

## PLUMBAGO ZEYLINICA LINN. AND PLUMBAGO ROSEA - REVIEW OF MICROPROPAGATION RESEARCH

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### ABSTRACT

The Ayurved cures the aging and its allied ill-effects by use of Rasayan (Rejuvenating / Antiaging) drugs. Chitrak (*Plumbago zeylinica*) is an age old Rasayan herb in traditional Ayurved. This herb has been well researched in recent times. However the other species of Chitrak have been neglected. Ayurvedic texts mention five different colored flowered Chitrak plants and the order of efficacy of the species is according to the color of flower. The major reason of lack of research in other species is their becoming rare or extinct. Thus the red flowered Chitrak (*Plumbago rosea*) is available in only in Sikkim and blue colored Chitrak (*Plumbago capensis*) is available only in some parts of Africa. There is no trace of yellow and black colored Chitrak today. This extinction sounds the danger bell for the continuance of *Plumbago rosea* and *Plumbago capensis*. The micro propagation is the best way to preserve these species. This Review Article researches the work done in this field of micro propagation of Chitrak species by tissue culture techniques. It has been found that almost all the research has been done on *Plumbago zeylinica* and a lone work on *Plumbago rosea*. A focused research needs to be conducted in this field that too in a way so that all Chitrak species are available in all the geographical regions.

**Keywords:** *Plumbago zeylinica*, Chitrak, Tissue culture, Micro propagation.

### 1. INTRODUCTION

#### 1.1 Chitrak

**1.2.1 Chitrak flowers:** According to Vag Bhatt's *Astang Hridayam*, Chitrak is found in Yellow, White and Black color flowers. Black color flower Chitrak is most effective Rejuvenator, the white color flower Chitrak (*Plumbago zeylinica*) is less effective than black color flower Chitrak but more effective than yellow color flower Chitrak.<sup>1</sup>

At present, both black and yellow color flower Chitrak are extinct.

**1.2.2 Chitrak flowers:** According to the *Bhavprakash Nighantu*, Chitrak is found with three different colored flowers viz. White (*Plumbago zeylinica*), red (*Plumbago rosea*) and blue (*Plumbago capensis*).

**Comparative Therapeutic Effects-** *P. capensis* is more effective than *P. rosea* which is more effective than *P. zeylinica* (more so as rasayan)<sup>2</sup>

यथास्त्वं चित्रकः पुष्पैर्ज्ञेयः पीतसितासितैः ।  
यथोत्तरं स गुणवान् विधिना च रसायनम् ॥ ६२ ॥

**A. White flowered Chitrak:**

English : Lead war  
Sanskrit : Agni, Vahni, Krishanu, Huashaa, Dahana, Hutabhuk

1. Bengali : Chita
2. Gujrati : Chitrakmula
3. Hindi : Chira, Chitra
4. Kannada : Chitramula, Vahni, Bilichitramoola
5. Kashmiri : Chitra, Shatranja
6. Malayalam : Vellakeduveli, Thumpokkoduveli
7. Marathi : Chitraka
8. Oriya : Chitamula, Chitoparu
9. Punjabi : Chitra
10. Tamil : Chitramoolam, Kodiveli
11. Telugu : Chitramulam

**B. Red flowered Chitrak:**

Colored Lead wart  
Botanical name- *Plumbago rosea* Linn  
Geographic distribution-Sikkim

**1.2.3 Names in other languages**

Hindi- Laal Cheeta, Laal chit-ur  
Bengali- Laal chitta, Rakto chito  
Marathi- Laal chitrak  
Kannad- Chitramool  
Telugu- Yerra chitramoolam

**C. Blue flowered chitrak-**

*Plumbago capensis* Thumb  
Geographic distribution-Africa



Plumbago zeylinica plant with flowers



Plumbago zeylinica roots

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Plumbago rosea plant with flowers



Plumbago rosea roots



Plumbago capensis plant with flowers



Plumbago zeylinica plant with live roots

**Traditional therapeutic benefits:** It is Rejuvenator, strengthener and enhances intelligence.<sup>3</sup>

छायाशुष्कं ततो मूल मासं चूर्णीकृतं लिहन् ।  
 सर्पिषा मधुसर्पिभ्यां पिबन् वा पयसा यतिः ॥ ६३ ॥  
 अम्भसा वा हितान्नाशी शतं जीवति नीरुजः ।  
 मेधावी बलवान् कान्तो वपुष्मान् दीप्तपावकः ॥ ६४ ॥

#### 1.2.4 THERAPEUTIC ACTIVITIES

Plumbago zeylinica is an important ayurvedic herb which has been found effective in many ailments by modern pharmacological research. Some such research is quoted below.

