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IN-VITRO PLANTLETS REGENERATION FROM CALLUS CULTURES OF CYCLEA PELTATA (Lam) Hooks & Thoms - A MEDICINAL PLANT

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ABSTRACT

The roots of Cyclea peltata (Lam) have great medicinal value and are used for medicinal purpose, both internally as well as externally. Organogenesis (Shoots &roots) induced from callus produced from stem cultured in M.S medium supplemented with 0.5mg/L kinetin and 2.00 Mg/ L 2,4-D. The callus transferred in to M. S medium containing 1.5mg/L and 0.5 M. g/L. 2,4-D started to produce stem and leaves. An average of 15 shots were produced at a time from the callus transferred to the M.S. medium having 2.0 M. g / L. Kinetin only. Rooted plantlets were hardened and transferred to soil where they established very well the soil. The protocol can be used for supply planting materials for local cultivars and callus utilized for secondary metabolite extraction. The whole plant is used in a lot of ayurvedic formulations.

KEY WORDS

Cyclea peltata, Organogenesis, Hardening, Medicinal plants etc.

INTRODUCTION

Patha is one of the herbs mentioned in all ancient scriptures of Ayurveda. It has various synonyms, mentioned in samhitas, like tiktapuspa, brhar tikta, dipani, atisaranasani, rasa, sthapani, vrkki, trisira, ambastha etc. portraying its peculiarities. The great sage Caraka has categorized patha as sandhaniya – a healing herb; stanyasodhana – lactodepu rant, jvarahara – alleviates fever. Maharishi Susruta has mentioned it as visaghna – anti – toxin (Susruta Samhita, Sutra, A-38) and also to be useful (especially, the leaves) in diabetic disorders of the skin, like boils and carbuncles. Two varieties of patha have been mentioned in the Ayurvedic texts, viz. brhat patha and laghu patha i.e. with large and small leaves, respectively. Both the varieties are, more or less, similar in the properties.

The plant grows throughout India and Sri Lanka, up to 800-900 metres elevation. It is a slender twining shrub, frequently climbing up on tall trees. The leaves are simple, alternate, heart shaped, 2.5-10 cm long and 2.5-3.75 cm broad, stipule 5-10 cm long and nerves 7-11. The flowers unisexual, pale yellow, in axillary panicles. The fruits are ovoid drupes, brown or scarlet in color. The seeds are covered. The roots are tuberous, cylindrical, irregularly curved, with grayish brown surface. The plant blooms in the rainy season.

The botanical name of raja-patha or brhat patha described above, is Cyclea peltata and it belongs to family Menispermaceae. The botanical name of the other variety, laghu patha is Cisampelos pareira. The root bark showed presence of 11 quaternary alkaloids, three of which were termed menismine, cissamine and pareirine in addition to know 1- bebeerines, hayatinin, hayatin and d- isochondrodendrine, fove more tertiary

alkaloids present in root bark. From the leavescycleanine, (-) bebeerines, hayatinin, hayatidin, hayatin and (+) bebeerines, hayatinin, hayatidin, hayatin and (+) bebeerines, hayatinin, hayatidin, hayatin and (+) querticol isolated.

Properties

Patha is pungent and bitter in taste, pungent in the post digestive effect and has hot potency. It alleviates all the three doshas. It possesses light and sharp attributes. It has bitter, digestant, antipyretic and astringent properties and is used in the diseases like fever, diarrhea, pruritus, dermatoses, worms, asthma, tumors, heart diseases and wounds.

Uses

The roots of patha have great medicinal value and are used for medicinal purpose, both, internally as well as externally. External application of the paste of its roots and leaves is extremely beneficial, in infected wounds, sinuses, and skin diseases like erysipelas and pruritus. The external application of this paste is said to be useful in serpant bite also. The root juice is salutary in headache, as nasal drops. The roots have anti-inflammatory activity and hence alleviated the edema. Patha is a valuable wound healer and antidermatosis herb.

Internally, patha alleviates all the three doshas, but predominantly kapha and vata. In fever, allociated with rigors, the decoction of roots works well, with pepper powder. It also alleviates the burning sensation of the body. Patha is a keen stimulant for digestive system and endows the actions like appetizer, digestant, astringent, vermicide, hence, is used in anorexia, dyspepsia, diarrhea, dysentery, worms and abdominal pain etc. In diarrhea, the roots mashed with buttermilk, are beneficial. The roots are also very rewarding in urinary ailments like dysuria and cystitis. Patha is one of the best herbs used as a blood purifier. It is salubrious in heart diseases, associated with edema and blood diseases, as well. It is commonly used as a bitter tonic. The roots are given along with the butter-milk or jaggery, in diarrhea and hemorrhoids of vata and kapha types. It is an effective panacea for kaphaja stanyadusti, as lactodepurant. In menstruation with blood clots, the decoction of patha, trikatu (Sunthi, marica and pippali) and kutaja is benevolent.

