EFFECT OF THIDIAZURON (TDZ) ON *IN VITRO* PROPAGATION OF VALUABLE MEDICINAL PLANT: *URARIA PICTA* (JACQ.) DESV. EX DC.

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ABSTRACT

Thidiazuron (TDZ) is a known plant growth regulator, highly used in tissue culture techniques for shoot organogenesis and increased axillary bud proliferation. Effect of TDZ on *in vitro* propagation of *Uraria picta* from nodal explants was studied during September-October 2013 in the Department of Botany, Gujarat University, Ahmedabad, Gujarat, India. Among the various concentrations (0.5, 1, 1.5, 2, 2.5, 3 mg/l) of TDZ alone and with combination of $AgNO_3$ (2 mg/l) were analyzed to establish shoot induction. TDZ at low concentration (0.5 mg/l) showed maximum morphogenetic response producing an average of 10 shoots per plant. Root induction *in vitro* on generated shoots was achieved on half-strength MS medium supplemented with IBA (1.5 mg/l). Average nine roots per shoot having an average length of 6.95 cm were generated on this medium formulation. Through this procedure about 356 plantlets were generated from single nodal explant over three sub-cultures. Micropropagation would be useful to generate large number of clonal propagules.

KEYWORDS: ***Uraria picta;*** **medicinal plant; *In vitro* multiplication; tissue culture; thidiazuron; silver nitrate; India.**

INTRODUCTION

Thidiazuron (TDZ), chemically known as 1-phenyl-3-(1, 2, 3-thiadiazol-5-yl) urea is a synthetic plant hormone. It is well established that TDZ has cytokinin-like activity and enormous capacity to induce shoot organogenesis in a number of plant species including legumes (3, 14, 18). TDZ concentration is highly sensitive for the induction of shoot multiplication, growth and development of *in vitro* culture of plant. It is extensively used for *in vitro* micropropagation of many medicinal plants to meet the demand for planting materials. *Uraria picta* (Jacq.) Desv. ex DC. belonging to family Fabaceae is suffruticose herb which grows up to 1.5-2 m tall and found in dry grasslands, waste places, open deciduous forests and in all plains of India. It

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is also found in Ceylon, Malaysia and Philippines (20). It is one of the components of ayurvedic formulation “Dashmularisht”. Roots are aphrodisiac in nature, mainly used for fracture healing properties (8, 10). Its decoction is useful in cough, chills and fevers. In Southern Nigeria, leaves of *Uraria picta* were used for treatment of gonorrhoea. It is used as antidote (2) and in treatment of primary neurological disorders (8). In Ghana, the plant is used in heart troubles. It is also used in preparation of ‘Abana’, an ayurvedic drug remedy useful in treatment of hypertension, tachycardia and angina (15).

The existence of *U. picta* in natural population is highly under threat. It is naturally propagated by seeds. However, the seed set is poor and seed viability and percentage of germination is low (10). Large-scale production is a pre-requisite to meet the pharmaceutical needs and also for the effective conservation of this valuable medicinal plant. Tissue culture techniques can be applied to large scale propagation and biodiversity conservation especially for those species which either the roots/rhizomes or the whole plant is used in drug preparation (8, 10, 18). Micropropagation or clonal propagation is one of the approaches of plant tissue culture dealing with *in vitro* multiplication of plants. The application is to produce large number of identical, aseptic plants in limited period of time and space (21). Because of this potentiality, study was initiated to develop a protocol for multiplication of *U. picta* through nodal culture.

MATERIALS AND METHODS

Present study was carried out to analyse the effect of TDZ on *in vitro* propagation of *U. picta* using nodal explants during September-October 2013 in Department of Botany, Gujarat University, Ahmedabad, Gujarat, India. Plants of *U. picta* growing in botanical garden were used for clonal propagation purpose. Seeds were collected from the healthy plants. The seed coat was removed simply by soaking in 98 percent pure H_2SO_4 (HiMEDIA). After 30 minutes, seeds were washed under running tap water, then washed with 10-12 drops of antiseptic liquid soap in distilled water for 15 minutes with constant shaking and rinsed thoroughly under running tap water for 30 minutes. Finally, seeds were surface sterilized for 30 minutes with 0.1 percent $HgCl_2$; followed by extensive rinse (3-4 times) with DW (10). Then sterilized seeds were implanted on plain MS medium fortified with 3 percent sucrose and solidified with 0.8 percent agar-agar in *in vitro* condition. The seeds were germinated and produced seedlings within 4-5 weeks.

