In vitro Micropropagation and Cytomorphological Evaluation of Centella asiatica (L.) Urban (Mandukparni) from Himachal Pradesh, India - An Endemic, Endangered and Threatened Herb

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Key words: Centella asiatica, Indian pennywort, Memory enhancer, Cytomorphotypes

Abstract

The present research work reports the cytomorphological evaluation and an efficient protocol for in vitro micropropagation of an endemic, endangered and threatened herb Centella asiatica (L.) Urban (Mandukparni) from Himachal Pradesh, India. Plant species is a potent memory enhancer, antidiabetic, antioxidant, antimutagenic, anticancerous and also reported to have cardiovascular properties and used to cure chronic hepatitis disorders. Eight morphometric characters for each 10 different accessions were extensively studied, but no new morphotype was reported. As per the cytology, the present report (2n = 18) confirmed the earlier chromosome counts from India and abroad. As there is an important need to preserve this plant species and to make it available all over the year to pharmaceutical industries without causing loss of germplasm from the wild region, efficient in vitro micropropagation protocol through nodal explant has been developed. As per the results, the highest percentage of multiple shoot induction was 90.20 showing average 16.3 number of shoots on the medium augmented with 2.0 mg/l BAP + 0.5 mg/l Kn. Whereas, the combined concentration of 1.0 mg/l NAA + 1.0 mg/l IBA showed highest 92.2% root induction with average 16.5 number of roots per shoot. The survival rate of these plantlets under green house condition was 80%. This protocol can be used for further regeneration and genetic transformation studies in Centella

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asiatica (L.) Urban. This research communication provides first record about the cytomorphological status comprises with in vitro multiplication of C. asiatica (L.) Urban, from Himachal Pradesh.

Introduction

Centella asiatica (L.) Urban (Apiaceae) is a perennial prostrate, aromatic herb, creeping with long stolons, rooting at nodes, leaves simple, flowers pink or red, fruit ovoid. The herb distributed and found wild throughout tropical and sub-tropical regions or countries like Bangladesh, Nepal, India, China, Malaysia, Indonesia, Sri Lanka and South America (Satake et al. 2007, Zheng and Quin 2007). Commonly known in India as Indian pennywort, Mandukaparni, Jal Brahmi and Gotukola (Pullaiah 2006) and in Bangladesh it is well known as Thankuni (Huq 1986), herb is known as Pegagainin Malaysia, Gotukola in America, Kakikuda or Pegagan in Indonesia, Luie Gon Gen or Tung Chain in China (Tolkha 1999). In India, this species can be found up to an altitude of 500 to 1800 m above sea level (Patra et al. 1998).

It is well defined by the Botanical Survey of India (BSI) that the requirement of Centella asiatica (L.) Urban plant in India is met from the natural resources/populations, leading to their gradual depletion and due to over exploitation of this herb for medicinal purposes and the absence of organized cultivation practices, this important plant species has dwindled to such a critical level that a ban on its collection from the wild has been recommended (Nayar and Sastry 1987). Because of unrestricted exploitation of this valuable natural resource, its ever increasing demand by the Indian pharmaceutical industries, limited cultivation and insufficient attempts for its replenishment, the wild stock of this medicinally important plant species has been markedly depleted and now it is listed as threatened species by the International Union for Conservation of Nature and Natural Resources (IUCN) (Pandey et al. 1993) and an endangered species (Singh 1989, Sharma and Kumar 1998). This species is also considered as endemic to Western Ghats of South India (Nayar 1996).

