DOI:10.26524/krj200

Kong. Res. J. 4(2): 39-46, 2017 ISSN 2349-2694
Kongunadu Arts and Science College, Coimbatore.

PROPAGATION STRATEGIES OF *TRIBULUS TERRESTRIS* L. A PORTENTOUS MEDICINAL PLANT EMPLOYING TISSUE CULTURE TECHNOLOGY

Jamuna, S[1*]., B. Anjali[2] and K. Karthika[1]
[1]Department of Botany, Kongunadu Arts and Science College, Coimbatore-641029.
[2]Department of Biotechnology, SNDP Yogam College, Konni, Pathanamthitta, Kerala.
*E.mail: sjamunaphd@gmail.com

ABSTRACT

A protocol for micropropagation of *Tribulus terrestris,* an important medicinal herb was established using juvenile explants *viz.,* leaf, node and internode. All the explants were tested for callus induction on Murashige and Skoog's (MS) medium, supplemented with BAP, NAA and 2,4-D. Among the three explants leaf explant responded well (98%) for the callus induction in the MS medium composted with BAP and NAA (4.0 and 0.5 mg/L) followed by the nodal segments (58.75%) in the same medium. Maximum number of shoot induction from the callus of leaf derived explants (91.1%) was perceived on MS medium fortified with BAP 4.0 mg/L and NAA (0.5 mg/L). Moreover, root elongation and profuse rooting percentage (77.19%) were achieved when the well-grown shoots were cultured on MS media supplemented with IAA (2.0 mg/L) for leaf callus derived shoots. The regenerated plantlets were hardened and established at 80% survival rate in hardening media encompassed with red soil, sand and vermicompost in the ratio of 1:1:1 by volume.

Keywords: *Tribulus terrestris,* leaf, node and internodal explants, *in vitro* regeneration.

1. INTRODUCTION

The genus, *Tribulus* comprises about 20 species of creeping shrubs or herbs, of which *Tribulus terrestris* L. (Zygophyllaceae) is the most common. It is also known as 'puncture vine' native of Mediterranean region and that found in warm regions of Europe, Asia, America, Africa and Australia (Frohne, 1999). It is a tap rooted, prostrate, procumbent flowering plant, the stem grows up to 2cm long, leaves are opposite, 4-8 pairs, and spear shaped leaflets, presence of hairs on margin of the leaf. Flowers are yellow. Seeds enclosed in a woody star-shaped structure 5-7mm long and 5-6mm wide (carpels). The leaf parts of *T. terrestris* are used to cure renal problems by the tribals of Al-Rass province, Saudi Arabia (Gamal *et al.,* 2010).Juice of fresh leaves is given to animals in case of colic and chronic cough by the rural farmers and traditional herbal healers from Tikamgarh District of Bundelkhand, Central India (Verma, 2014). The traditional practitioners of Palakkad District prescribed the decoction of dried seeds for diuretic and antilithiatic activity (Smitha *et al.,* 2014). The native communities of Terai forest of Western Nepal were stipulated the entire plant is given orally for the treatment of urinogenital tract infection (Anant Gopal *et al.,* 2012).

Conventionally the plant is propagated through seeds, but it yields very low percentage of germination under natural and laboratory conditions (Raghu, 2005; Raghu and Mohanan, 2006). Due to its high medicinal value and increasing demand, *in vitro* propagation strategies have great importance to reacclimatize the plant in a large-scale is vital in current scenario. Hence, the present study was aimed at to regenerate the plantlets of *T. terrestris* using the juvenile leaf, node and internodal explants and to guard against its overexploitation.

2. MATERIALS AND METHODS

2.1. Collection of the plant materials

Healthy and immature leaf, node and internodal segments of the study species, *Tribulus terrestri* (Fig. 1) were collected foot hills of Maruthamalai, Coimbatore district and used as explants.