Single-node explants were isolated from 37 days old seedlings. The nodal explants (1-2 cm long) were implanted on MS medium fortified with 3 percent

sucrose and solidified with 0.8 percent agar-agar. Media was supplemented with six concentrations of TDZ (0.5, 1, 1.5, 2, 2.5, 3 mg/l) in combination with and without $AgNO_3$ (2 mg/l) (Table 1). Sub-cultures were made at 2 weeks interval and results were recorded.

For induction of roots, *in vitro* shoots were treated with range of auxins (IBA and NAA) at various concentrations in ½ strength MS medium (Table 2). The pH of media was adjusted to 5.8 prior to autoclaving. The cultures were incubated in a culture room with 25±1°C and 16 hours photoperiod was provided by white fluorescent tubes (1000 lux). Regenerated shoots were transferred on medium augmented with distinct plant hormone with appropriate concentration and supplements (0.5 mg/l TDZ and 2 mg/l $AgNO_3$) in which maximum number of shoots obtained.

The developed plantlets were washed gently in water to remove adhering media and dipped in 1 percent (w/v) bavistin for 10 minutes. The treated plantlets were then planted in thermocol glasses containing a mixture comprising soil, sand and compost (1:1:1). Humidity was maintained around transplanted plants by covering the plastic glasses for two weeks and regular spray with water. After four weeks, plantlets were transferred to a shade-area (covered with jute sheet) where humidity was maintained by keeping the jute sheet wet with water. By this technique, plantlets were successfully acclimatized without mist-house/greenhouse facilities. Then well developed plants were transferred in earthen pots.

RESULTS AND DISCUSSION

Seed germination is dependent on various factors soaking treatment of seeds in concentrated H_2SO_4 was helpful for (about 100 percent) seeds germination. Sterilization treatment with 0.1 percent mercuric chloride ($HgCl_2$) was done to protect seeds from any type of contamination.

Explants grown on MS medium lacking plant hormones did not prove effective for shoot multiplication and did not show any morphogenetic response. Whereas sprouting and shoot proliferation was observed within one week of culture on MS medium fortified with various concentrations of TDZ. Effect of TDZ with $AgNO_3$ (2 mg/l) on axillary shoot proliferation was also evaluated (Table 1). TDZ is known for inducing shoot multiplication in many plants (23, 24, 26, 27). TDZ induces shoot induction in high rate by stimulating the biosynthesis of endogenous auxins and cytokinin (7, 16) or may mimic an auxin response (29, 9) or modifies endogenous auxin

metabolism (12, 24). Silver nitrate proved to be an important inhibitor of ethylene action and improved the growth of *in vitro* cultures (7). Addition of silver nitrate in tissue culture medium inhibits secretion of phenolic compounds from explant, protects the medium from bacterial contamination and allowed the improvement of regeneration process (5, 7).

Table 1. Effect of different combinations of TDZ and $AgNO_3$ (2 mg/l) on multiplication of *U. picta* from nodal explants.

TDZ [mg/ l]	$AgNO_3$ [mg/l]	Response %	No. of Multiples
0.5	-	100	10.33 ± 1.2
1	-	85	9.16 ± 0.67
1.5	-	75	6.21 ± 0.85
2	-	30	5.5 ± 2.21
2.5	-	30	3.3 ± 0.25
3	-	25	2.75 ± 0.25
0.5	2	100	2.33 ± 0.42
1	2	75	3.5 ± 0.72
1.5	2	30	2.4 ± 0.51
2	2	25	1.71 ± 0.28