The substances of therapeutic interest are the saponin containing triterpene acids, asiatic acid, madecassic acid, asiaticosides as A and B (Kirtikar and Basu 1975). The plant acts as potent memory enhancer which contains glycosides as brahmoside, brahminoside, indocentelloside and leaves are rich in carotenoids, vitamin B and C (Silviya 2010). Vellaerine is the active component present in the leaves, is an oleaginous white crystalline substance considered advantageous in cognitive impairment (Bakhru 2003). Leaves extract inhibits the growth of human uterine carcinoma, human gastric carcinoma and mirine melanoma cells in vitro (Yoshida et al. 2005). Extract of whole plant is reported to have
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The whole plant is used in anxiety, neurosis, memory enhancer, jaundice, leprosy and skin diseases. The plant is also used as antidote to cholera, against rheumatism, elephantiasis, hydrocele, in tonics, bronchitis, asthma, gastric problems, leucorrhoea, kidney troubles and dropsy. Oral administration of different extracts showed anticonvulsant activity (Sudha et al. 2002) and aqueous extract of leaves has shown wound healing activity (Patil and Mandewgade 2003). Plant is used in the therapy of fever, measles, haematemesis, epistaxis, diarrhea, dysentery, rheumatism, spasms, ulcers, tuberculosis, syphilis, acne, allergies, convulsions, eczema, gonorrhoea and jaundice (Hanida and Kapoor 1988; Hausen 1993, Inamdar et al. 1996). Plant is also used as a component in one of the drug ‘Geriforte’ used to cure senile pruritus (Anon. 1992). It is a brain tonic, psycho-physical regenerator and blood purifier (Jorge and Jorge 2005). Plant is rich in minerals such as calcium, magnesium, potassium, phosphorus and aluminium (Herbert et al. 1994, Brinkhaus et al. 2000). This herb used as a raw material for preparation of pharmaceuticals, dermaticals and aroma therapeutical products. The plant is extensively used in Indian Herbal Pharmacopoeia, German Homeopathic Pharmacopoeia, European Pharmacopoeia and the Pharmacopoeia of the People’s Republic of China (Brinkhaus et al. 2000).

As per earlier cytological studies on genetic divergence among the accessions of *C. asiatica* (L.) Urban were restricted to India, cytotype with (2n = 18) was reported by different researchers (Mitra and Datta 1967, Datta and Maiti 1968a, Sinha and Sinha 1978, Ahmad and Koul 1980, Prasad and Janakiammal 1985, Subramanian 1986, Krishnanappa and Basappa 1988, Hamal and Koul 1989, Das and Mallick 1991, Trivedi and Trivedi 1992). The intraspecific polyploidy within the species is well reported from India and other parts of the world such as: (22 + 1 - 2B) by Joshi and Raghuvanshi (1970) and (2n = 22) by Raghuvanshi and Joshi (1968). Therefore, there is great need to check the ploidy level of this astonishing herb and to establish a link between cytomorphological aspects with phyto-biochemical relatedness.
In recent years, *in-vitro* culture techniques have been gaining popularity due to mass multiplication and germplasm conservation of rare, endangered and threatened medicinal plants (Sahoo and Chand 1998, Prakash et al. 1999, Rao 2004). As we know that tissue culture techniques can play a significant role in the rapid multiplication of the elite genotypes or clones and germplasm conservation of *C. asiatica*. There are a number of research publications previously reported on *in-vitro* regeneration of *C. asiatica* (L.) Urban through callus culture from leaf explants (Patra et al. 1998, Banerjee et al. 1999, Naidu et al. 2014), from stem node explants (Hossain et al. 2000), stem segments (Patra et al. 1998), through somatic embryogenesis (Martin 2004), from nodal segments (Karthikeyan et al. 2009), regeneration of plant from non-embryonic cell lines (Bibi et al. 2011), from shoot tips explant (Tiwari et al. 2000; Nath and Alak 2003, Rahman et al. 2008, Moghaddam et al. 2011, Chaturvedi et al. 2013). Nowadays, the common method employed for the micropropagation involves the propagation of shoots via a solid or semi-solid system. Such systems have successfully contributed to improve multiplication yield and more important in improving productivity and reducing the time taken for multiplication of plant. Therefore, it is important to develop an efficient micropropagation technique for this most important herb. Although extensive research has already been done on this herb, but present communication, reports a reproducible and rapid method for *in vitro* multiplication of *C. asiatica* through high frequency axillary shoot proliferation from nodal explants followed by successful establishment of regenerated plants in soil. In here we describe a protocol for high frequency plant regeneration from nodal explants of *C. asiatica* (L.) Urban for the first time from Himachal Pradesh (India).

Even though the genus is medicinally so important, it has failed to attract the attentions of the researchers regarding the combined and comparative study of both cytomorphological and micropropagation parameters on different populations of *C. asiatica* from Himachal Pradesh. Therefore, as a part of our investigation on selected medicinal and aromatic plants from North India, this research communication is a pioneer attempt to provide first record about the cytomorphological status comprises with *in vitro* multiplication of *C. asiatica* (L.) Urban, a naturally grown medicinally important plant species from Himachal Pradesh, which were previously undescribed from this study region.

