Micropropagation of an important medicinal plant henna
(Lawsonia inermis.linn)


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INTRODUCTION

Biotechnology has the potential of improving the value of medicinal plants through modern methods of cultivation and improving the quality and quantity of drug content. Most plants cells are totipotent. Mitosis divides the genetic material into identical complements. Some frequently encountered types of plant tissue culture are callus culture, cell suspension culture, organ culture, meristem tip culture, and protoplast culture. Many important Chinese medicinal herbs have been successfully propagated in vitro either by organogenesis or by somatic embryogenesis. The use of tissue culture technology for biosynthesis of secondary metabolites, particularly in plants of pharmaceutical significance, holds the controlled production of plant constituents. Techniques such as meristem culture and hot water treatment of explants before in vitro culture have been used to produce plants free from pathogen. The technique of tissue culture has potential to offer possible solution to some of these problems. Plant tissue culture techniques are now being used globally for the multiplication of medicinally important species and also monitoring for the secondary metabolites. The success of plant tissue culture as a means of plant propagation is greatly influenced by the nature of the culture medium used. For healthy and vigorous growth, intact plants need to take up from the soil relatively large amounts of some inorganic ions (the so-called major plant nutrients - salts of nitrogen, potassium, calcium, phosphorus, manganese, zinc, boron, copper, molybdenum and cobalt) plant tissue culture media provide not only these major (macro-) and minor (Micro-) nutrients, but also a carbohydrate, usually sucrose, to replace the carbon, which the plant normally fixes from the atmosphere by photosynthesis. Improved results are obtained by providing trace amounts of certain organic compounds notably vitamins, amino acids and plant growth regulators. 

Lawsonia inermis. Linn belongs to the family Lythraceae common name is Henna Plant, Indian Name is Mehandi. It is globous much branched under shrub, fast growing upto 20 feet tall. It has opposite elliptic, lanceolate leaves 0.5 to 2" long and 0.4 to 1.2" wide. Propagation is by cutting or seed. The plant has numerous fragrant white,pink or red flowers terminal panicles in summer and has commercial value. It has got undehiscent fruit. The medicinal properties are antidiarrheal, antisyphetic, astringent, emmenagogue, liver tonic, affungual and ultra violet screening activity, antihemorrhagic, cardio inhibitory, hypotensive and

Abstract

Lawsonia inermis. Linn is an important medicinal plant used in traditional Indian systems of medicine. The plant is available in many parts of Tamilnadu. The present investigation was undertaken to explore the in vitro response of various explants cultured on MS medium supplemented with PGR’s. The explants cultured were shoot tips, axillary buds, stem and leaf discs. The explants were obtained from healthy plants growing in the field or maintained in the green house, were cultured on MS medium, was supplemented with IAA, 2,4-D, 2,4,5-T, NAA, IBA, BAP, KIN and 2ip. These were added individually or in various combinations and also with coconut milk. Callus was induced in all the explants, however, caulogenesis was observed only in the nodal explants. Among the 3 explants nodal explants responded profusely in all the treatments. Multiple shoots and shoot growth was promoted in cytokinin rich and auxin low concentration treatment.

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sedative effects. It has also been used as a folk remedy against amoebiasis, head ache, jaundice and leprosy.

**Aim of the present investigation**

The major objective of this study is to determine a suitable in vitro culture technique for a rapid multiplication and regeneration of *Lawsonia inermis*. To screen for an ideal explants and to determine its morphogenetic potential. To find out the morphogenetic potential of explants like leaf disc, stem, axillary buds and seeds. To determine the effect of different plant growth hormones like 2, 4-D, 2, 4, 5-T, NAA, BAP, Kinetin and 2ip or the combination of both auxins and cytokinins on various explants of *Lawsonia inermis*.

**MATERIAL AND METHODS**

**Plant Material**

Healthy plants of *Lawsonia inermis*. were collected from the field. Young healthy and disease free portion of the branches were selected and used as the primary source of fresh explants for the in vitro propagation and regeneration studies.

**Surface Sterilization**

Healthy and young explants like shoot tips, axillary buds, internodes, petioles and leaf disc were selected for sterilization. Then the excised explants were cut into small pieces of about 1 to 1.5 cm length. The levels of external micro-organisms were reduced by first by washing the material under running tap water for several hours. Surface sterilization is always necessary and it was carried out with several different reagents. Sodium hypochlorite (0.5 - 2% w/v) or calcium hypochlorite (a filtered 5 - 10% w/v solution) is now most commonly employed. Penetration is considerably assisted by a short immersion in 70% ethanol, by adding a non-phytotoxic wetting agent to the sterilant solution (0.05% of tee pol or Lissapol F, 0.01% of tween 20 or tween 80, 0.1% of 7, and 0.2% Alconox.

- Ca 20 minutes in 5% Ca hypochlorite.
- 5 - 15 minutes in 0.5 - 1% Na hypochlorite.

