



CRUCIAL ROLE OF TDZ IN THE QUICK REGENERATION OF MULTIPLE SHOOTS OF CLITORIA TERNATEA L

Najma Ismail, Uzma Rani and Amla Batra

Plant Biotechnology lab. No.5, Department of Botany, University of Rajasthan, Jaipur, India.

*Corresponding author's E-mail: najmaismail@yahoo.co.in

Accepted on: 30-11-2010; Finalized on: 10-02-2011.

ABSTRACT

An efficient and rapid micropropagation technique was developed for rare medicinal plant *Clitoria ternatea*. The crucial role of TDZ was investigated on *in vitro* shoot proliferation from nodal explants of *Clitoria ternatea*. Murashige and Skoog (MS) medium containing TDZ (0.05- 2.5 μ M) was evaluated to develop multiple shoot buds and maintaining high rates of shoot multiplication on hormone free MS medium. The maximum number of shoots (15-20) with (80%) response was obtained on MS medium supplemented with (2.50 μ M) TDZ. *In vitro* developed shoots were rooted on half strength MS medium supplemented with IBA (2.0 μ M). Complete plantlets were then hardened, acclimatized and finally transferred to natural conditions where they exhibited 85% survivability.

Keywords: *Clitoria ternatea*, TDZ, Regeneration.

INTRODUCTION

Clitoria ternatea L. also known as "butterfly pea" in English is a multipurpose forage legume belongs to family Fabaceae. It is distributed in tropical Asia, the Philippine Islands and Madagascar¹. The root, stem and flowers are recommended for the treatment of snake bite and scorpion sting². Traditionally, the root of this plant has laxative, diuretic, anti-inflammatory and anthelmintic properties due to which, it is useful in the treatment of dysentery, severe bronchitis, asthma and hectic fever³. Also, the root extract is helpful in improving learning and memory in rats⁴. It is used as a tonic to cure ulcers of the cornea and tuberculosis⁵.

The wild stock of this important plant species has been rapidly diminished due to overexploitation and no efforts for its replenishment has been undertaken till date. Thus, this species has been listed as a rare plant species by the International Union for Conservation of Nature and Natural Resources (IUCNNR)⁶. However, propagation of *C. ternatea* L. through seeds is unreliable due to poor seed germination rate and less survivability under natural conditions¹ to fulfill the increasing demand of this potent medicinal plant, *in vitro* culture is an alternative method for conservation of this diminishing plant population. Sparse reports on *in vitro* studies of *C. ternatea* L. using a variety of explants such as leaf, immature embryo, nodal segments and excised root⁵⁻⁹, respectively are available. Pre-existing regeneration protocols of this plant species are not much efficient, repetitive and take lot of time for *in vitro* propagation. Therefore, the present study provides an efficient and repetitive protocol for micropropagation of *C. ternatea* L.

MATERIALS AND METHODS

Young shoots of *C. ternatea* were collected from the botanical garden of the Rajasthan University, Jaipur, India. The shoots were first washed under running tap water and then treated with a detergent, teepol 5%(v/v) for 10 min. followed by 3-4 washing with sterile distilled water (DW). The plant material was surface sterilized with 0.1% (w/v) HgCl₂ for 4 min following repeated washes with sterile distilled water. 0.5-1.0 cm sized nodal segments were excised aseptically and cultured on sterile shoot induction medium.

Media and culture conditions

The nutrient medium used in all the experiments was prepared, using the MS salts and vitamins (Murashige and Skoog 1962) with 3% (w/v) sucrose and (0.8%) bacteriological grade agar, and the pH of the medium was adjusted to 5.8 before autoclaving at 121^o C for 15 min. All the culture vials were kept in culture chamber at 25 \pm 2^o C under 16/8 hr (light/dark) photoperiod with a light intensity of 50 μ Mol⁻²S⁻¹ supplied by cool white fluorescent tube and with 60-65% relative humidity.

Shoot induction medium

MS basal medium containing different concentration of TDZ (0.05, 0.10, 0.50, 1.00 and 2.50, 5.0, 10.0 μ M) was used for shoot proliferation and multiplication. MS medium lacking growth regulator served as control. After an induction period of 3 weeks these were subcultured on hormone free MS medium. The frequency of explants producing elongated shoot was escalated after 2 weeks of sub-culturing (Table 1).