2.2. Surface sterilization of the explants

The leaf, node and internodal explants were washed with running tap water and they were cut into small pieces. Apparently, the dust particles on the surface were reduced with a surfactant, tween-20 (5%w/v). After 5 minutes they were rinsed with double distilled water. Likewise, the elimination of fungus on the surface of the explant were done by a fungicide, bavastin (5%w/v) for 5 minutes and rinsed with double distilled water for 3-4 times. Furthermore, screening of bacterial contamination on the explants was carried out with ampicillin (5%w/v) for 5 minutes followed by 3-4 rinses in double distilled water. Finally, removal of toxic chemicals were carried out by dipping the explants

into 0.1% $HgCl_2$ for 5 minutes and then it was rinsed with autoclaved double distilled water, within the Laminar air flow chamber.

2.2. MS media and culture conditions

After surface sterilization the leaf, node and internodal (0.5cm) explants were inoculated on MS media (Murashige and Skoog, 1962) containing 30% sucrose solidified with 8% agar (Tissue culture grade, Hi-Media, India). The pH of the medium was adjusted to 5.6-5.8 with 0.1 N NaOH or 0.1 N HCl prior to the addition of agar. It was autoclaved at 121°C and 15lbs/$inch^2$ pressure for 15 minutes. The cultures were maintained under white fluorescent light having 2000 lux light intensity. The incubation temperature was adjusted to 25±2°C with 16 hours light and 8 hours dark period in every 24 hours cycle.

2.3. Callus induction

The leaf, node and internodal explants were inoculated on MS medium supplemented with different concentrations and combinations of growth regulators *viz.,* BAP, NAA and 2,4-D. The days required for callus formation, percentage, colour and nature of the callus was perceived.

2.4. Shoot regeneration

For the shoot induction the *in vitro* derived callus was transferred to MS medium containing different concentrations and combinations of growth regulators such as BAP, NAA and 2,4-D. The percentage of explants responding, number of shoots per callus and length of the shoots were recorded after 40 days of culture.

2.5. Root induction and acclimatization

The thrived shoots were shifted to MS medium fortified with various concentrations of IBA, NAA and 2,4-D for root formation. The rooting attributes *viz.,* per cent of shoots responding for rooting, number of roots per shoot and the length of the roots were observed. The rooted plantlets were transferred to the hardening medium containing various combinations of hardening mixtures and the rate of survivability was determined.

2.6. Statistical analysis

In vitro regeneration of the study species were done using thirty explants and were repeated thrice. Data were subjected to statistical analysis (ANOVA) and means of different experiments were compared by using Duncan's Multiple Range Test ($p<0.05$) (Duncan, 1955).

3. RESULTS

The number of days required for callus induction after the inoculation of explants was varied between 12 and 30 for the study species, *T. terrestris* (Table 1). A higher percentage of callus formation (98.97%) in leaf segments were perceived in the MS medium containing the growth regulator, BAP and NAA at 4.0 and 0.5mg/L respectively (Fig. 2, a and b). Next to the leaf explant the nodal explants showed higher degree (58.75%) of callus induction in same concentration. On the other hand, intermodal segments produced very poor response (39.36%) of callus formation in the above said concentration. From the results two well responded explants (leaf and node) were taken for further shoot and root regeneration.

Table 2 shows the shoot induction (91.1%) of leaf derived callus explants of the species, *T. terrestris.* The best shoot induction (91.1%) was noticed in the MS medium fortified with BAP (4.0mg/L) and NAA (0.5mg/L). It also displayed the higher number of shoots (11.56 shoots/callus) and shoot length (6.5cm) (Fig. 2, c). Table 3 and Fig. 3, a and b exhibits the shoot induction from the nodal explants of the study species. The maximum percentage of shoot induction (65.53%) was viewed in the MS medium fortified with BAP (4.0 mg/L) and NAA (0.5 mg/L). The greater number of shoots (12.66 shoots/callus) and shoot length (6.9cm) were also observed in the same medium.

Root induction of leaf derived callus of the species, *T. terrestris* is depicted in Table 4 and Fig.2, d. The MS medium composed with IAA (2.0 mg/L) alone unveiled maximum root proliferation than the other concentration and combinations tried. It also produced higher number of roots (9.37 roots/shoot) and root length (7.3cm). The root induction of the node derived shoot explants of study species is given in Table 5 and Fig. 3, c. The higher percentage (60.67%) of root induction was identified in the MS medium composed with IAA (2.0 mg/L) alone and this concentration also revealed higher number of roots (12.67 roots/shoot) and the root length (7.5 cm).