After exposure to sterilant solutions, explants were washed and / or soaked in several changes of sterile water to remove all traces of the sterilizing agent.

**MS Media Preparation:**

The various components of the medium, i.e. micro, macro, nutrients, vitamins, growth regulators, etc., were added in required proportions. Prior to the addition of agar, pH of the medium was adjusted to 5.8. 3% of sucrose was added as carbon source and 0.8% agar was added for solidification. It was then slightly warmed and 10 ml of medium was dispensed into culture vials and then autoclaved at 15 lbs for 15 minutes. To this MS medium, PGR’s like 2, 4-D, 2, 4, 5-T, NAA, BAP, IAA, IBA, 2ip, KIN, Coconut Milk + KIN, BAP + KIN, Coconut milk + BAP, 2, 4-D + 2, 4, 5-T + KIN, Coconut milk + KIN, BAP + KIN, Coconut Milk + BAP, 2, 4-D + 2, 4, 5-T + KIN, were added at different concentrations and in different combinations.

**Inoculation**

Prior to inoculation all the implements and vials were kept inside the laminar air flow chamber in the presence of UV light for about 30 minutes. The explants were first kept on sterile tissue paper in order to remove water from the surface. The explants were transferred to the cutting board and the explants were further trimmed to 0.5 - 1 cm and planted on the culture medium. The mouth of the culture vials was held closed to the flame and sealed immediately after inoculation with sterile heavy duty aluminium foil and labeled. The inoculated culture vials were incubated in dark and light conditions at 24 ± 2oC. Light conditions were provided by cool - white fluorescent lights of 3000 lux.

**Temperatures**

The average temperature employed in a large sample of experimental reports to be 25oC (with a range between 17o and 32oC).

**Light**

Cultures are generally grown in diffuse light in the range 100 - 1000 lux.

**Humidity**

A moist atmosphere was used as it is important for the growth of callus.

**Parameters observed and recorded:**

The cultures were observed at regular intervals for growth changes and differentiation based on visual observation.

**RESULTS AND DISCUSSIONS**

In this investigation of explants of leaf, stem, axillary buds and seeds were cultured on Murashige and skoog’s basal medium with various PGR’s such as 2,4-D, 2,4,5-T, IAA, NAA, PAA, kin, 2ip and BAP individually or in different combinations or with coconut milk. In the current study, leaf disc were inoculated on MS medium supplemented with 2, 4-D, 2,4,5-T, kin, 2,4,5-T+2, 4-D, Kin+NAA, Kin+BAP, 2,4,5-D+kin. Proliferation of callus was high in 2, 4-D+2,4,5-T and Kin+BAP. Leaf disc explants induced rhizogenesis within 12 – 14 days when treated with 2, 4-D and 2,4,5-T.
Callus induction in general was suppressed due to endogenous hormone level and culture conditions. Stem explants induced callus and shoots in 2,4-D 10^-5 M, Kin 10^-5 M, BAP 10^-5 M and 2,4-D 10^-5 M + 2,4,5-T 10^-5 M. Caulogenesis was indirect in Kin 10^-5 M and direct in other treatments. Shoot inductions were observed at the elongated and at the stem region.

Nodal explants were inoculated on MS medium supplemented with 2,4-D, 2,4,5-T, Kin, BAP, 2,4,5-D+2, 5-T, IAA+BAP, 2ip+2,4,5-T. Callus was generally induced at the cut end of the stem touching the medium. Callus did not induced any shoot in 2,4-D and 2,4,5-T treatments.
The axillary bud induced multiple shoots in cytokinin or in combination with auxins. The shoot growth achieved 4 – 6 cm in about 25 – 30 days. The seeds were sterilized and trimmed and inoculated in the medium supplemented with 2,4-D 10-5 M, 2,4,5-T 10-5 M, 2,4-D 10-5 M + 10% coconut milk. Callus induced were green and compact in auxin treatment and friable in coconut milk treated medium.

**Figure: 8 Axillary bud induced multiple shoots**

This study also shows the callus induction is promoted by auxin and shoot of multiple shoots were induced by cytokinin and cytokinin rich and auxin low treatments. The potential of each explants has been analysed using various PGR's for morphogenesis. Further studies may be conducted to identify potential explants for micro propagation and tissues / cells can be identified for production of therapeutic agent and viable chemical for cosmetic and dyeing industries.

**Conclusion**

The various explants were cultured on MS medium supplemented with IAA, 2, 4-D, 2,4,5-T, NAA, IBA, BAP, KIN and 2ip. In all explants, callus production occurred on all surfaces. Multiple shoots induced in apical and axillary meristems were derived from matured explants of *Lawsonia inermis* Linn. The rate of multiplication was higher when the cultures were incubated under the continuous light rather than dark. Rhizogenesis occurred in all the explants, however, leaf disc explant proved to be the best system for rhizogenesis. Multiple shoots derived from shoot tips and auxiliary buds could be induced to produce roots.

**Reference**