**a. Immunomodulation:** The research data indicate that plumbagin augments the macrophage bactericidal activity by potentiating the oxyradical release at low concentration whereas at the higher concentration it has inhibitory activity.<sup>4</sup>



**b. Anti oxidant activity:** In conclusion, these studies reveal that extracts of *P. zeylanica* and its active ingredient plumbagin have significant antioxidant abilities that may possibly explain some of the reported therapeutic effects.<sup>5</sup>

**c. Anti Cancer:** These results indicate that plumbagin is a potent inhibitor of the NF-kappaB activation pathway that leads to suppression of NF-kappaB-regulated gene products. This may explain its cell growth modulatory, anticarcinogenic, and radiosensitizing effects previously described.<sup>6</sup>

**d. Anti Allergic properties:** These findings demonstrate that Ethanolic extract of *Plumbago zeylanica* inhibits mast cell-dependent immediate allergic reactions, which is probably mediated by reducing the release of mediators such as histamine from mast cells via elevating intracellular cAMP level and weakening the inflammatory action of mediators.<sup>7</sup>

**e. Cholesterol lowering activity:** Plumbagin feeding brings about a definite regression of atheroma and prevents the accumulation of cholesterol and triglycerides in liver and aorta.<sup>8</sup>

**f. Anti Inflammatory activity:** The results revealed that while some of the fractions haemagglutinated red blood cells, others provided effective antioxidant and anti-inflammatory activities.<sup>9</sup>

**g. Memory enhancing and reversal of Amnesia:** The *Plumbago zeylanica* at dose 200mg/kg. has shown promising memory enhancing effect in mice. Furthermore, the extracts significantly reversed the amnesia induced by scopolamine (0.4mg/kg i.p.)<sup>10</sup>

**h. Hepatoprotective activity:** The *Plumbago zeylanica* extract had marked inhibition effects on HBeAg and HBsAg which expressed by 2.2.15 cells.<sup>11</sup>

**i. CNS Stimulant activity:** These behavioural and biochemical results indicated stimulatory properties of the extract of the root of *P. zeylanica*, which may be mediated by dopaminergic mechanisms in the rat brain.<sup>12</sup>

**j.** The effects of the ethanol extract of the root of *Plumbago zeylanica* on key enzymes of

glycolysis and other biochemical parameters were studied in the rat.<sup>13</sup>

**k. Anti Malarial activity:** The following five species seems to be of special interest for further antimalarial studies, *Casearia elliptica*, *Holarrhena pubescens*, *Pongamia pinnata*, *Soymida febrifuga*, and *Plumbago zeylanica*<sup>14</sup>

## 2. Extinction of Chitrak species

**2.1** The red flowered Chitrak (*Plumbago rosea*) is available in only in Sikkim.

Blue colored Chitrak (*Plumbago capensis*) is available only in some parts of Africa.

There is no trace of yellow and black colored Chitrak today. The black flowered Chitrak is described to be most potent species.

This extinction sounds the danger bell for the continuance of *Plumbago rosea* and *Plumbago capensis*.

## 3. Preservation of plants and their species

Micropopagation is one of the best ways to preserve the plants.

### 3.1 Tissue culture<sup>15</sup>

**3.1.1 Introduction:** Plant tissue culture is the technique of growing plant cells, tissues and organs in an artificial prepared nutrient medium static or liquid, under aseptic conditions.

It has advanced the knowledge of fundamental botany, especially in the field of agriculture, horticulture, plant breeding, forestry, somatic cell hybridization, phytopathology and industrial production of plant metabolites, etc.

### 3.1.2 Importance of Tissue Culture

**Technique :** Propagation of valuable economic plants through tissue culture based on the principle of totipotency (every cell within the plant has potential to regenerate into a whole plant).

In plant breeding, embryo, ovary and ovule culture as well as in vitro pollination have been employed to overcome morphological and physiological sterility and incompatibility.