Hardening experiments were conducted for two explants of the study species, *T. terrestris* by using various hardening media to determine the survivability rate of plantlets (Tables 6 and 7). The survivability rate of leaf callus derived plantlets was significantly higher (80.46%) in the hardening medium comprised by garden soil, sand and vermicompost in the ratio of 1:1:1 by volume followed by 75.24% survivability rate was obtained from decomposed coir waste, perlite and compost in

the ratio of 1:1:1 by volume (75.24%) (Table 6 and Fig. 2, e). On the other hand, it was noticed that the nodal callus derived plantlets registered higher survivability (70.56%) in the hardening medium encompassed by vermiculate, coir waste and forest litter in the of 1:1:1 by volume followed by 66.45% of survivability was seen in decomposed coir waster, perlite and compost in the ratio of 1:1:1 by volume (Table 7 and Fig. 3, d). The study divulged that all hardening media except red soil combined with sand were suitable for maintaining the higher rate of plantlet survivability, above 50%.

Table 1. Effect of different concentrations of growth regulators on per cent callus induction from leaf, node and internode explants of the species, *Tribulus terrestris.*

Growth regulators (mg/L)			Days required for callus formation after inoculation			Callus formation (%)		
			Explants			Explants		
BAP	NAA	2,4-D	Leaf	Node	Internode	Leaf	Node	Internode
0.5	0.5	0.0	23	15	-	65.31±0.82^{i}	45.34±1.63^{h}	11.31±0.14^{i}
1.0	0.5	0.0	23	14	12	70.22±1.63^{g}	51.23±0.82^{d}	19.00±1.63^{g}
1.5	0.5	0.0	27	22	13	72.34±0.82^{f}	49.45±0.82^{e}	28.32±0.82^{ef}
2.0	0.5	0.0	15	23	15	73.44±1.63^{cd}	55.00±0.82^{b}	25.19±1.63^{f}
2.5	0.5	0.0	26	26	14	77.00±1.63^{b}	51.85±0.82^{de}	32.42±1.63^{bc}
3.0	0.5	0.0	28	27	16	75.18±0.82^{cd}	47.45±1.63^{g}	32.17±0.82^{b}
3.5	0.5	0.0	18	15	17	76.56±1.60^{c}	19.00±1.63^{l}	10.00±1.63^{ij}
4.0	0.5	0.0	19	12	-	98.97±0.63^{a}	58.75±1.63^{a}	-
0.5	0.0	0.5	21	13	16	63.76±1.63^{kl}	34.31±0.41^{i}	16.24±2.45^{h}
1.0	0.0	0.5	17	17	18	61.46±1.63^{m}	47.26±1.63^{f}	23.18±0.82^{g}
1.5	0.0	0.5	23	16	17	64.00±0.82^{k}	54.47±0.82^{c}	39.36±0.82^{a}
2.0	0.0	0.5	18	14	13	65.21±1.63^{ij}	31.57±0.33^{j}	9.14±1.63^{jk}
2.5	0.0	0.5	20	15	14	69.64±0.82^{h}	49.89±0.82^{ef}	10.67±0.82^{j}
3.0	0.0	0.5	22	23	17	72.77±1.63^{de}	54.16±1.63^{bc}	28.17±0.24^{de}
3.5	0.0	0.5	23	27	15	76.17±1.63^{bc}	57.00±0.82^{ab}	31.34±0.82^{cd}
4.0	0.0	0.5	25	30	-	73.43±0.82^{b}	24.11±1.63^{k}	-

Mean values in columns are followed by different letter (s) are significant to each other at 5% level accordance to DMRT.

Table 2. Effect of different concentrations of growth regulators on shoot initiation, shoot number and shoot length after the subculturing of leaf derived callus of the species, *Tribulus terrestris.*