**Materials and Methods**

Plant materials for cytomorphological studies were collected from wild region of Sirmour District (Himachal Pradesh) and cultivated in agricultural experimental farms and for *in vitro* micropropagation of *Centella asiatica* (L.) Urban, cultivated
accessions were collected during the months of June-July, 2014 from agricultural experimental farms and analysed in Biotechnology Laboratory of Eternal University, Baru Sahib, Himachal Pradesh (India). Plants specimens were identified by the Department of Botany, the Punjabi University, Patiala (Punjab), India.

On the basis of eight morphometric characters, namely plant height (cm), leaf colour, leaf size (cm), number of leaves/branch, number of branches/node, length of stolon between nodes, flower colour, primary root length (cm), root diameter (cm) (2 cm below base) on 10 plants were extensively studied from the study area for each accession of *C. asiatica* and the average results were taken into consideration to find out new morphotypes (Table 1).

Immature floral buds were collected for each accession and fixed in Carnoy’s fixative for 24 hrs and preserved in 95% alcohol at 4°C for further use. For meiotic studies smear was made using standard aceto-carmine technique to analyze cytological variability. A number of slides were carefully examined for cytological analysis, chromosome counts were made and abnormalities were recorded in each population. Pollen analysis was made by mounting pollen grains from mature flowers in 50% glycerol-acetocarmine and by taking well filled pollen grains with stained nuclei as apparently viable while shriveled and unstained pollen grains were taken as sterile. The photomicrographs of the chromosomes count were made from permanent and freshly prepared slides with the help of Leica Qwin Digital Imaging System.

Nodal explants/shoot tips were collected from the young sprouts of the stock plants and thoroughly washed under the running tap water for 30 min and treated with 10% (v/v) Teepol (Himedia, Ltd. India) for 15 min and followed by repeated rinsing with running tap water for 10 min. The explants were washed thrice with double distilled water and further sterilization was done under aseptic conditions in a Laminar Airflow Hood/Chamber (Popular Traders, India). The explants were surface sterilized with 0.1% (w/v) HgCl₂ for 8 min and thoroughly washed with sterile/autoclaved double distilled water several times to remove traces of sterilant (HgCl₂) before inoculation.

The shoot tips were cut into appropriate size and cultured on MS basal medium containing 3% (w/v) sucrose in all experiments. The pH of the medium was adjusted to 5.8 prior to autoclaving and addition of 0.8% (w/v) agar. The molten medium was dispensed in 15 ml aliquots into culture tubes (25 × 150 mm) and capped with non-absorbent cotton plugs. Along with that 50 ml molten medium was also dispensed in different glass jam jars (400 ml). The medium was autoclaved at 1.1 Kg/cm² pressure and 121°C for 15 min. The cultures were
Table 1. Average morphometric characters of 10 accessions of *C. asiatica* from Himachal Pradesh.

<table>
<thead>
<tr>
<th>Name</th>
<th>Locality/altitude</th>
<th>Acc. Collection No.</th>
<th>Plant height (cm)</th>
<th>Leaf colour</th>
<th>Leaf size (cm)</th>
<th>No. of leaves/branch</th>
<th>No. of branches/node</th>
<th>Length of stolon (cm)</th>
<th>Flower colour</th>
<th>Primary root length (cm)</th>
<th>Root diam. (2cm below the base)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. asiatica</em> (L.) Urban Baru Sahib (H.P.)* (754 m)</td>
<td>10002</td>
<td>13.5</td>
<td>Green</td>
<td>4.0 x 2.0</td>
<td>Single</td>
<td>3</td>
<td>6.0</td>
<td>Pinkish</td>
<td>5.2</td>
<td>1.1</td>
<td></td>
</tr>
</tbody>
</table>

* H.P.: Himachal Pradesh.

Table 2. Cytologically worked out *C. asiatica* wit's chromosome number, locality, altitude, habit and previous chromosome number reports.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Name</th>
<th>Locality/altitude</th>
<th>Habit</th>
<th>Accession number</th>
<th>Present report (n)</th>
<th><strong>Previous chromosome number reports</strong></th>
</tr>
</thead>
</table>

incubated in the culture room maintained at 25 ± 2°C and 50 - 70 % RH under a photoperiod of 16 hrs at 3000 lux light intensity provided by cool white florescent tubes (Philips Ltd, India).