One of the most significant developments in the field of plant tissues culture during recent years are the isolation, culture and fusion techniques which have their special importance in studies of plant improvement by cell modification and somatic hybridization.

**3.1.3 Plant Tissue Culture:** The technique has developed around the concept that a cell is

totipotent that is has the capacity and ability to developed into whole organism .The principles involved in plant tissue culture are very simple and primarily an attempt , whereby an explant can be to some extent freed from interorgan, inter-cellular interactions and subjected to direct experimental control. The most common culture in plant tissue is callus, which is wound tissue composed of undifferentiated, highly vacuolated and unorganized cells.

**Callus Culture:** For raising the callus tissues, a tissue culturist must have clear understanding of some basic principles. A cell from any part of the plant like shoot apex, bud, leaf, mesophyll cells, epidermis, cambium , anthers , pollen, fruit etc., when inoculated in a suitable medium under aseptic laboratory conditions can able to differentiate and multiply .This results into the formation of an amorphous mass of cells known as callus , which can induced to re-differentiate on appropriate medium to develop embryoids which directly develop into the plantlets, eventually giving rise to a whole viable plant.

#### Meristem culture

When a meristem is cultured in vitro, then it produces a small plant bearing 5 or 6 leaves. This could be obtained within a few weeks. Then the stem is cut into 5-6 small micro cuttings , which under favourable conditions, become fully grown plants.

#### Organ culture

A body of higher plants has complex inter-relationships between different organs like root, shoot, apical meristem, leaf primordia, floral buds, ovary, ovule, anther lobes, fruit, seed, etc. In this method a particular organ is isolated and cultured under laboratory condition in a chemically defined media where they retain their characteristic structure and other features and continue to grow as usual. In organ culture, organs are not induced to form callus, therefore, it differ from the callus culture where the organisation of the intact tissues is lost.

#### Culture media

The success in cell, tissue and organ culture technology is related to the selection or development of the culture medium. As no single medium will support the growth of all tissues.

A nutrient medium generally contains inorganic salts, vitamins, growth regulators, carbon source

and gelling agent. Other components added for specific purpose including organic nitrogen compounds- Hexitols, amino acids, antibiotics and plant extracts.

The Murashige-Skoog medium (MS), Linsmaier and Skoog (LS), Woody plant medium , Somatic embryogenesis medium and derivatives of these media have wide application for different plant species and for different culture objectives.

**Media components:** Plant growth regulators, Inorganic salts, Carbon source, Gelling Agent, Amino Acids and Amides, Antibiotics and Natural Complexes

#### Plant Growth Regulators:

The four classes of growth regulators are commonly used in tissue culture media:

- \*Auxins
- \*Cytokinins
- \*Gibberellins
- \*Abscisic acid

The type of growth regulators and concentration used will vary according to the cell culture purpose.

**Carbon source:** The carbohydrates in form of sucrose or glucose (2-5%W/V) , as a carbon source are essentially required in tissue culture as cells or tissues are generally not photosynthetically active . Examples are Hexitol, Sugars.

**Amino acids and Amides:** The amino acids and amides are very important for morphogenesis in tissue culture. Example of Amino acids and amides are – L-cysteine, L – serine, L-glutamine, L-tyrosine etc.

**Natural complexes :** The natural complexes such as coconut endosperm, milk (CM), yeast extract ( YE), fish emulsion, malt extract, potato extract, tomato juice etc, are used in tissue cultures for various purposes.

#### 4. Tissue culture research on Chitrak:

The tissue culture research has been done mainly on *P. zeylinica*. Only one research work has been found on *P. rosea*.

**4.1** This study, conducted in Jinju, Korea, uses explants from stem and leaves. *Agrobacterium*

*rhizogenes* was used for hairy roots development.<sup>16</sup>

It investigated the development of the efficient protocols for adventitious and hairy root cultures of *Plumbago zeylanica*. Adventitious roots were initiated from leaf and stem explants cultured on MS medium with different concentrations and combinations of auxins. The highest number of roots was obtained when the explants were cultured on MS medium with 1.0 mg-L<sup>-1</sup> IBA and 0.5 mg-L<sup>-1</sup> NAA.