Growth regulators (mg/L)			Culture response (%)	No. of shoots/callus	Shoot length (cm)
BAP	NAA	2,4-D			
0.5	0.5	0.0	20.2±0.81^{m}	1.57±0.82^{j}	1.4±0.82^{h}
1.0	0.5	0.0	24.6±0.96^{k}	3.52±1.63^{hi}	2.6±0.82^{g}
1.5	0.5	0.0	34.1±0.99^{j}	5.23±0.82^{g}	3.0±1.63^{ef}
2.0	0.5	0.0	38.4±1.34^{ij}	6.42±1.63^{ef}	3.5±0.82^{de}
2.5	0.5	0.0	50.0±2.23^{h}	5.18±1.63^{fg}	2.6±0.82^{fg}
3.0	0.5	0.0	22.4±0.84^{l}	4.00±0.82^{h}	3.2±1.63^{e}
3.5	0.5	0.0	32.5±1.43^{k}	6.65±0.820^{e}	3.8±1.63^{d}
4.0	0.5	0.0	91.1±1.33^{a}	11.56±1.63^{a}	6.5±0.82^{a}
0.5	0.0	0.5	54.8±1.75^{f}	9.38±2.45^{bc}	5.9±1.63^{ab}
1.0	0.0	0.5	66.2±2.29^{d}	8.76±1.63^{c}	4.2±0.82^{cd}
1.5	0.0	0.5	53.4±1.26^{g}	7.47±1.63^{d}	5.3±163^{b}
2.0	0.0	0.5	58.2±1.22^{ef}	9.98±0.16^{b}	4.7±0.82^{bc}
2.5	0.0	0.5	69.5±1.50^{c}	8.66±0.82^{cd}	4.2±1.63^{c}
3.0	0.0	0.5	77.7±1.49^{b}	6.22±1.63^{f}	3.8±0.19^{d}
3.5	0.0	0.5	58.1±1.00^{e}	4.16±1.63^{gh}	2.7±0.82^{f}
4.0	0.0	0.5	39.4±1.07^{i}	3.18±0.33^{ij}	1.8±0.82^{gh}

Mean values in columns are followed by different letter (s) are significant to each other at 5% level accordance to DMRT.

Table 3. Effect of different concentrations of growth regulators on shoot initiation, shoot number and shoot length after the subculturing of node derived callus of the species, *Tribulus terrestris.*

Growth regulators (mg/L)			Culture response (%)	No. of shoots/callus	Shoot length (cm)
BAP	NAA	2,4-D			
0.5	0.5	0.0	43.24±1.63^{fg}	4.78±0.82^{gh}	1.9±0.82^{j}
1.0	0.5	0.0	51.98±0.82^{d}	5.45±0.8^{g}	2.5±0.82^{h}
1.5	0.5	0.0	55.00±1.63^{c}	6.36±2.45^{fg}	2.8±1.63^{gh}
2.0	0.5	0.0	46.65±1.63^{e}	4.25±1.63^{hi}	4.1±0.82^{d}
2.5	0.5	0.0	34.76±0.82^{i}	2.00±0.82^{i}	3.4±0.82^{f}
3.0	0.5	0.0	28.54±0.82^{l}	7.67±16.3^{de}	1.9±0.82^{jk}
3.5	0.5	0.0	32.34±1.63^{j}	6.97±1.63^{f}	3.2±1.63^{g}
4.0	0.5	0.0	65.53±0.82^{a}	12.66±0.82^{a}	6.9±0.82^{a}
0.5	0.0	0.5	21.58±0.82^{m}	10.73±1.63^{b}	4.1±0.82^{d}
1.0	0.0	0.5	43.34±0.24^{f}	9.86±0.82^{bc}	2.5±0.82^{hi}
1.5	0.0	0.5	38.67±0.82^{h}	9.67±1.63^{cd}	5.5±1.63^{b}
2.0	0.0	0.5	46.14±0.82^{ef}	7.47±0.16^{e}	4.3±1.63^{cd}
2.5	0.0	0.5	41.00±1.63^{g}	9.81±0.82^{c}	4.8±0.16^{c}
3.0	0.0	0.5	57.45±0.82^{bc}	7.15±1.63^{ef}	4.0±0.82^{e}
3.5	0.0	0.5	58.88±0.82^{b}	4.66±0.82^{h}	5.3±0.82^{bc}
4.0	0.0	0.5	29.62±1.63^{k}	8.75±0.24^{d}	3.9±1.63^{ef}

Mean values in columns are followed by different letter (s) are significant to each other at 5% level accordance to DMRT.