MATERIALS & METHODS:

Explants were collected from Arya Vaidya Sala, Kottakkal, Malappuram,Kerala.It has planted in the green house of the college for frequent collection of explants. The healthy shoots were collected and washed thoroughly in running tape water and second wash with 5% solution of extran followed by 2% solution of Potassium hypochlorate. Finally, surface sterilization has done by using 0.1% HgCl2 solution for 2 minutes, again washed with double distilled water thrice, to remove the traces of HgCl2.

M.S medium (Murashige and Skoog 1962) was prepared by using Standard analytical grade chemicals (High media) and double distilled water. All ingredients for the M.S. medium has prepared as separate stock solutions. Pipetted out required quantity of stock solutions into beaker and the final made up as 200 M.L. After adjusting the medium pH at 5.6 and added Agar powder to the medium to solidify the medium.

Explants inoculated in M. S Medium (1962) supplemented with different combinations of 2, 4-D and kinetin (Table-1). Inoculated cultures were incubated at 25±2ºC and 16-8 photoperiod (1000 -2000 Lux). Measurements were taken at regular intervals of 15 days. Sufficiently grown plantlets were transferred to pots containing mixture of sand and soil (1:1) and were kept under room temperature. Humidity was maintained by covering each pot with polythene bags (Das. 1993). The established plantlets were transplanted to the soil.

RESULT AND DISCUSSION

C. pelteta is a well-known medicinal herb used in ayurvedic and other traditional medicines for their effectiveness against wide range of diseases including skin infections due to the advantage of the diversity of secondary metabolites responsible for their antibacterial activity.

The induction of callus using auxin alone or in combination with cytokinin in MS medium was reported in different explants of various plant species viz. cotyledon explants of *Mucuna pruriens* (L.) DC. in the medium with 6.7µM of 2, 4 – D (Vibha *et al.*,2009) and leaf explants of *Phyllanthus amarus* in the medium with 2.26µM of 2, 4 - D and 2.32µM of Kn (Nitnaware *et al.*, 2011). In the present study, the stem explants of *C. peltata* responded well when cultured on the medium supplemented with combinations of auxin and

cytokinins selected in the study. This suggests that the callus induction and adventitious shoot regeneration from the selected explants depended on the genotype and combinations of growth regulators as reported by Zhang *et al.,* (2004).

Antitoxin activity of aqueous extract of *Cyclea peltata* root against *Naja naja* venomand of already reported (Sivaraman *et al.,* 2017).

Table1. Effect of kinetin and auxin in the stem explants of *Cyclea peltata*

Cytokinin (mg/L)	Auxin (mg/L) (2-4, D)	Callus production/ shoot induction	% of Response	Nature of callus
00	0.5	……	Nil	No callus development
0.10	1.00	++	40	Very little callus
0.25	1.50	+++	50	Moderate callus
0.50	2.00	++++	70	Profuse callus
0.75	2.50	+++	60	Moderate callus
1.00	3.00	++	50	Moderate callus
1.00	0.25	++	50	shooting
1.50	0.50	+++	70	Shooting and rooting
2.00	0.00	++++	60	Very well shooting and rooting.

++= little, +++ = Moderate, ++++ = Excellent

MS +3% Sucrose +0.8% Agar; Data taken after 60 days

M.S. medium supplied with 0.5 M. g/L. 2, 4-D only not produced any response. Synergetic activity of kinetin along with 2, 4-D showed increased callusing property of explants. When the M.S medium supplied with 0. 25M.g/L. kinetin along with 1.5 M. g/L. 2, 4-D, has produced moderate callus the stem explants. Again, the quantity of kinetin and 2, 4-D slightly increased, the explants produced more amount of callus. The maximum amount of callus obtained the M.S supplied with 0.5M. g/L. kinetin and 2.0M. g/L.2,4-D (Fig-1 A). The induction of callus from other members of Menispermaceae viz. *Stephania cepharantha,* Hayata (Susuki *et al*.,1992), *Coscinium fenestratum* (Nair *et al*. 1992) and *Tinospora cordifolia* (Chintalwar *et al*., 2003, Jyothi & Dennis 2012) were reported when cultured on MS medium supplemented with 2, 4 – D + Kn/BAP.All these repots explained the effect of combined action of 2,4-D and kinetin. All reports favor the M.S medium was the best one for the Menispermacea members. Here also M.S. medium showed best result (Figure 1 A, B, C, &D).

