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Invitro Propagation of Podophyllum Hexandrum

Ahmad Rizwan*, Sharma Saurabh, Kumar Vishal, Verma Chhavi, Bari Darakhshan G
Vivek College of Technical Education, Bijnor (UP) India

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ABSTRACT: Podophyllum hexandrum Royle, a source of highly valued podophyllotoxin has been subjected to heavy collection from the wild. The ever-increasing demand of podophyllum is mainly due to two semi synthetic derivatives of podophyllotoxin that is etoposide and teniposide, which are used in the treatment of various types of cancer. The anti cancer lignan derivative podophyllotoxin in Podophyllum hexandrum is biosynthesized at very low quantities in intact plant, so the biotechnological production of podophyllotoxin has been considered essential. Callus cultures have been established from root explants of aseptically grown Podophyllum hexandrum seedlings. A fully defined MS medium supplemented with Naphthalene acetic acid and 6-benzylaminopurine were effective for both initiation and sustained growth of callus tissue.

Keywords: Podophyllum hexandrum, Tissue culture, Podophyllotoxin, HPTLC, HPLC.

I. INTRODUCTION:

Podophyllum hexandrum Royle (Berberidaceae) also known as the Indian podophyllum is a perennial herb, growing on the lower slopes of the Himalayas in scrub and Forest from Afghanistan eastwards to central china[1, 2, 3, 4]

The rhizomes of Podophyllum hexandrum are known to contain several lignans which are dimerisation product of Phenypropanoid pathway intermediates linked by central carbons of their side chain[5,6,7]The lignans occurring in Podophyllum posses anti-tumor properties, Podophyllotoxin being the most active cytotoxic contains 4.3% Podophyllotoxin on a dry weight basis. Its insecticidal and phytotoxic activities are also reported[8,9,10]. However these lignans are too toxic for the treatment of neoplastic disease in humans. Nevertheless, Podophyllotoxin is used as starting compound for the chemical synthesis of etoposide and teniposide both being applied successfully as antitumor agents[11]. Their cytotoxic action is based on inhibition of topoisomerase II, while Podophyllotoxin acts as an inhibitor of the microtubule assembly. These semi synthetic analogues are indicated for small lung cell cancer, testicular cancer, neuroblastoma, hepatoma and other tumor disease[11,12].

The limited availability of Podophyllum hexandrum plant due to its long juvenile phase and poor fruit setting ability as well as the time consuming collection of the plants results in shortage of Podophyllum resin[13,14,15]. Moreover, because of the non-optimal yield after extraction, Podophyllotoxin is an expensive starting compound for the chemical synthesis of its derivatives. Therefore, the biotechnological production of Podophyllotoxin using plant cell culture derived from Podophyllum hexandrum may be an attractive alternative[16,17,18].

This study demonstrates the potentiality of static culture in production of Podophyllotoxin.

II. MATERIAL AND METHODS:

Seeds and Seedlings

Mature fruits of Podophyllum hexandrum Royle were collected during the month of October from G.B Pant Institute of Himalayan Environment and Development Kosi-Katarmal, Almora and High altitude plant physiology research Center, Srinagar .Seeds were separated from pulp, washed under running tap water for 20 min.dried under shade and stored at 4˚c until used. Seeds were cut with a scalpel blade, removing a section of seed coat with two incisions around the seed hilum region, and then maintained in dark, sterile conditions on moist filter paper at 27˚C until emergence of the radical. Germinated seeds was placed on solid nutrient agar slabs (full strength MS medium, 0.8% agar, pH 5.8) in sterile culture tubes and transferred to growth room conditions, with diffuse light day/night regime (16/8 hr) as well as in the dark.

Callus initiation and growth:

Aseptically germinated seed embryo were washed with double distilled sterile water and surface was disinfected with aqueous solution of

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sodium hypochlorite for 8 min. followed by repeated washing (4 times) in sterilized double distilled water under aseptic conditions so as to remove traces of sodium hypochlorite. The explants 3- 4 mm were carefully excised and transferred into MS media with various concentrations of BAP (0.5-2.5µM), NAA (0.5-5µM) and GA_3 (0.5µM-1µM). Specific growth regulator combinations referred to below:

Table -1. Effect of different supplement on growth of callus of Podophyllum hexandrum.