For shoot proliferation MS supplemented with various concentrations of plant growth regulators, different concentrations of BAP 1, 2, 3 mg/l combined with fixed concentration of Kn (0.5 mg/l) were used for culture initiation and multiplication of shoots. In addition, different concentrations of Kn (1, 2, 3 mg/l) and BAP (1, 2, 3 mg/l) were also used for shoot induction. All the cultures were transferred to the fresh medium after 2 - 3 weeks. The mean number of shoots and lengths were evaluated after 4 weeks of inoculation.

*In vitro* raised shoots (3 - 6 cm) were excised and transferred to full strength MS containing 3% (w/v) sucrose solidified with 0.8% (w/v) agar. The medium was further supplemented with different concentrations of NAA and IBA (1, 2, 3 mg/l) and combined concentration of both NAA and IBA (0.5 + 0.5, 1.0 + 1.0, 1.5 + 1.5 mg/l). Data of the number of roots were recorded after four weeks.

Well-developed rooted plantlets were removed carefully from the culture vessels, washed gently under running tap water to remove the remains of agar and transferred to pots containing sterilized mixture of sand, soil and farmyard manure in the ratio of 1 : 1 : 1. The potted plantlets were kept under transparent polythene sheets for 2 weeks to ensure high humidity and then kept in open diffused light for hardening. After 20 days the surviving plants were transferred to pots containing garden soil and maintained in the greenhouse for acclimation.

**Results and Discussion**

We have extensively studied eight morphometric characters namely: Plant height; leaf colour, leaf size, number of leaves/branch, number of branches/node, length of stolon between nodes, flower colour, primary root length (cm), root diameter (2 cm below base) for each 10 different accessions of *C. asiatica*, this extensive screening revealed that there is no new morphotype reported on the basis of study conducted. For all the accessions the average plant height (13.5 cm) was recorded, the leaf color was green in all the accessions, leaves are simple, orbicular-reniform, puberulous and single per branch. We have recorded that every node has an average of 3 branches/nodes, well established rooting was recorded at every node with average primary root length (5.2 cm) along with root diameter (1.1 cm), the average length of creeping long stolons between two nodes (6.0 cm) (Figs A, B and C), pinkish colored flowers in axillary fasciculate umbels were recorded in all the accessions (Table 1.).
The present meiotic study was made in wild population collected from Baru Sahib (H.P.) region and cultivated in the Departmental greenhouse. The study revealed the presence of 9 bivalents in all the PMCs at M-I (Figs D and E). PMCs at A-I showed 9 chromosomes at each pole (Fig. F) and another PMC showed 9 chromosomes at each pole with a laggard (Fig. G). Due to stickiness (Fig. H.) the well spread M-I were difficult to observe. No further meiotic abnormality was observed at any stage of meiosis. The pollen fertility was 87 per cent. The present report confirms many earlier chromosome counts from different parts of India and abroad. Beside present report of (2n = 18), another cytotype with (22 + 1 - 2B) has also been reported from India (Raghuvanshi and Joshi 1968). However, from outside India, diploid, tetraploid and hexaploid cytotypes were also reported (Table 2.).

Thus intraspecific polyploidy on x = 9 is well represented in the species. In the present study the results show that the nodal explants cultured on MS supplemented with different concentrations and combinations of BAP and Kn have regenerated and gave best response in terms of multiple shoot production. The nodal explants cultured on MS showed signs of bud break in 15 days (Fig. I) followed by production of multiple shoots within 25 - 40 days (Figs J and K). Among two cytokinins, BAP was found to be superior than Kn in terms of better shoot development and number of shoots per node. The highest percentage of multiple shoot induction was 90.20% showing an average 16.3 number of shoots on the medium fortified with 2.0 mg/l BAP + 0.5 mg/l Kn (Graphs 1 and 2), followed by 75.30% shoot induction and an average of 9.5 shoots in the medium supplemented with 3.0 mg/l BAP + 0.5 mg/l Kn. The individual concentration 3.0 mg/l BAP showed 70.50% of shoot induction with an average of 4.1 number of shoots, followed by 58.23% shoot induction with an average of 2.8 shoots in the medium containing 2.0 mg/l BAP, whereas, Kn can also induce 56.75% shoots and an average of 2.3 shoots per node, when used individually with a concentration of 3.0 mg/l Kn (Table 3).