Hairy root culture of *P. zeylanica* was obtained by infecting leaf explants cultured *in vitro* with *Agrobacterium rhizogenes* MTCC 532. The highest frequency of explant transformation was about 93%. The developed culture exhibited fast growth and high lateral branching on growth regulator free MS medium. The root cultures obtained were inoculated into B5, MS and SH media supplemented with different carbon sources with or without auxins and were placed on a rotary shaker 80 rpm for 35 days under dark or light conditions. Of the three media tested, MS medium sustained better root growth than others and sucrose proved to be the best carbon source. The biomass in hairy root culture was higher than in nontransformed root culture.

**4.2** This study has been conducted in Oklahoma, USA, using germinated seeds and agrobacterium and the resultant plants were transplanted in greenhouse.<sup>17</sup>

*Plumbago zeylanica* is a unique model for studying flowering plant gametogenesis, heterospermy, and preferential fertilization, yet understanding the control of related molecular mechanisms is impossible without efficient and reproducible regeneration and stable genetic transformation.

This study found three key factors for enhancing successful regeneration:

- (1) tissue source of explants,
- (2) combination and concentration of growth regulators, and
- (3) culture conditions.

The highest frequency of shoot regeneration was achieved using hypocotyl segments cultured on MS basal medium supplemented with BA 2.0 mg/l, NAA 0.75 mg/l, adenine 50 mg/l and 10% (v/v) coconut milk under subdued light at 25±2°C; under these conditions, each hypocotyl segment produced over 30 shoots,

arising primarily through direct organogenesis after 3 weeks of culture.

Regenerated shoots rooted easily on half-strength basal MS medium and were successfully established in the greenhouse. Using this tissue culture protocol, reporter gene *GUS* under the constitutive CaMV 35S promoter was introduced into *P. zeylanica* cells of petiole, cotyledon and hypocotyl with *A. tumefaciens* strains AGL1 and LBA4404. Transient expression was observed in all recipient tissues. Stable transgenic calli originating from petiole were obtained.

**4.3** This study was conducted in Ibadan, Nigeria, using embryos and nodal cuttings as explants.<sup>18</sup>

The root of *Plumbago zeylanica* is widely used by traditional Yoruba healers in Ibadan, Southwestern Nigeria, in the management and treatment of various infections and diseases. The plant is mainly harvested from the wild. The indiscriminate collection of the roots and non-cultivation of the plant has many implications for biodiversity. The plant is becoming scarce due to increasing demand for its use in ethnobotanical practice. These factors necessitate the study of micropropagation of *P. zeylanica* via tissue culture to ensure its sustainability. The embryos and nodal cuttings of *P. zeylanica* were used to evaluate the effect of culture media and growth regulators on the *in-vitro* shoot production and growth. The embryos were significantly viable on Nitrogen - Phosphorus Potassium (NPK) basal media. The highest multiplication rate of the explants was obtained using Murashige and Skoog (MS) medium supplemented with naphthalene acetic acid (NAA) (0.01 - 0.05 mg/l) and benzyl amino purine (BAP) (2.0 -4.5 mg/l). The single nodes of established plantlets were repeatedly sub-cultured on MS-NAA-BAP media at 4 week intervals for six months; the media enabled multiple shooting, rooting and mass multiplications without decline. The phytochemicals found in the *in-vitro* plantlets were saponins and tannins. The rooted plants which were successfully acclimatized in a greenhouse, then transferred to soil, showed a normal growth.

**4.4** In this study, conducted in Chennai, leaf callus culture was used and the plants were transplanted in soil.<sup>19</sup>

In this research, the morphogenic potential of the leaf callus cultures of the *Plumbago zeylanica*

was investigated to develop reliable protocols for the shoot generation and somaclonal variation. Maximum callus proliferation was obtained in the Murashige and Skoog (MS) Medium, supplemented with 2 mg BAP per Litre.

The maximum shoot regeneration (15.79 to 16.81) was achieved in five weeks of culturing callus on MS medium containing 0.75 mg BAP, 1mg IAA and NAA each per Litre. Regenerated shoots were rooted on half strength MS medium supplemented with 0.5 mg NAA per Litre.

The rooted plantlets were successfully established in soil. Calli derived from leaf explants cultured on MS medium fortified with 2 mg BAP per Litre when subcultured on MS medium fortified with 2mg BAP, 1.5 mg Kin and 1mg NAA per Litre induced somclonal variation.

