Journal of Biology, Agriculture and Healthcare
ISSN 2224-3208 (Paper) ISSN 2225-093X (Online)
Vol.4, No.9, 2014

In Vitro Micropropagation of *Trichopus Zeylanicus* Gaetn. Through Seed Explant

Shubha[1]* D.Anusuya[2]
1.Department of Botany, Government First grade College, Vijayanagara, Bengaluru,560 104, Karnataka, India
2 Department of Botany, Bangalore University, Bengaluru-560 056, Karnataka, India.
*E-mail of the Corresponding author: ashurajkashi@yahoo.com

The research is funded by The University Grants Commission, New Delhi, India under Faculty Improvement Program.

Abstract
Trichopus zeylanicus Gaertn. belonging to family Trichopodaceae is a rare herb with extraordinary medicinal properties found in the Agasthyar hills in Thiruvananthapuram of Kerala. *Trichopus zeylanicus* Gaertn. was regenerated *in vitro* on MS medium supplemented with different concentrations and combination of auxins and cytokinins. The effect of phytohormones on *in vitro* establishment of explants, production of multiple shoots and rooting of established shoots were studied. MS medium supplemented with 4.44 µM BAP produced maximum of 32 multiple shoots from seed explant. MS medium supplemented with 2.46 µM IBA induced maximum rooting. The rooted plantlets were acclimatized with a survival rate of 75 per cent to 80 per cent.
Key words: Micropropagation, *Trichopus zeylanicus* Gaertn., Multiple shoots, Seeds.

1.Introduction

Trichopus zeylanicus Gaertn is commonly known as "Arogyapacha" (giver of evergreen health) which is grown in the Agasthyar hills of Kerala. The plant is a small erect perennial herb. Stem several from a nodose rhizome, slender, leaves ovate to lanceolate to broadly triangular ovate. Flowers 4 to 5 in cyme, perianth dark brown. Fruit 3 winged. Seeds are oblong and dorsally grooved.

The Kani tribe of Kerala has preserved the herb and the knowledge about its use. This kani tribe knew about the anti-fatigue property of young fruits of arogyapacha which they often ate during their strenuous trekking through the hilly tracts of Western Ghats. This herb with high tonic qualities can be comparable to Korea's ginseng (Pushpangadan *et al.* 1995). The fruits and leaves are used by Khani tribals as a rejuvenating tonic (Pushpangadan *et al.* 1988) and investigations have revealed its strong antifatigue, antihepatotoxic, antitress, staminaboosting and immunomodulatory properties (Pushpangadan *et al.* 1995).

The antihepatotoxic and chloretic properties of *Trichopus zeylanicus* was reported for the first time by Subramaniam *et al.* (1998). Subramanian *et al.* (1997) reported the aphrodisiac property of *Trichopus zeylanicus* extract in male mice. Administration of *Trichopus zeylanicus* leaf extract in ethanol in male mice increased the aphrodisiac activity. Tharakan, B. *et al.* (2006) studied the antioxidant property of *Trichopus zeylanicus* and reported that NADH, polyphenols and sulphydryl compounds present in it have the ability to scavenge reactive oxygen species suggesting that the antioxidant property may be an important mechanism of action of the plant to combat fatigue. Subramanian *et al.* (2000) reported that active fraction of *Trichopus zeylanicus* stimulated macrophage phagocytosis in mice. Subramaniam *et al.* (2002) studied the effect of the antifatigue activity of *Trichopus zeylanicus* leaf (alcohol extract) on energy metabolism. Chauhan (2003) reported anti-HIV property of *Trichopus zeylanicus*.

Since the plant is endangered and has a great medicinal potential, micropropagation was tried.

1.1 Research method

Plant material and explant preparation: Mature fruits were collected from Agasthyar hills and seeds were used as explants. Fruits were first washed in running tap water for an hour. Fruit wall was removed and seeds were washed with 5 per cent teepol solution for 10 minutes followed by 10 per cent sodium hypochlorite solution for 15 minutes followed by several washes in sterile double distilled water before taking to LAF bench. Further the seeds were sterilized with 70 per cent alcohol for 30 sec and washed in distilled water. The seeds were then transferred to a beaker containing 0.1 per cent mercuric chloride solution for 2 minutes, followed by a thorough washing in sterile distilled water. Streptomycin (0.1 per cent) was used to control bacterial contamination for 30 seconds. Excess water was removed from the seeds by blotting them on a sterilized filter paper discs. Finally explants were transferred to MS medium supplemented with different combinations of auxins and cytokinins at different concentrations.