Table 4. Effect of different concentrations of growth regulators on rooting percentage, root number and root legth after the subculturing of leaf derived shoot from callus of the species, *Tribulus terrestris.*

Growth regulators (mg/L)			Shoots rooted (%)	No. of roots/shoot	Root length (cm)
IBA	IAA	2,4-D			
0.5	0.5	0.0	42.36±0.82^{kl}	3.35±0.82^{i}	3.1±0.41^{hi}
1.0	0.5	0.0	47.79±1.63^{j}	4.76±0.41^{g}	2.8±0.65^{jk}
1.5	0.5	0.0	53.59±0.82^{gh}	5.48±1.63^{ef}	3.0±0.82^{j}
2.0	0.5	0.0	57.43±0.41^{f}	4.25±0.82^{hi}	4.8±0.65^{k}
2.5	0.5	0.0	61.90±0.16^{e}	6.46±0.82^{cd}	5.4±0.33^{ef}
3.0	0.5	0.0	63.00±0.82^{d}	5.56±1.63^{e}	4.0±0.82^{g}
0.0	0.5	0.0	44.75±0.65^{k}	4.26±0.41^{h}	3.0±0.82^{ij}
0.0	1.0	0.0	53.47±1.63^{gh}	5.38±0.82^{f}	5.8±1.63^{e}
0.0	1.5	0.5	75.38±0.82^{b}	8.76±0.82^{b}	6.5±0.82^{bc}
0.0	2.0	0.5	77.19±1.63^{a}	9.37±1.63^{a}	7.3±0.82^{a}
0.0	2.5	0.5	66.46±0.82^{c}	6.65±0.41^{c}	6.5±0.41^{b}
0.0	3.0	0.5	54.57±0.41^{g}	5.98±0.82^{d}	5.8±0.82^{c}
0.0	0.0	0.5	35.86±1.63^{n}	2.43±1.63^{k}	2.3±1.63^{k}
0.0	0.0	1.0	41.45±0.82^{lm}	3.23±0.82^{ij}	3.3±0.82^{h}
0.0	0.0	1.5	48.34±1.63^{ij}	4.49±1.63^{gh}	4.9±1.63^{f}
0.0	0.0	2.0	49.64±0.82^{i}	5.75±0.41^{d}	5.5±0.41^{cd}
0.0	0.0	2.5	38.28±1.63^{m}	3.00±1.63^{j}	3.0±1.63^{j}
0.0	0.0	3.0	30.17±0.82^{o}	1.37±0.82^{l}	1.7±0.82^{h}

Mean values in columns are followed by different letter (s) are significant to each other at 5% level accordance to DMRT.

Table 5. Effect of different concentrations of growth regulators on rooting percentage, root number and root legth after the subculturing of node derived shoot from callus of the species, *Tribulus terrestris.*

Growth regulators (mg/L)			Shoots rooted (%)	No. of roots/shoot	Root length (cm)
IBA	IAA	2,4-D			
0.5	0.0	0.0	43.24±1.63^{h}	4.78±0.82^{k}	1.9±0.82^{l}
1.0	0.0	0.0	51.98±0.82^{f}	5.45±0.82^{i}	2.5±0.82^{k}
1.5	0.0	0.0	55.00±1.63^{e}	6.36±2.45^{gh}	2.8±1.63^{jk}
2.0	0.0	0.0	46.65±1.63^{g}	4.25±1.63^{kl}	3.1±0.82^{hi}

2.5	0.0	0.0	34.76±0.82^{l}	2.00±0.82^{n}	3.4±0.82^{g}
3.0	0.0	0.0	28.54±0.82^{o}	7.67±1.63^{e}	2.9±0.82^{j}
0.0	0.5	0.0	32.34±1.63^{m}	6.97±1.63^{g}	3.2±1.63^{h}
0.0	1.0	0.0	42.53±0.82^{i}	9.66±0.82^{cd}	5.9±0.82^{c}
0.0	1.5	0.0	21.58±0.82^{p}	10.73±1.63^{b}	6.1±0.82^{bc}
0.0	2.0	0.0	43.54±0.24^{hi}	9.86±0.82^{bc}	6.5±0.82^{b}
0.0	2.5	0.0	60.67±0.82^{a}	12.67±1.63^{a}	7.5±1.63^{a}
0.0	3.0	0.0	46.14±0.82^{gh}	7.47±0.16^{ef}	4.3±1.63^{e}
0.0	0.0	0.5	59.00±1.63^{bc}	9.81±0.82^{c}	4.8±0.16^{cd}
0.0	0.0	1.0	57.45±0.82^{d}	7.15±1.63^{f}	4.0±0.82^{ef}
0.0	0.0	1.5	58.88±0.82^{c}	9.66±0.82^{d}	3.3±0.82^{gh}
0.0	0.0	2.0	29.62±1.63^{l}	8.75±0.24^{de}	3.9±1.63^{f}
0.0	0.0	2.5	41.00±0.82^{ij}	5.16±0.82^{ij}	4.5±1.63^{de}
0.0	0.0	3.0	59.45±0.24^{b}	4.00±0.82^{l}	4.7±033^{d}