Thus, from the present results BAP was found to be effective and superior for shoot induction if used in combination with Kn (Figs L, M and N). Earlier, it was reported that BAP individually acts as a better agent for shoot induction. These findings are also consistent with the earlier reports on different medicinal plants (Chirangini et al. 2005, Ghanti et al. 2004, Karthikeyan et al. 2007, Lal et al. 1998, Sharma et al. 2014). Well-developed shoots were isolated and cultured on MS having different concentrations of NAA and IBA. The frequency and number of roots per shoot varied with individual and combined concentrations of the NAA and IBA. Among all the concentrations, the combined concentration of 1.0 mg/l NAA + 1.0 mg/l IBA showed highest 92.2% root induction with an average 16.5
number of roots per shoot (Graphs 3 and 4), whereas, among all individual concentrations, the highest 83.9% root induction with average 12.5 number of roots per shoot in the medium containing 2.0 mg/l IBA was achieved, followed by 76.2% root induction with an average 9.5 number of roots per shoot in the medium containing 2.0 mg/l NAA (Table 4).

Table 3. Effect of BAP and Kn on shoot multiplication from the nodal explants of C. asiatica on MS.

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>BAP (mg/l)</th>
<th>Kn (mg/l)</th>
<th>Mean number of shoots/node</th>
<th>Mean length of shoots (cm)</th>
<th>% of culture response</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>1.0</td>
<td>0.0</td>
<td>1.3</td>
<td>1.2</td>
<td>33.20</td>
</tr>
<tr>
<td>2.</td>
<td>2.0</td>
<td>0.0</td>
<td>2.8</td>
<td>1.6</td>
<td>58.23</td>
</tr>
<tr>
<td>3.</td>
<td>3.0</td>
<td>0.0</td>
<td>4.1</td>
<td>1.9</td>
<td>70.52</td>
</tr>
<tr>
<td>4.</td>
<td>0.0</td>
<td>1.0</td>
<td>1.1</td>
<td>2.1</td>
<td>26.30</td>
</tr>
<tr>
<td>5.</td>
<td>0.0</td>
<td>2.0</td>
<td>1.8</td>
<td>2.5</td>
<td>39.42</td>
</tr>
<tr>
<td>6.</td>
<td>0.0</td>
<td>3.0</td>
<td>2.3</td>
<td>3.1</td>
<td>56.75</td>
</tr>
<tr>
<td>7.</td>
<td>1.0</td>
<td>0.5</td>
<td>6.2</td>
<td>2.9</td>
<td>65.80</td>
</tr>
<tr>
<td>8.</td>
<td>2.0</td>
<td>0.5</td>
<td>16.3</td>
<td>4.6</td>
<td>90.20</td>
</tr>
<tr>
<td>9.</td>
<td>3.0</td>
<td>0.5</td>
<td>9.5</td>
<td>3.4</td>
<td>75.30</td>
</tr>
</tbody>
</table>

Table 4. Effect of NAA and IBA on rooting of in vitro raised shoots of Centella on MS.

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>NAA (mg/l)</th>
<th>IBA (mg/l)</th>
<th>Mean number of roots/shoot</th>
<th>Mean length of roots (cm)</th>
<th>% of root induction</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>1.0</td>
<td>0.0</td>
<td>6.3</td>
<td>1.2</td>
<td>31.5</td>
</tr>
<tr>
<td>2.</td>
<td>2.0</td>
<td>0.0</td>
<td>9.5</td>
<td>2.3</td>
<td>76.2</td>
</tr>
<tr>
<td>3.</td>
<td>3.0</td>
<td>0.0</td>
<td>4.6</td>
<td>1.5</td>
<td>34.2</td>
</tr>
<tr>
<td>4.</td>
<td>0.0</td>
<td>1.0</td>
<td>3.4</td>
<td>2.4</td>
<td>41.8</td>
</tr>
<tr>
<td>5.</td>
<td>0.0</td>
<td>2.0</td>
<td>12.5</td>
<td>6.6</td>
<td>83.9</td>
</tr>
<tr>
<td>6.</td>
<td>0.0</td>
<td>3.0</td>
<td>5.4</td>
<td>3.4</td>
<td>54.5</td>
</tr>
<tr>
<td>7.</td>
<td>0.5</td>
<td>0.5</td>
<td>6.8</td>
<td>3.2</td>
<td>51.6</td>
</tr>
<tr>
<td>8.</td>
<td>1.0</td>
<td>1.0</td>
<td>16.5</td>
<td>6.8</td>
<td>92.2</td>
</tr>
<tr>
<td>9.</td>
<td>1.5</td>
<td>1.5</td>
<td>6.1</td>
<td>3.5</td>
<td>56.3</td>
</tr>
</tbody>
</table>