Rooting of elongated shoots and acclimatization

After proper shoot induction the plantlets were carefully removed from the medium and washed with sterilize



double distilled water properly, so that there should not be any trace of medium on roots. *In vitro* regenerated and elongated shoots (5-6 cm. long) were excised and transferred onto the rooting media containing ½ MS supplemented with IBA and strong *in vitro* rooting response was achieved on IBA (2.0 µM) (Table 2; Fig. E). After proper elongation these rooted plantlets were transferred to soilrite. These were uncovered after 2 weeks and subsequently transferred to green house for acclimatization.

Statistical Analysis

All the experiment was repeated thrice with 10 explants for each treatment. The data was analyzed using SPSS version 10 (SPSS Inc., Chicago, USA) and means were compared using Tukey's tests at the 5% level of significance. All means are presented with ± SE.

RESULTS AND DISCUSSION

The nodal explants placed on control medium did not produce any morphogenetic response. MS medium fortified with different concentration of TDZ individually stimulated axillary shoot sprouting within 1 week of culture. MS medium containing TDZ greatly influenced the frequency of multiple shoots regeneration. Among the various concentration of TDZ tested, 2.50µM was the most effective in inducing highest percentage 90% with maximum shoot multi (13.6±0.67) (Table1, Fig A) number of shoots increases with the increase in TDZ concentration, after reaching an optimum level (2.50 µM) number of shoots decreases. Reduction in number of shoots generated from each node at a concentration higher than the optimum level was also reported from several medicinal plants^{10,11}. The stimulating effect of TDZ on bud breaking and invitro multiple shoot formation has

been reported earlier for several plant species including woody taxa^{12,13}.

In the present investigation, frequency of multiple shoot was high in comparison to other reports on *Clitoria ternatea*^{8,9}. When other cytokinin were not effective than TDZ (0.1 nm -10µM) is found to be effective for multiple shoot bud induction in many species¹⁴. The maximum number (16-20) of shoots was obtained from nodal segment cultured on MS medium containing 2.50µM TDZ for four weeks prior to their transfer to hormone free MS medium (Fig C) continued presence of TDZ in their media causes deleterious effect by forming fasciated and distorted shoots^{15,16} (Fig B), to overcome this problem the cultures were transferred to hormone free MS medium¹³. In contrast, efficiency of BA and NAA on TDZ exposed explants for maximum shoot induction and was also evaluated in *Liquidambar Styraciflua*¹⁷ and *vitex nugundo*¹⁰. *In vitro* regenerated and elongated shoots (5-6 cm. long) were incised and transferred onto the rooting media containing half strength of MS medium supplemented with IBA and strong *in vitro* rooting response was achieved on IBA (2.0 µM) after 15 days of sub-culturing (Table 2, Fig.D). The number of roots per shoot varied with different concentrations of IBA. However, root development was slow at higher concentrations of IBA than that of optimum concentration (2.0 µM) along with diminished growth at lower concentrations of IBA (Table 2). The success of IBA in promoting efficient root induction has also been reported earlier in *C. ternatea*⁷, and *Withania somnifera* (Dunal) L.¹⁸ In contrast to this, efficient roots were induced on MS medium fortified with IAA in *Cardiospermum halicabum* and *Azadiractha indica*^{19,20}, respectively and NAA was also proved to be best for *in vitro* rooting in *C. ternatea* L.⁹

Table 1: Effect of different concentration of TDZ supplemented with MS medium on shoot induction in *Clitoria ternatea*

TDZ (µM)	% Response	No of Shoot buds / Ex plant
0	0%	0
0.10	50%	7.4±0.50
0.50	60%	4.8±0.58
1.00	80%	9.8±0.80
2.50	90%	20.6±0.27
5.0	70%	6.6±0.67
10.0	40%	4.6±0.40