**4.5** This study was conducted in Bhuvaneshwar and uses nodal culture. The plants were transplanted in greenhouse and later in soil.<sup>20</sup>

An efficient protocol was developed for *in vitro* clonal propagation of *Plumbago zeylanica* Linn. through nodal culture. Multiple shoots were induced from nodal explants of *P. zeylanica* on Murashige and Skoog's (1962) medium supplemented with 0.5 mg L<sup>-1</sup> to 1.0 mg.L<sup>-1</sup> 6-benzyladenine and 3% (w/v) sucrose. Inclusion of IAA (0.01mg L<sup>-1</sup>) in the culture medium improved the frequency of production of multiple shoots. Rooting was readily achieved upon transferring the shoots onto half-strengthMS medium supplemented with 0.25 mg L<sup>-1</sup> IBA and 2% (w/v) sucrose. Micropropagated plantlets were hardened in the greenhouse and successfully established in soil.

**4.6** This research project was carried out in Coimbatore, using nodal explants with total success in establishing plants in soil.<sup>21</sup>

An effective protocol for *in vitro* shoots multiplication and plant regeneration of *Plumbago zeylanica* L. was reported here. A rapid shoot proliferation was observed on the nodal explants of *P. zeylanica* in MS medium supplemented with 1.0mg/L BA and 1.0 mg/L GA3. The highest length of shoot (5.88±0.44) was achieved after 1 week of incubation. Regenerated shoots were rooted on half strength MS medium supplemented with 1.0mg/L BA and 0.5 mg/L IAA. The rooted plantlets were successfully established in soil with 100 percent survival rate.

**4.7** This research project was carried out in Varodara, Gujrat.<sup>22</sup>

Protocols for plant propagation through axillary bud proliferation and organogenesis were established for Chitrak - *Plumbago zeylanica* Linn. (Plumbaginaceae). MS medium with 4.4 mg/l BA and 1.4 mg/l IAA elicited the maximum number of shoots (12 multiple shoots) from nodal explants. Leaf based callus differentiated into more than 30 shoots on MS with 160 mg/l adenine sulphate. The regenerated shoots were rooted on MS with 1.2 mg/l IBA within ten days. Almost, 96% of the rooted shoots survived hardening when transferred to the field. The regenerated plants did not show any morphological change and variation in levels of secondary metabolite when compared with the mother stock.

**4.8** This is the only study on *P. rosea* and was conducted in Thailand.<sup>23</sup>

Root cultures of *Plumbago rosea* Linn. were established from young leaf explants on solid Gamborg's B5 (B5) medium supplemented with the combination of  $\alpha$ -naphthalene acetic acid (NAA) and kinetin in the concentration ranges of 0.5-2.0 mg/l and 0.1-0.5 mg/l, respectively. The production of plumbagin, determined by TLC-densitometry was higher [ $0.016 \pm 0.0030\%$  dry weight (DW)] in cultured roots obtained from B5. medium supplemented with 1.0 mg/l NAA and 0.1 mg/l kinetin. Plant selection increased the plumbagin production to  $0.129 \pm 0.0139\%$  DW, while variation of sucrose and nitrogen (as (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>) concentration in B5 media slightly increase the plumbagin synthesis to  $0.023 \pm 0.0017$  and  $0.020 \pm 0.0015\%$  DW, respectively.

## CONCLUSION

The major reason of lack of research in other species is their becoming rare or extinct. Thus the red flowered Chitrak (*Plumbago rosea*) is available in only in Sikkim and blue colored Chitrak (*Plumbago capensis*) is available only in some parts of Africa. There is no trace of yellow and black colored Chitrak today. This extinction sounds the danger bell for the continuance of *Plumbago rosea* and *Plumbago capensis*.

The micro propagation is the best way to preserve these species. This Review Article researches the work done in this field of micro propagation of Chitrak species by tissue culture techniques. It has been found that almost all the research has been done on *Plumbago zeylanica* and a lone work on *Plumbago rosea*. No



research project was found on *P. capensis*. Also no research uses root as explants in *P. zeylanica*. A focused research needs to be conducted in this field that too in a way so that all Chitrak species are available and preserved in all the geographical regions.

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