Mean values in columns are followed by different letter (s) are significant to each other at 5% level accordance to DMRT.

Table 6. Effect of different composition of hardening medium on survivability rate of leaf callus derived plantlets of the species, *Tribulus terrestris.*

Hardening medium composition (w/v)	No. of plantlets Under hardening	No. of plantlets survived	Survivability (%)
Red soil: sand (1:1)	50	22^{e}	44.31^{e}
Garden soil: sand: vermicompost (1:1:1)	50	42^{a}	80.46^{a}
Decomposed coir waste: perlite: vermicompost (1:1:1)	50	36^{b}	75.24^{b}
Vermicompost : red soil (1:1)	50	30^{e}	65.58^{c}
Red soil: sand: vermicompost (1:1:1)	50	28^{d}	60.11^{d}

Mean values in columns are followed by different letter (s) are significant to each other at 5% level accordance to DMRT.

Table 7. Effect of different composition of hardening medium on survivability rate of node callus derived plantlets of the species, *Tribulus terrestris.*

Hardening medium composition (w/v)	No. of plantlets Under hardening	No. of plantlets survived	Survivability (%)
Red soil: sand (1:1)	50	17^{e}	34.86^{e}
Garden soil: sand: vermicompost (1:1:1)	50	40^{a}	70.56^{a}
Decomposed coir waste: perlite: vermicompost (1:1:1)	50	36^{b}	66.45^{b}
Vermicompost : red soil (1:1)	50	26^{d}	50.25^{d}
Red soil: sand: vermicompost (1:1:1)	50	34^{c}	55.63^{c}

Mean values in columns are followed by different letter (s) are significant to each other at 5% level accordance to DMRT.

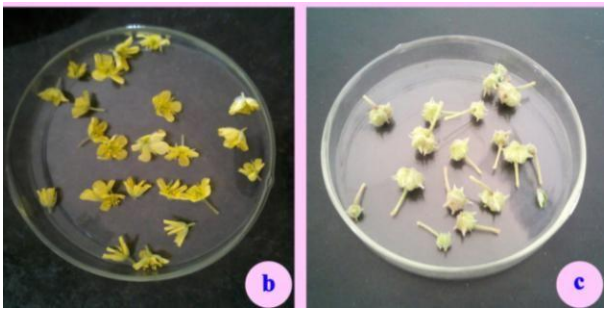

Fig. 1. Habit of the study species, *Tribulus terrestris*. a-Habit; b-Flowers; c-seeds.