Combination of TDZ with silver nitrate did not found effective for shoot multiplication in *U. picta* (Table 1). TDZ alone could stimulate the regeneration of nodal explants. Shoot regeneration and multiplication in medium supplemented with $AgNO_3$ in combination with TDZ exhibited poor response (Fig. 1B). In all the concentrations of TDZ supplemented medium, frequency of shoot bud sprout was in abundance and exhibited healthy growth (Fig. 1C-E). TDZ at low concentration (0.5 mg/l) showed maximum multiplication, producing an average of 10 shoots per explant (Fig. 1C). Most of the shoots regenerated on TDZ comprising medium remained stunted with swelled basal ends and poorly developed leaves (Fig. 1 C & E). So, elongation of shoots were achieved by transfering the *in vitro* generated axillary buds on MS medium fortified with 0.5 mg/l TDZ (Fig. 1F). The earlier studies on micropropagation of various plant species such as *Hedychium coronarium* (14), *Cleome viscosa* (28), *Aerva lanata* (26), *Cardiospermum halicacabum* (13), broccoli and cabbage (23) proved that TDZ alone and in combination with auxins (17, 28, 23) is the most effective for inducing adventitious and axillary shoot proliferation. TDZ also found effective to produce *in vitro* flowers in *Aerva lantana* (26), induce somatic embryogenesis in pigeonpea (25) and delayed the onset of leaf senescence in *Pelargonium* (19).

Table 2. Influence of various types of auxins in half-strength MS medium on root induction of *in vitro* generated shoots of *U.picta*.

Growth hormones [mg/l]		Response [%]	No. of roots	Root length [cm]
IBA	NAA			
-	-	25	1.5 ± 0.49	2.33 ± 1.07
0.5	-	75	5.66 ± 1.32	2.05 ± 0.71
1	-	100	4.33 ± 2.7	5.84 ± 1.14
1.5	-	100	9 ± 1	6.95 ± 2.08
2	-	75	8 ± 0	3.62 ± 0.55
2.5	-	80	4 ± 0	6.33 ± 2.22
-	0.5	25	4 ± 0.57	0.78 ± 0.17
-	1	50	5.8 ± 1.37	1 ± 0.37
-	1.5	75	7 ± 3	0.42 ± 0.14
-	2	70	7 ± 1.29	0.92 ± 0.35

Means ± SE of 10 replicants.

Earlier studies on micropropagation of *U. picta* reported about 7-19 shoots per explant on medium containing benzyladenine (BA) alone (4, 10) or BA along with IAA and adenine sulfate (22). The results of present work revealed that shoot induction efficacy of *U. picta* can be positively modified by low concentration of TDZ. Excision as well as sub-culture of *in vitro* regenerated shoots provide a right path to increase the rate of shoot proliferation. So, *in vitro* generated clusters of multiple shoots were sub-cultured on MS medium augmented with 0.5 mg/l TDZ.

In vitro regenerated shoots with 2-3 cm length having three to four leaves were used for rooting. MS media with half-strength supplemented with various concentrations of IBA (Indole-3-butyric acid) and NAA (naphthalene acetic acid) were used for rooting of *in vitro* generated shoots. Results differed depending upon the concentration, nature and strength of auxins employed. Half-strength MS medium enriched with IBA (1.5 mg/l) induced maximum number of roots (an average of nine roots per shoot) having an average length of 6.95 cm (Table 2). The roots were healthier and much branched with numerous root hairs (Fig. 1G).

Plantlets were successfully acclimatized without mist/greenhouse facilities (Fig. 1H). A high humidity was maintained by a regular spray with water. After four weeks, plantlets were transferred to a shade-area (covered with jute sheet) where humidity was maintained by keeping the jute sheet wet with water. After one month of hardening, the acclimated plantlets were transferred to the field showing normal features without any morphological variation (Fig. 1I). The result described here, demonstrate successful and reproducible clonal propagation of a valuable medicinal plant *U. picta*.