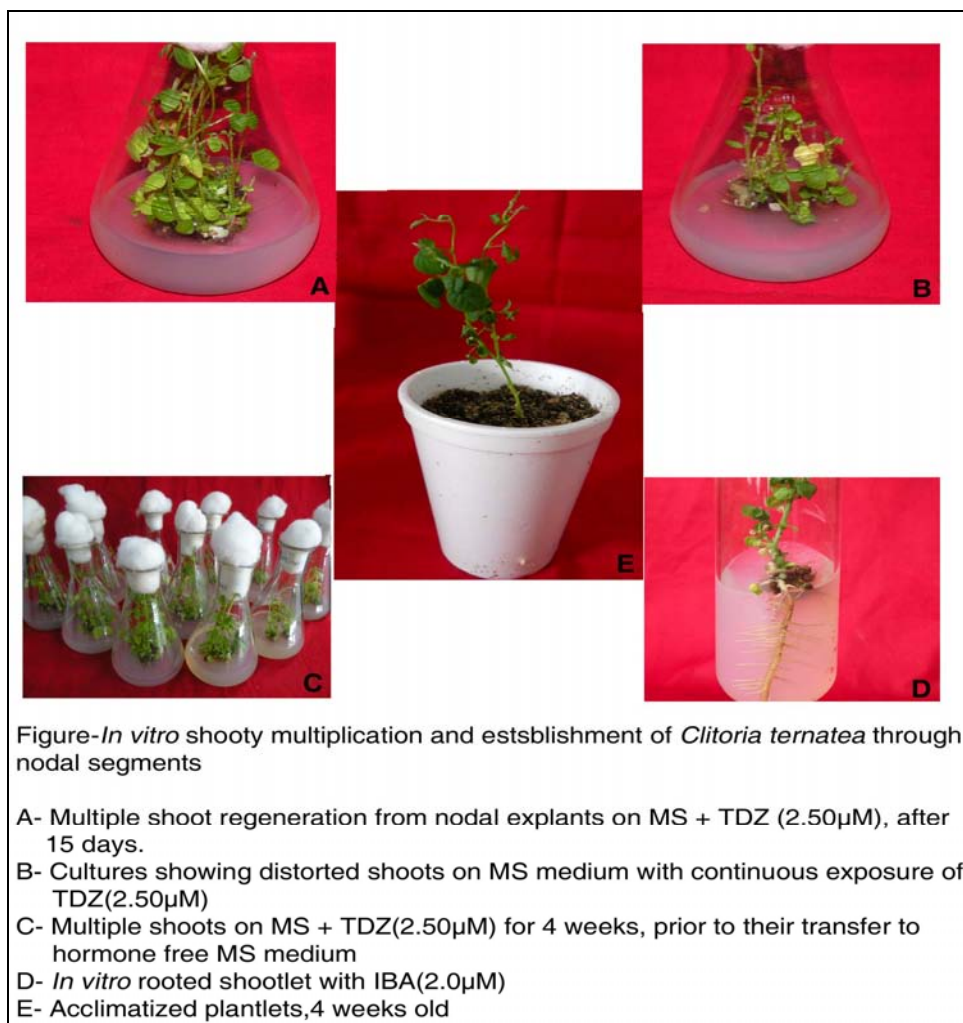
Values represent mean ± SE of 28 replicates per treatment in three repeated experiments. Mean values with the column followed by the same letter are not significantly different by the Turkey's test at 0.05% probability level.

Table 2: Effect of different concentration of IBA supplemented with ½ strength of MS medium on root induction in *Clitoria ternatea*

Medium Composition	% Rooting	No of Roots/ Shoot
1/2MS+IBA (0.5µm)	10	1.13
1/2MS+IBA (1.0µm)	30	1.76
1/2MS+IBA (2.0µm)	60	3.95
1/2MS+IBA (3.0µm)	20	2.57

Values represent mean ± SE of 28 replicates per treatment in three repeated experiments. Mean values with the column followed by the same letter are not significantly different by the Turkey's test at 0.05% probability level.





After the development of roots, the plantlets were taken out from the culture vial and washed with sterilized distilled water to remove adhering agar medium, so that the chances of contamination could be stopped. Then these juvenile plantlets were transferred to the earthen pots containing sterilized soil and sterilized soilrite and kept in plant growth chamber for their hardening at optimized natural conditions like temperature, light and humidity. After 22-28 days, these plantlets were simultaneously exposed to natural environment, where 85% plantlets survived well in the field (Fig. E).

CONCLUSION

In conclusion, an efficient protocol was developed for successful micropropagation and multiple plant regeneration of an important medicinal plant *C. ternatea* L. In this study, we reported *in vitro* propagation from nodal segments of *C. ternatea* L using TDZ. The protocol reported in this study can be used for rapid and large scale propagation of *C. ternatea* L.