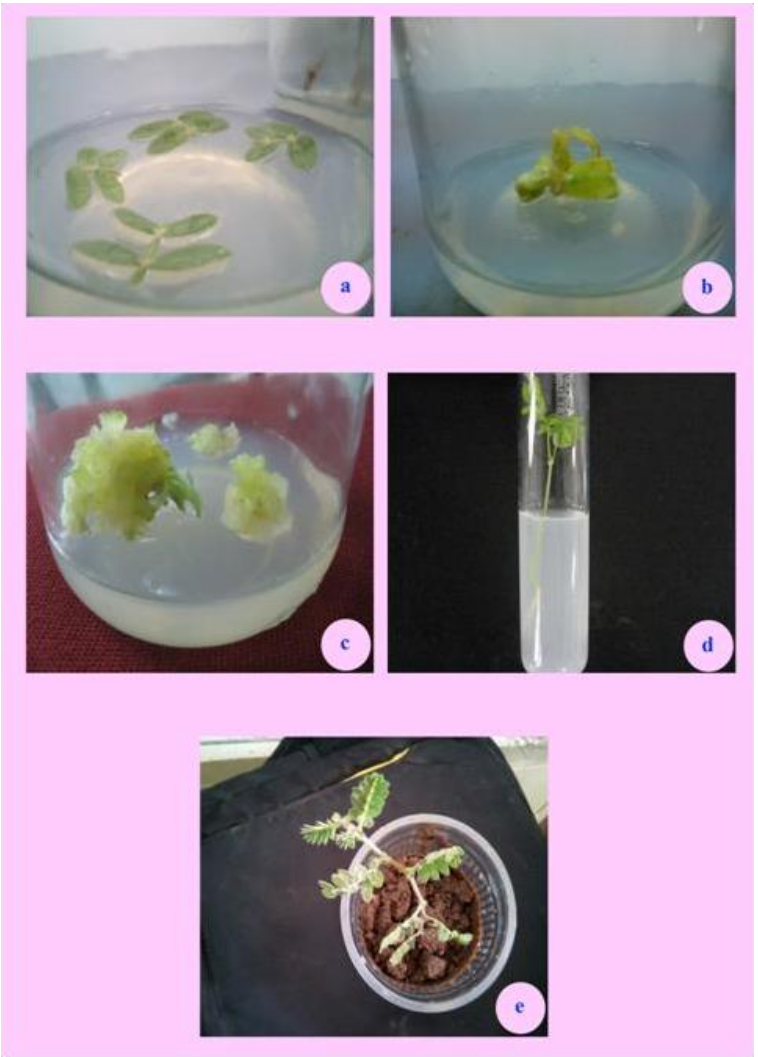

Fig. 2. *In vitro* regeneration of *Tribulus terrestris* using leaf explant.

a - Inocluation of leaf explant on MS medium with BAP and NAA (4mg/L and 0.5mg/L). b - Callus induction of the leaf explant on MS medium with BAP and NAA (4mg/L and 0.5mg/L). c – Shoot induction of *in vitro* derived callus explants in the MS medium with BAP and NAA (4mg/L and 0.5mg/L). d – Root induction of *in vitro* cultured shoots from the leaf derived callus in the MS medium fortified with IAA 2mg/L. e – Acclimatization of *in vitro* plantlets.

Fig. 3. *In vitro* regeneration of *Tribulus terrestris* using nodal explant.

a - Inocluation of nodal explant on MS medium with BAP and NAA (4mg/L and 0.5mg/L).

b - Shoot induction of nodal explants in the MS medium with BAP and NAA (4mg/L and 0.5mg/L). d – Root induction of *in vitro* cultured shoot from the node derived shoot in the MS medium fortified with IAA 2mg/L. e – Acclimatization of *in vitro* plantlets.

4. DISCUSSION

Plant tissue culture technology is being widely used for large scale plant multiplication. Apart from their use as a tool of research, plant tissue culture technique has in recent years, become of major industrial importance in the area of plant propagation, disease elimination, plant improvement and productivity of secondary metabolites. Small pieces of tissues (named explants) can be used to produce hundreds and thousands of plants in a continuous process. Endangered, threatened and rare species have successfully, been grown and conserved by micropropagation because of high coefficient of multiplication and small demands on number of initial plants and space.

Due to its high medicinal value and increasing demands, *in vitro* studies have great importance in the propagation of the study species, *Tribulus terrestris.* In the present study, callogenesis and shoot proliferations of the study species were witnessed in MS medium supplemented with higher concentration of cytokinin (BAP 4mg/L) and low concentrations of auxins (NAA 0.5mg/L). In accordance with Singh and Goyal (2016), the best callogenesis and shoot regeneration of *T. terrestris* were also observed in higher concentrations of cytokinin (BAP 2.0 mg/L) and low concentration of auxin (NAA 2.5 mg/L). The study also suggested that the balance of auxin and cytokinin is a decisive morphogenetic factor. The present results exhibited that high concentration of BAP and low concentration of NAA were efficient for the induction of callus and subsequently shoot regeneration. Reports of auxin and cytokinin combinations were supporting the organogenesis differentiation in other species have been well documented by Jamuna and Paulsamy, 2014 (a and b); Jejurker *et al.,*2016).