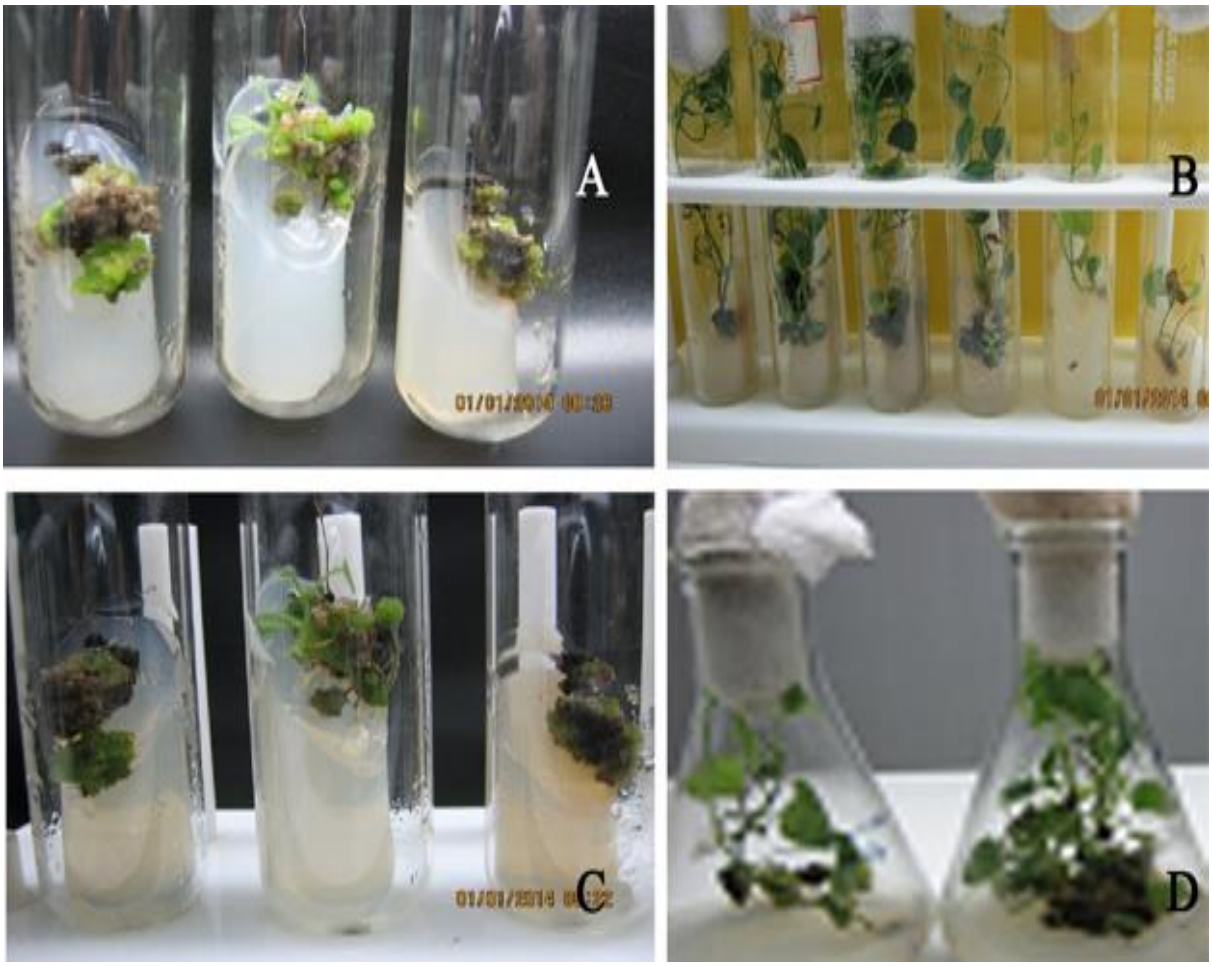
Another earlier report says that, the M.S. medium supplied with 2.0M. g/L NAA alone was the best one induction of callus from stem explants of *Cyclea peltata* (Bhagya and Chandrashekar 2013). In *Tinospora cordifolia*, the leaf explants were cultured in M.S. supplied with NAA or 2, 4-D alone could produce very good callus from the leaf explants (Singh *et al*. 2009). This can be corroborating with this work. Again, Deepa and Shad (2016) against view of this result and they says that 2.0 M. g/L. NNA along with M.S. medium was very effective for in *Cissampelos pariera,* this may be due to genus or species difference.

The result can be compare with the findings of Anand (2014) and Callus transferred to medium having low concentration of 2, 4-D and high concentration of kinetin, it started caulogenesis (Fig.1 C). The synergetic activity of Kinetin or BAP were explained by Anand while working with *Kaempferia rotunda* a monocot rare medicinal plant. Later the auxine completely removed by kinetin, well developed shoots and roots induced from the callus culture (Figure.1 D). Furthermore, this callus later used for the extraction antioxidants and secondary metabolites like keto-carotenoid spheroidenone (MW-582, $C_{41}H_{58}O_2$) (Sivaraman *et al*., 2017, Bhagya *et al*., 2012, Shine *et al*., 2009).

This method can be widely used for the conservation this medicinal plant having wide use.

Figure 1. Effect of kinetin and auxin in the stem explants of *Cyclea peltata*

A- Effect of M.S having 2.0mg/l 2,4-D and 0.5mg/l Kinetin; B- Effect of M.S having 2.0mg/l kinetin; C- Effect of M.S. having 1.5mg/l Kinetin and 0.25mg/l 2,4-D; D- Cultures grown in M.S. having 1.5mg/l Kinetin and 0.25mg/l 2,4-D were transferred to culture flasks



CONCLUSION AND SUGGESTION

This protocol is highly exploited for the production of seed plant lets for the supply of local cultivars and callus induction via suspension method is useful for the production of secondary metabolites (Vibha *et al.*, 2009)

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