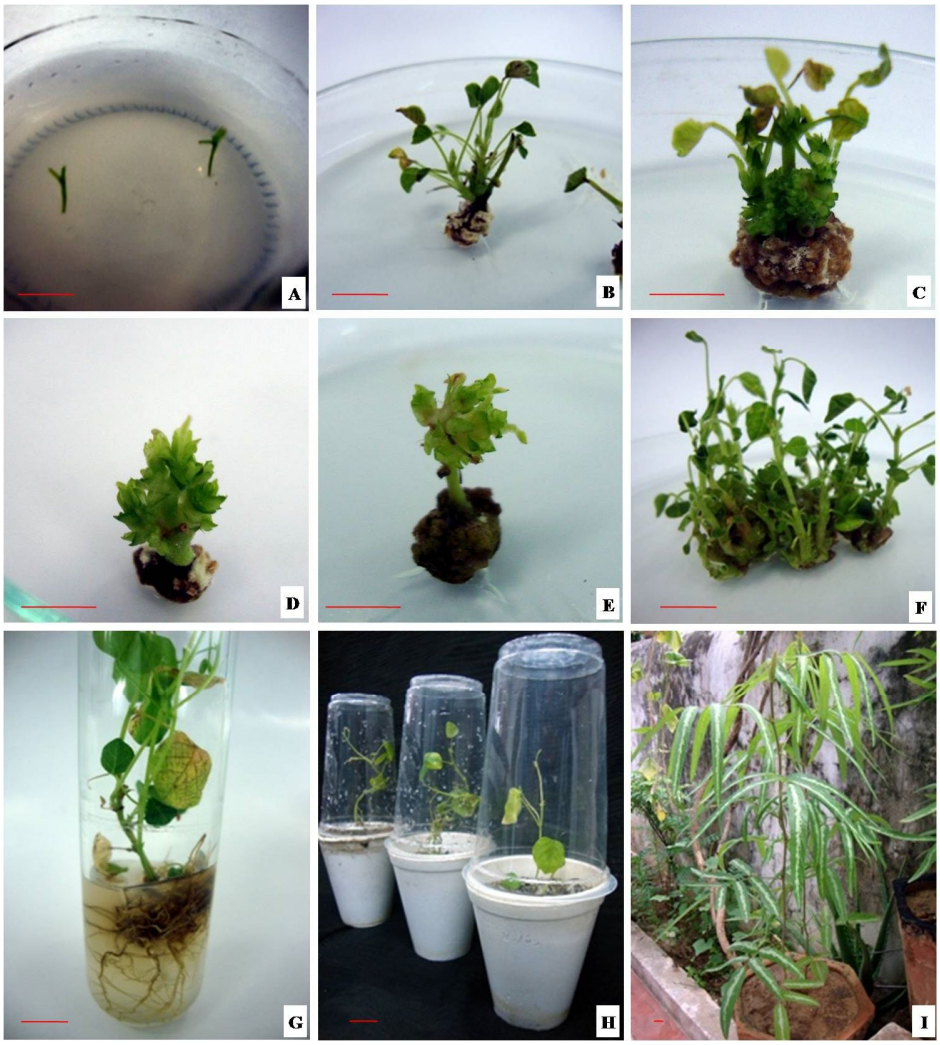

Fig. 1. Effect of TDZ on multiplication of *Uraria picta* A- Nodal explant; B-Sprouting of nodal buds on MS medium supplemented with TDZ (1mg/l) + AgNO3 (2 mg/l); C-E-Sprouting of nodal buds on MS medium supplemented with TDZ (0.5, 1 and 2 mg/l subsequently); F-Elongation of shoot buds on MS medium fortifield with 0.5 mg/l TDZ; G-Roorting on *in vitro* generated shoots; H-Hardening of *Uraria* plantlets; I-Plant in the earthen pot.
Note: Horizontal bar in each photograph is equal to 1 cm.

CONCLUSION

The findings of present work revealed that low concentration of TDZ is highly effective for shoot proliferation of *Uraria picta*. MS medium fortified with 0.5

mg/l TDZ is an optimized formulation for *Uraria picta* micropropagation. These optimized protocols for shoot proliferation of *Uraria picta* can be used to fulfill the industrial demand without destroying the natural sources.

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