Therefore, it is clear that the combined concentration of 1.0 mg/l NAA + 1.0 mg/l IBA is an ideal treatment for root induction. As we know from the previous reports that IBA was the most suitable auxin for rooting in many plant species (Parveen and Shahzad 2010, Raghu et al. 2006, Shahzad et al. 2007). Again IBA was found to be efficient in inducing rooting in higher altitude Himalayan plants viz., *Aconitum atrox* (Rawat et al. 1992), *Podophyllum hexandrum* (Nadeem et al. 2000), *Cedrus deodara* (Nandi et al. 2002). After 4 weeks, well rooted plantlets
Fig. 1. Centella asiatica. A. Plant’s bed, B. Shooting and rooting at each node, C. Creeping of long stolons between nodes, D-E. PMC at M-I with 9n, F. PMC at A-I with 9 chromosomes at each pole, G. PMC at A-I showing 9 chromosomes at each pole with laggard, H. PMCs showing stickiness of the chromosomes. I. Nodal explant/shoot tips cultured in MS + BAP. J. Multiple shoots initiation on MS + BAP. K-N. Elongation of in vitro shoots on the medium. O. Acclimatization of in vitro regenerated plants to natural conditions/pot containing sterilized mixture of sand, soil and farmyard manure in the ration of 1 : 1 : 1 (Scale = 10 μm).
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Graph 1. Shoot regeneration/induction percentage by using different concentrations of BAP and Kn.

Graph 2. Average/mean number of shoots per node by using different concentration of BAP and Kn.

Graph 3. Root regeneration/induction percentage by using different concentrations of NAA and IBA.

Graph 4. Average/mean number of roots per shoot by using different concentrations of NAA and IBA.
were obtained and their morphometric characters were studied. These plantlets were removed from the culture vessels, washed gently under running tap water and planted in small pots (plastic cups) containing a potting mixture of sand, soil and farmyard in the ratio of 1 : 1 : 1 (Fig. 1O). The potted plantlets were covered by transparent polythene sheet to maintain suitable humidity. After proper acclimation, the survival rate of these plantlets under greenhouse condition was 80%. It has been already reported that the nodal explants are the best source for multiple shoot induction in medicinal plants viz., *Rauwolfia serpentina*, *Vitex negundo*, *Emblica officinalis* (Roy et al. 1995; Vadawale et al. 2006; Rahaman et al. 1999). A number of medicinal plants were also cultured for conservation and multiplication by using various tissue culture techniques viz., *Heracleum candicans*, *Plumbago zealanica*, *Cassia alata*, *Solanum trilobatum*, *Eclipta alba*, *Ocimum sanctum*, *Bacopa monniera*, *Solanum nigrum* etc. (Wakhlu and Sharma 1998, Chaplot et al. 2006, Hasan et al. 2008, Jawahar et al. 2004, Tiwari et al. 1998, Vijayakumar et al. 2010, Preethi et al. 2011, Lim et al. 2009, Baskaran and Jayabalan et al. 2005, Sridhar and Naidu 2011).

The above protocol can be exploited for commercial propagation and conservation of valuable medicinal plants which are considered as endangered, endemic and threatened herbs.

In the present report, we have improved the protocol and optimized the combinations for better and superior shoot and root induction in *C. asiatica* (L.) Urban. The nodal explant culture technique and micropropagation reported here offer an effective method of multiplication and conservation of this important medicinal plant species. Cytomorphological studies play a great role in understanding and evaluating the processes as to how some plant species create new cyto- and morphotype providing material for future research involving this potential medicinal herb.

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