Nutrient medium preparation and conditions: To the full strength MS medium 0.9 per cent tissue culture grade agar, 3 per cent sucrose were added. Required concentration of auxins and cytokinins were put into this solution and it was made upto 1000 ml by DD water. After the addition of all the chemicals p^H of the medium

was adjusted to 5.8 before autoclaving at 121^{0}C and 15 lb pressure for 20 minutes. All the culture tubes and flasks were labeled and transferred to the culture room which was maintained under controlled condition of light, temperature and humidity. The temperature was kept at a constant of 25 ± 2^{o}C. The light source was fluorescent tubes with an intensity of illumination of 2000-3000 lux. The cultures were exposed to 16h of light and 8h of darkness. The cultures were subcultured regularly and results were recorded periodically. Each treatment consisted of 10 replicates and the experiment was repeated thrice.

Influence of combination of IBA and BAP at different concentrations on *in vitro* production of multiple shoots was studied. The successfully established explants were used in this experiment. The explants were excised by trimming the unwanted tissue and subcultured to know the effect of different combination of NAA and BAP with full strength MS medium supplemented with 3 per cent sucrose and gelled with 0.9 per cent agar.

Influence of MS medium supplemented with different concentrations of IBA and NAA on *in vitro* rooting was studied. A combination of different concentrations of NAA and BAP was also studied. The cultures were examined frequently and the data were recorded.

1.1.2 Result analysis

Influence of different concentrations of IBA and BAP combination on *in vitro* establishment of explants was carried out. The full strength MS medium was supplemented with 3 per cent sucrose and gelled with 0.9 per cent agar along with IBA and BAP at different concentrations. Influence of NAA and BAP on *in vitro* establishment of explants revealed following facts. It is evident from table that per cent establishment of explants was excellent at 4.44 µM BAP (89 per cent). Intensity of growth was maximum at 4.44 µM BAP. Per cent establishment of explants was minimum at 0.54 µM NAA + 0.44µM BAP.

Table also shows that a minimum of 32 days were taken for the initiation of explants at 5.37 µM NAA + 8.88 µM BAP followed by 2.69 µM NAA + 13.32 µM BAP and 2.69 µM NAA + 11.1 µM BAP. A maximum of 45 days was taken for initiation at 0.44 µM NAA + 0.49 µM BAP.

According to Table 28, Plate 23c and 23d maximum of 32 shoots were produced on MS medium supplemented with 4.44 µM BAP and took around 5 weeks for initiation whereas at 5.37 µM NAA + 8.88 µM BAP a minimum of 33 days were taken for initiation of multiple shoot buds .Next best was MS medium supplemented with 6.66 µM BAP.

Early root initiation was observed on MS medium supplemented with 2.69 µM NAA. At 2.69 µM NAA intensity of root growth was maximum and took minimum number of days (24 days) for root initiation. Next best concentration of hormone for better growth was IBA at 1.97µM (Table 3).Maximum number of roots were observed in 2.46 µM IBA followed by 2.69 µM NAA.

The rooted plantlets were planted in net pots containing sand, soil, vermiculite in the ratio 1:1:1 along with sterilized enriched compost in green house for acclimatization with a success rate of 75 percent to 80 per cent.

TABLES

Table-1 Influence of NAA and BAP in combination with MS medium supplemented with 3 per cent sucrose on *in vitro* establishment of explants of *Trichopus zeylanicus* Gaertn.

Treatments (µm)		Days taken for initiation	Intensity of growth	Per cent of explants established (Mean±SE)
NAA	BAP			
0.54	0.44	45	+ +	48± 0.57
1.07	0.88	41	+ +	64±0.21
1.61	1.33	41	+ +	65±0.27
2.69	1.78	36	+ +	65±1.34
2.69	4.44	35	+ + +	68±0.4
2.69	6.66	35	+ + + +	69±0.033
2.69	8.88	34	+ + + +	69±0.17
2.69	11.1	33	+ + +	68±1.21
2.69	13.32	30	+ +	72±0.23
5.37	8.88	32	+ + + +	76±0.57
0.0	4.44	34	+ + +	89±0.11
0.0	6.66	34	+ + +	78±0.34
5.37	0.0	35	+	50±0.11
8.06	0.0	35	+	49±0.02

\+ Poor
++ Good
+++ Very good

++++ Excellent

Table 2. Influence of NAA and BAP with MS medium supplemented with 3 per cent sucrose on *in vitro* regeneration of multiple shoots from seed explants of *Trichopus zeylanicus* Gaertn.