REFERENCES

1. Anonymous (1988). The wealth of India: a dictionary of Indian raw materials and industrial products, New Delhi: Publication and Information Directorate, CSIR 11
2. Morris JB (1999) Legume genetic resources with 'Value added' industrial and pharmaceutical use. In: Janick J (ed) Perspective on new crops and new uses. Alexandria, VA: ASHS Press pp 196-201.
3. Kumar KP, Soniya EV, Lawrence B, Nair GM (1993) Micropropagation of *Clitoria ternatea* L. (Papilionaceae) through callus regeneration and shoot tip multiplication. 527 Journal of Species and Aromatic Crops 2:41-46.
4. Rai KS, Murthy KD, Karanth KS, Nalini K, Rao MS, Srinivashan KK (2002) *Clitoria ternatea* root extract enhances acetylcholine content in rat hippocampus. Fitoterapia 73,658-689.
5. -171 Malabadi RB, Nataraja K (2001). Shoot regeneration in leaf explants of *Clitoria ternatea* L. cultured in vitro. Phytomorph. 51: 169.
6. Panday NK, Tewari KC, Tewari RN, Joshi GC, Pande VN, Pandey G (1993) Medicinal plants of Kumaon,

- Himalaya strategies for conservation. In: Dhar U (ed) Himalayan Biodiversity Conservation Strategies. No.3 Publications, Nanital, India.
7. Lakshman KK, Dhanalakshmi S (1990) Callus, organogenesis and plant formation in tissue cultures of *Clitoria ternatea*. Annals of Botany 66:451-445.
 8. Rout, G.R. (2005). Effect of cytokinin and auxin on micropropagation of *Clitoria turnatea* L. Biot. Lett., 41: 21-26.
 9. Shahzad A, Faisal M, Anis M (2007) Micropropagation through excised root culture of *Clitoria ternatea* L. and comparison between in vitro regenerated plant and seedlings. Annals of Applied Biol 150 (3): 341-349.
 10. Ahmed N. and Anis M. (2007) Rapid clonal multiplication of a woody tree, *Vitex negundo* L. through shoots proliferation, Agroforest Syst (2007) 71:195-200.
 11. Faisal M., Siddique I., Anis M (2006) In vitro rapid regeneration of plantlets from nodal explants of *Mucuna pruriens* – a valuable medicinal plant Ann app Biol. 148:1-6.
 12. Ahmad N, Siddiqui I, Anis M(2006) Improved plant regeneration in chilli using thidiazuron. Biol Plant 50:701-704.
 13. Faisal M, Ahmad N, Anis M(2005) Shoot multiplication in *Rauvolfia tetraphylla* L. using thidiazuron. Plant Cell Tiss Org Cult 80: 187-190.
 14. Huetteman CA, Preece JE (1993) Thidiazuron: a potent cytokinin for woody plant tissue culture. Plant Cell Tiss Org Cult 33:105-119.
 15. Bohmer P, Meyer B, Jacobson HJ(1995) Thidiazuron induced high frequency of shoot induction and plant regeneration in protoplast derived pea callus. Plant Cell Rep. 15:26-29.
 16. Murthy BNS, Victor J, Singh RPS, Fletcher RA, Saxena PK(1996) In vitro regeneration of chickpea (*Cicer arietinum* L.) stimulation of direct organogenesis and somatic embryogenesis by thidiazuron. Plant Growth Reg. 19: 233-240.
 17. Kim MK, Sommer HE, Bongarten BC, Markle SA(1997) High frequency induction of adventitious shoots from hypocotyls segments of *Liquidambar styraciflua* L. by thidiazuron. Plant Cell Rep 16:536-540.
 18. Sharma MM, Batra A (2006) High frequency Plant Regeneration Indian Ginseng: *Withania somnifera* L.(Dunal). Physiol Mol Biol Plants 12(4) :289-293.
 19. Jahan AA, Anis M (2009) In vitro rapid multiplication and propagation of *Cardiospermum halicabum* L. through axillary bud culture. Acta Physiol Plant 31:133-138.
 20. Shekhawat SG, Mathur S, Batra A (2009) Role of Phytohormones and nitrogen in somatic embryogenesis induction in cell culture derived from leaflets of *Azadirachta indica*. Biol Plant 53(4):707-710.

About Corresponding Author: Mrs. Najma Ismail



Mrs. Najma Ismail is Post graduated and graduated from Aligarh Muslim University, India. She has taken specialization in Plant Biotechnology and Plant Tissue Culture. She is currently pursuing her Ph.D under the guidance of Prof. Amla Batra, University of Rajasthan, Jaipur, India. She is very thankful to Miss Uzma Rani for her great help in micropropagation of *Clitoria ternatea* L.