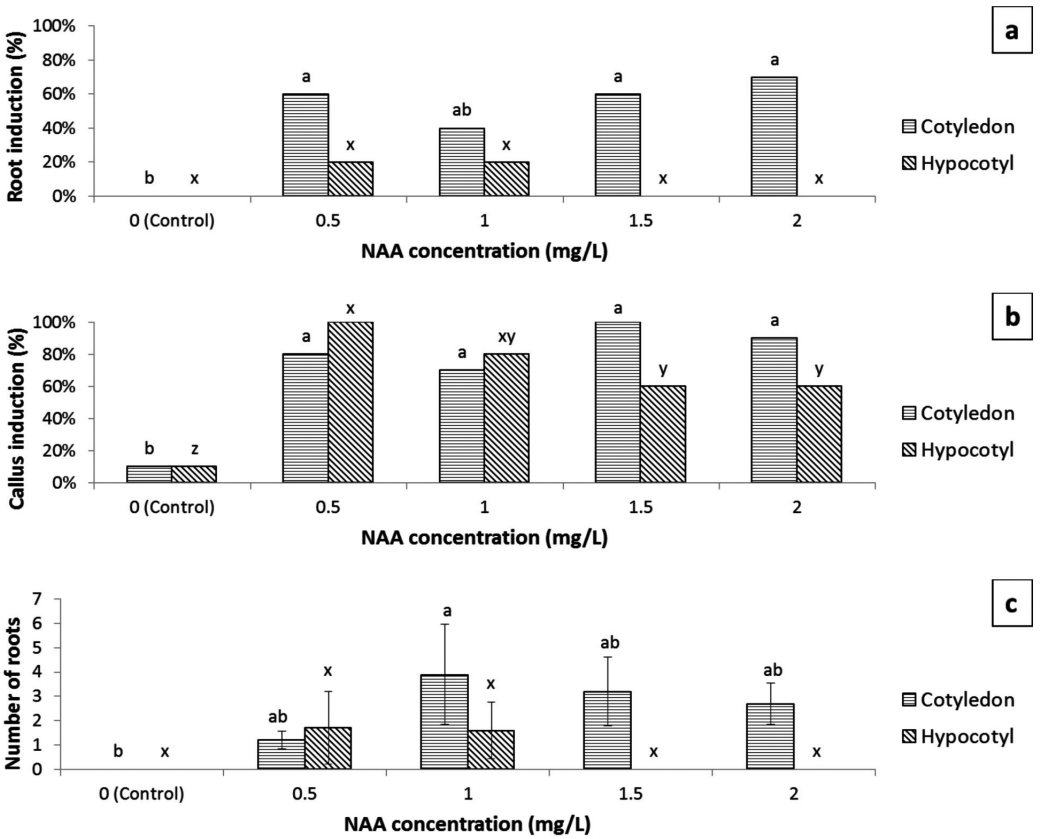

Figure 3: The effect of 1-Naphthaleneacetic acid (NAA) on a) root induction; b) callus induction; c) number of roots induced from cotyledon and hypocotyl explants of *C. ternatea* after 8 weeks of culture

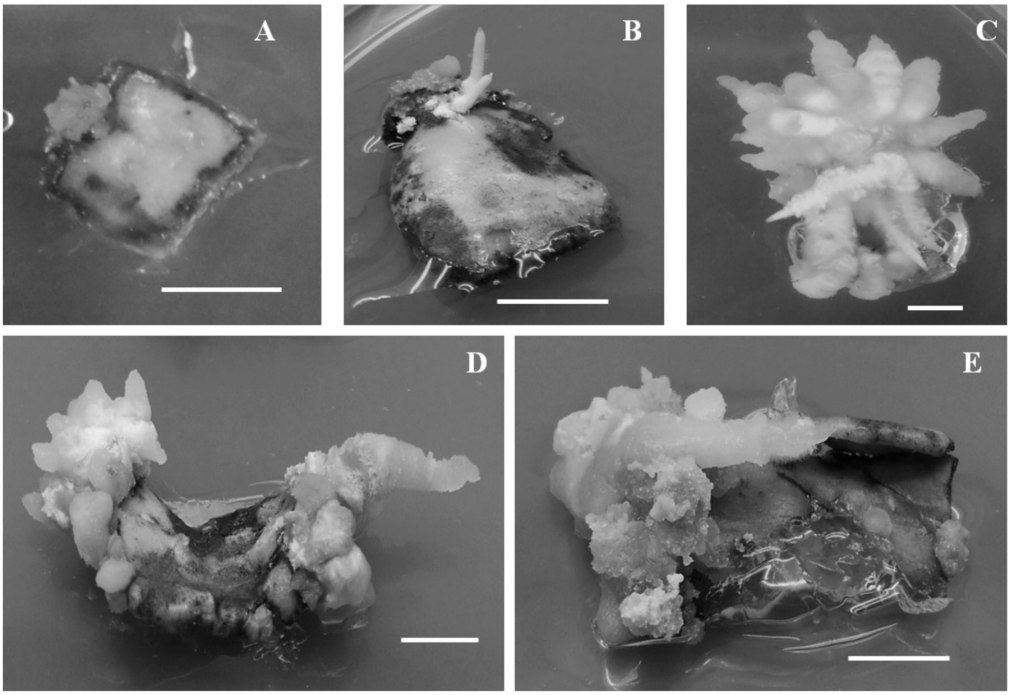

Figure 4: Root and callus induction of cotyledon explants of *C. ternatea* inoculated on half-strength MS medium supplemented with different concentrations of 1-Naphthaleneacetic acid (NAA) after 8 weeks of culture. A) control; B) 0.5 mg/L; C) 1 mg/L; D) 1.5 mg/L and E) 2 mg/L. The scale bars represent 0.5 cm

0.5 mg/L NAA. This study served to establish understanding on the selection of auxins for root induction in *C. ternatea*.

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