Treatments (µm)		Days taken for initiation	Intensity of growth	No. of shoots (Mean±SE)
NAA	BAP			
0.54	0.44	44	+	2±0.29
1.07	0.88	42	+	2.4±0.23
1.61	1.33	39	+	2.8±0.11
2.69	1.78	37	+ +	3.2±0.86
2.69	4.44	35	+ +	3.2±0.57
2.69	6.66	35	+ + +	3.2±1.09
2.69	8.87	33	+ + +	5±0.28
5.37	8.87	34	+ + +	6±1.15
2.69	11.10	35	+ + +	5±1.44
2.69	13.32	35	+ + +	5±0.92
0.0	4.44	36	+ + + +	32±0.63
0.0	6.66	35	+ + +	22±0.28
5.37	0.0	35	+ +	1±0.57
8.06	0.0	35	+ +	1±0.28

\+ Poor
++ Good
+++ Very good
++++ Excellent

Table3. Influence of IBA and NAA with MS medium on *in vitro* formation of roots of *Trichopus zeylanicus* Gaertn.

Treatments (µm)		Days taken for initiation	Root intensity	No. of roots (Mean±SE)
IBA				
0.49		38	+	1.2 ± 1.73
0.98		38	+ +	1.3±1.44
1.48		38	++	1.3±0.08
1.97		36	+ + +	1.3±0.29
2.46		34	+ + + +	2.6±0.23
NAA				
0.54		39	+ +	1.2±0.46
1.07		38	+ +	1.2±0.65
1.61		39	+ +	1.3±0.51
2.15		30	+ +	1.3±1.44
2.69		24	+ + + +	2.4±0.11
3.22		31	+ +	2.2± 1.73
NAA	BAP			
0.54	0.49	38	+	1± 0.92
0.54	0.98	39	+	1±0.11
1.07	0.98	39	+ +	1.4±1.09
1.61	0.49	38	+ +	1.5±0.63
1.61	0.98	38	+ +	1.5±0.28
0.0	0.44	42	+ +	1.1±0.57
0.0	0.88	42	+ +	1.1±1.09
0.0	1.78	43	+ +	1.0±0.57

\+ Poor
++ Good
+++ Very good
++++ Excellent

1.1.3 Discussion

In vitro propagation of *Trichopus zeylanicus.* Gaertn has not been fully explored and it is the need of the hour as

it is critically endangered. The importance of this plant from medicinal point of view and its exploitation will lead to decline in its population. Tissue culture technology could be successfully applied in the propagation of plants with poor and uncertain response to conventional propagation and for those plants which are on the verge of extinction (Rout *et al*. 2006; Tsay *et al*. 2006; Sundara kumari *et al*. 2005). Therefore, in the present investigation, efforts were made for the development of *in vitro* regeneration protocol for *Trichopus zeylanicus* Gaertn.

MS medium supplemented with 4.44 µM BAP/l produced maximum number of multiple shoots from seed explant. Krishnan *et al*. (1995) reported the micro propagation of *Trichopus zeylanicus* Gaertn. on WPM medium from shoot tips supplemented with 8.88µM BAP. BAP proves to be the best cytokinin for multiple shoot regeneration (Geetha and Gopal, 2007).

2.46 µM IBA induced maximum rooting and this is in accordance with Sivasubramanian *et al*. (2002) in *Plectranthus vetiveroide* and Johnson *et al*. (2002) in *Rhinacanthus nasutus.* Kiranmai *et al*. (2007) reported that IBA was the most effective rooting hormone for *Pergularia daemia.*

This *in vitro* regeneration protocol worked out can be used to save *Trichopus zeylanicus. Gaertn* from extinction and to facilitate germplasm conservation. Further work is needed for this plant to safe guard it from becoming extinct

References

Chauhan, D.K.2003. Some potential herbal drugs for AIDS. Recent progress in medicinal plants. Ethnomedicine and pharmacognosy.V.K. Singh(Eds). Sci Tech publishing house,Texas. 231-241.

Geetha, R. and Gopal, G.V. 2007. Direct regeneration in *Glossocardia bosvallea*: A medicinal plant. Plant cell biology and molecular biology. **8** (3&4) 119-124.

Johnson,M.,Vallinayagam,S.,Manickam,V.S.andseeni,S.2002.Micropropagation of *Rhinacanthus nasutus*(L.) Kurz-A medicinally important plant.Phytomorphology.**52** (4).331-336.