Rhizogenesis of the study species from the leaf derived callus and nodal explants were noted at MS medium contained with IAA alone 2 mg/L. The result was harmony with previously reported species such as Indrias *et al.,* 2016; Roberto *et al.,* 2016. IAA is generally regarded as the major auxin, universally found in higher plants, that plays a center role in adventitious root formation (Davis and Sankla, 1988).

5. CONCLUSION

In the present study, an efficient regeneration through leaf and nodal explants were achieved by *in vitro* technique. This *in vitro* proliferation method is used for the enhancement the natural stock of plants in wild population. The present protocol will enable the propagation and conservation of *T. terrestris* in an efficient manner.

REFERENCES

Anant Gopal, S., A. Kumar and D.D. Tewari, (2012). An ethnobotanical survey of medicinal plants used in Terai forest of western Nepal. *J Ethnobiol Ethnomed* **8**:19.

Davis, H.B.E. and N. Sankhla, (1988). Adventitious root formation in culturings P: 315. Advances in Plant Sciences Series, Vol. 2, Dioscorides Press, Portland, Ore.

Duncan. D.B. (1955). Multiple range and multiple F tests. *Biometrics* **11**: 1-42.

Frohne, D. (1999). Ein neues Dopingmittel? Dtsch. *Apoth Ztg* **139**: 4752- 4754.

Gamal, E.G., K.S. Al-Khalifa, G.A. Saleem and E.M. Abdallah, (2010). Traditional medicinal plants indigenous to Al-Rass province, Saudi Arabia. *J. Med. Plants Res* **4**(24): 2680-2683.

Indrias, T., S. Teshome, T. Soromessa and T. Feyissa, (2016). Development of an efficient *in vitro* propagation protocol for *Satureja punctata* - A rare aromatic and medicinal plant. *Taiwania* **61**(1): 41-48.

Jamuna, S. and S. Paulsamy, (2014a). *In vitro* propagation and evaluation of antioxidant properties of the medicinal plant species, *Hypochaeris radicata* L. (Asteraceae) distributed in Nilgiris, the Western Ghats. *Asian J. Biomed. Pharmaceu Sci* **4**(31): 29-37.

Jamuna, S. and S. Paulsamy, (2014b). Micropropagation of medicinal herb, *Hypochaeris radicata* L. via indirect organogenesis. *Res. Biotechnol* **5**(3): 1-9.

Jejurker, S.H., U.R. Tanpure, S.D. Pawar, R.R. Joshi and M.S. Wadikar, (2016). Microprapogation of callus from *Syzygium cumini* explant. *Int. J. Pure App. Biosci* **4** 1): 291-295.

Murashige, T. and F. Skoog, (1962). A revised medium for rapid growth and bioassays with tobacco cultures. *Physiol. Plant* **15**: 473-497.

Raghu, A.V. and K.V. (2006). Seed germination studies on *Lughau panchamula* group of medicinal plants. *J. Non Timber For. Prod* **13**(4): 227-229.

Raghu, A.V. and V. Mohananan, (2005). Studies on variability, conservation and propagation of *Dasamula* group of medicinal plants, Ph.D.,

Thesis, University of Calicut, Kerala, India, pp. 115-120.

Roberto, C., S. Sabbadini and B. Mezzetti, (2016). The use of TDZ for the efficient *in vitro* regeneration andorganogenesis of strawberry and blueberry cultivars. *Sci. Hortic* **207**: 117–124.

Singh, M. and S.C. Goyal, (2016). Callus induction and regeneration from nodal explants in *Tribulus terrestris* Linn., an important medicinal plant. *World J. Pharm. Pharmaceut. Sci* **5**(4): 2254-2262.

Smitha, P.K., A. Latheef and A.B. Remashree, (2014). Ethnobotanical survey of diuretic and antilithiatic medicinal plants used by the traditional practitioners of Palakkad District. *Int. J. Herbal Med* **2**(2): 52-56.

Verma, R.V. (2014). An ethnobotanical study of plants used for the treatment of livestock diseases in Tikamgarh District of Bundelkhand, Central India. *Asian Pac. J. Trop. Biomed* **4**(1): S460-S467.