TREATMENT OF SUPPLEMENT(µM)				TYPES OF RESPONSE		
S.NO.	**NAA**	**BA**	**GA_3**	**CALLUS**	**COLOUR**	**NATURE**
1	0	1.5	0	+ +	Brownish	Compact
2	0	2.0	0	+	Brownish	Compact
3	1.5	0	0	++	Brownish	Compact
4	2.0	0	0	+	Brownish	Compact
5	0	0	0.5	+	Brownish	Compact
6	0.5	2.5	0	+++	Greenish	More Friable
7	1.0	2.0	0	+++	Greenish	More Friable
8	1.5	1.5	0	++	Greenish yellow	Less friable
9	2.0	1.0	0	++	Greenish yellow	Less friable
10	1.0	0	0.5	+++	Greenish	More Friable
11	1.5	0	0.5	+++	Greenish	More Friable
12	0.5	0	0.5	+ +	Greenish yellow	Less friable

(+): Poor callusing (+ +): Average callusing (+ + +): Good callusing (-): No response

The P^H of the medium was adjusted to 5.8. The cultures were maintained at 25±2°c in 16 hr. light and 8 hr. dark cycle and were transferred to fresh MS medium after a period of fourteen(14) days.

III. RESULT &DISCUSSIONS:

In present study experiments were carried out on tissue culture of (Podophyllum hexandrum (Berberidaceae).Tissue culture study starting from aseptic seed germination, standardization of media, callus initiation and growth study followed by Extraction, and estimation of Podophyllotoxin content in the callus cultures, and from cultivated roots of the plant.

Seed and Seedlings

Establishment of a routine protocol for tissue culture of Podophyllum hexandrum proved to be Difficult due to erratic seed germination, problems with the sterilization of explants, and the poor response of most plants to the culture regimes tested. Sterilization of explants, especially root material from soil-grown plants was unsuccessful[10,15], and germination of seeds under aseptic Conditions proved the most satisfactory approach[18]. Germination was dependent on storage Conditions and appropriate Pregermination treatment. A successful procedure was developed

Involving aseptic seed germination, light day/night regime (16/8 hr) condition at 27°C.

For aseptic germination of seeds they were treated with 70% ethanol for 15 seconds followed by 3% sodium hypochlorite for 5 minutes. They were then washed several times with sterile double distilled

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water to remove excess of sterilants. Various methods were taken up for the aseptic germination .The result showed that seed germination was within 6 days in petridish method. The sprouted seedling in the immature stage showed better proliferation and growth in M.S. basal medium with 100 µM solution of Gibberrllic acid is better whereas it remained stagnated if allowed to stay in cotton bed method for long.

Callus initiation

For callus initiation, full strength MS (Murashige and skoog) gave better results [14,15]and was therefore adopted for further studies. Root explants from seedlings derived from single plant were tested on hormone grids. No individual auxin or cytokinin initiated callus at the concentrations tested, With the exception of NAA where some response was observed at 0.5-5µM after four weeks. High concentrations of the BAP (2.5µM) in the presence of NAA
(0.5µM) enhanced callus initiation. Development of a friable callus was particularly associated with BAP in combination with NAA.

Optimum growth response was achieved with combined GA_3 NAA treatment, which appeared independent of GA_3 concentration in the range tested (0.5-1.0µM), but dependent on the NAA concentration(0.5-1.0µM). A similar high response was observed in the presence of BAPconcentration (0.5-1.0µM). Higher concentration of BAP (upto 2.5µM) were found to facilitate growth when NAA levels were low, but retard growth when NAA levels were high. Callus growth was more effectively supported on a MS medium with full strength, rather than at half strength MS medium[16]. Overall, a medium based on the combined use of NAA, BAP was suitable for both the initiation and sustained growth of Podophyllum hexandrum callus tissue.

IV. CONCLUSIONS

Podophyllum hexandrum has been considered a rare and threatened species, large scale removal of its underground parts still continues at rates well over natural regeneration. Therefore special Attention needs to be given for its propagation and conservation. The use of seeds/plant material from different population would help to ensure the high Podophyllotoxin content by tissue culture techniques. The combined and sustained effort with seed germination, in vitro culture and estimation is required to reduce the pressure on natural population.

Study was successful in standardizing the media for tissue culture, aseptic germination, callus Initiation, Extraction and estimation of Podophyllotoxin from Callus and cultivated Podophyllum hexandrum extract.

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