Kiranmai, C.,Aruna,V., Karuppusamy, S. and Pullaiah,T.2007. *In vitro* propagation of *Pergularia daemia* (Forsk.) Chiov. Plant cell biotechnology and molecular biology.**8** (1 & 2): 67-72.

Krishnan,P.N., Sudha,C.G. and Seeni,S.1995.Rapid propagation through shoot tip cultures of *Trichopus zeylanicus* Gaertn., a rare ethnomedicinal plant. Plant cell reports. **14**: 708-711.

Pushpangadan, P., Rajasekharan, S., Ratheeshkumar ,P.K., Jawahar ,C.R., 1988. 'Arogyapacha' (Trichopus zeylanicus, Gaertn) the Ginseng of Kani tribals of Agasthyar hills(Kerala) for evergreen health and vitality.*Acient Science of Life*.**,8**: 13-16.

Pushpangadan, P., Rajasekharan, S., Subramaniam, A., Latha, P. G., Evans D. A.,Valsaraj R,1995.
Furtherstudies on the pharmacology of *Trichopus zeylanicus*. Ancient science of life .**14**: 127-135.

Rout, G.R., Samanantray,S. and Das, P.2006. Manipulation and propagation of medicinal plants. Biotechnology Ad.**18**:91-120.

Subramanian, A., Madhava Chandran, V., Rajasekharan,S. and Pushpangadan, P.1997. Aphrodidiac property of *Trichopus zeylanicus* extract in male mice. Journal of Ethnopharmacology. **57**(1): 21-27.

Sivasubramanian,S.,Vallinayagam,S.,Raja,D.Patric and Manickam,V.S.,2002. Micropropagation of *Plectranthus vetiveroides(Jacob)*-A Medicinal plant.Phytomorphology,**52**(1):55-59.

Subramanian, A. D.A. Evans., Rajasekharan,S. and Pushpangadan, 2000. Effect of *Trichopus zeylanicus* Gaertn. (Active fraction) on phagocytosis of by peritoneal macrophages and humoral immune response in mice.
Indian Journal of Pharmacology 32: 221-225.

Subramanian, A., Evans O.A. Rajasekharan,S and Pushpangadan, P.1998. Hepatoprotective activity of *Trichopus zeylanicus* extract against paracetemol induced hepatic damage in rats. Indian Journal of Experimental Biology. **36**(4): 385-389.

Subramanian, A., Rajasekharan,S and Pushpangadan, P. 2002. Effect of *Trichopus zeyalnicus* leaf extract on the energy metabolism in mice during exercise and at rest.. Indian Journal of Pharmacology.**34**(1):32-37.

Sundarakumari,C.,Zhang, C.L., Martin, K.P., Slater,A. and Madhusudan, P.V.2005.Effects on auxins on indirect *in vitro* morphogenesis and expression of gusA transgene in lactenaceous medicinal plant, *Euphorbia nivulia* Buch-Ham. In vitro cell Dev.Biol.Plant. **41**:695-699.

Tharakan, B., Muralikrishnan. D., Brow-Borg, H.M. and Manyam, B. V. 2006. *Trichopus zeylanicus* combats fatigue without amphetamine mimetic activity. Phytotherapy Research. **20**(3):165-168.

Tsay, H.S.,Lec, C.Y., Agarwal, D.C. and Basker, S.2006. Influence of ventilation closure, gelling agent and explant type on shoot bud proliferation and hyperhydricity in *Scrophularia yoshimuae* a medicinal plant. Invitro Cell. Biol. plant **42**:445-449

The IISTE is a pioneer in the Open-Access hosting service and academic event management. The aim of the firm is Accelerating Global Knowledge Sharing.

More information about the firm can be found on the homepage:
http://www.iiste.org

CALL FOR JOURNAL PAPERS

There are more than 30 peer-reviewed academic journals hosted under the hosting platform.

Prospective authors of journals can find the submission instruction on the following page: http://www.iiste.org/journals/ All the journals articles are available online to the readers all over the world without financial, legal, or technical barriers other than those inseparable from gaining access to the internet itself. Paper version of the journals is also available upon request of readers and authors.

MORE RESOURCES

Book publication information: http://www.iiste.org/book/

Recent conferences: http://www.iiste.org/conference/

IISTE Knowledge Sharing Partners

EBSCO, Index Copernicus, Ulrich's Periodicals Directory, JournalTOCS, PKP Open Archives Harvester, Bielefeld Academic Search Engine, Elektronische Zeitschriftenbibliothek EZB, Open J-Gate, OCLC WorldCat, Universe Digtial Library , NewJour, Google Scholar