A plant regeneration protocol via somatic embryogenesis was developed using immature anthers of *H. brasiliensis* (clone RRII 105). Modified MS medium supplemented with 2,4-D, NAA and Kn as plant growth regulators was found to be ideal for embryogenesis. This protocol could be applied to several Indian genotypes of *Hevea* for production of large-scale plants. The present regeneration system can also be used for developing transgenic plants either by Agrobacterium-mediated gene transfer or by particle bombardment with desirable genes.

---

**Table 3. Influence of NAA and Kn on the number of somatic embryos formed in *H. brasiliensis***

<table>
<thead>
<tr>
<th>NAA (mg/l)</th>
<th>0.5</th>
<th>0.6</th>
<th>0.7</th>
<th>0.8</th>
<th>0.9</th>
<th>1.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>0.1</td>
<td>4.67 ± 1.75</td>
<td>10.83 ± 1.33</td>
<td>15.33 ± 1.63</td>
<td>11.83 ± 1.72</td>
<td>5.67 ± 0.82</td>
<td>0.7 ± 0.82</td>
</tr>
<tr>
<td>0.2</td>
<td>10.0 ± 1.67</td>
<td>18.0 ± 2.37</td>
<td>23.83 ± 1.72</td>
<td>20.67 ± 2.58</td>
<td>10.3 ± 1.75</td>
<td>3.5 ± 2.74</td>
</tr>
<tr>
<td>0.3</td>
<td>2.83 ± 1.79</td>
<td>9.0 ± 1.79</td>
<td>14.17 ± 2.93</td>
<td>11.83 ± 1.72</td>
<td>4.67 ± 1.51</td>
<td>1.0 ± 0.89</td>
</tr>
<tr>
<td>0.4</td>
<td>0.50 ± 0.84</td>
<td>3.17 ± 1.47</td>
<td>6.0 ± 1.26</td>
<td>3.83 ± 1.47</td>
<td>1.33 ± 1.75</td>
<td>0.0 ± 0.0</td>
</tr>
</tbody>
</table>

Mean number of embryos (± SD) were recorded from pooled data after 4–5 months of culturing in embryo induction medium.  
F = 10.92**; CD (0.05) = 1.95.  
**indicates significant at P < 0.01.

Acknowledgements. We thank Dr N. M. Mathew, Director, RRI for his encouragement to carry out this work.

Received 26 November 1998; revised accepted 24 February 1999.

---

**In vitro regeneration of a medicinal plant – *Houttuynia cordata* Thunb. from nodal explants**

P. J. Handique* and Pranjal Bora

Department of Biotechnology, Guwahati University, Guwahati 781 014, India

*In vitro* regeneration of multiple shoots was achieved in nodal explant cultures of a medicinal plant *Houttuynia cordata* Thunb. (family Saururaceae) on Murashige and Skoog (MS) medium supplemented with 1 mg/l of N-6 Benzyladenine (BA). *In vitro* grown shoots were cultured for rooting on MS medium with 1 mg/l of indole acetic acid (IAA). The rooted plantlets were successfully established in soil.

*Houttuynia cordata* Thunb. (family Saururaceae) is a wild perennial herb with creeping rootstocks and swollen nodes. The species exists in two chemotypes – the Japanese chemotype, which smells like orange and the Chinese chemotype which smells like raw fish and fresh coriander leaves. The Chinese chemotype was used in this study. Mainly leaves and occasionally the whole plant of *H. cordata* are used as medicine. Leaves are used to treat cold, measles, dysentery, indigestion, and eye, urine, and skin ailments. The plant is used as a detoxicant, anti-inflammatory, antipyretic and diuretic agent in traditional medicinal practices of Assam and China. Two pyridine alkaloids, three aporphine-related alkaloids (viz. cepharonine, cepharanthine B and 7-chloro-6-demethyl cepharanthine B) and aristolactams have been isolated from *H. cordata*. It also yields essential oils.

Organized cultivation of *H. cordata* for extraction of medicinal compounds was reported from Vietnam. In Assam, these plants are collected from natural population which is not abundant. *In vitro* propagation of this species could help in raising disease-free healthy clones.
in a large scale for extraction of pure alkaloids. This paper presents the results of in vitro regeneration of *H. cordata* from nodal explants.

Healthy shoots of *H. cordata* Thunb. were collected from the plants grown in the greenhouse and washed with tap water several times. Shoots were excised at the internodes and soaked in Tween 20 (20%) for 15 min with occasional stirring. The shoot segments were then washed several times with sterile distilled water (SDW) in a laminar airflow cabinet and then surface sterilized in 0.1% mercuric chloride solution for about 3 min followed by a thorough rinsing in SDW. These surface sterilized shoot segments were cut into 6-8 mm pieces. The shoot pieces which consisted of a single node containing a small portion of the internode on either side were used as explants.

The explants were cultured on MS medium with 0.8% agar and 3% sugar, and with or without N-6 benzyladenine (BA), (0.2-1.0 mg/l) in 25 x 150 mm culture tubes. The pH of the medium was adjusted to 5.8 prior to autoclaving. The media were autoclaved at a pressure of 15 lb/inch² for 15 min. The cultures were maintained under 2000 lux light intensity provided by white fluorescent lamps for 16 h photoperiod at 25 ± 2°C. For each treatment 20 replicates were used. The growing explants were subcultured only once after 30 days. After 4 weeks of subculture, the growing shoots were cultured for rooting on MS medium (pH 5.8) containing indole acetic acid (IAA) (0.2-1.0 mg/l) keeping the incubation conditions same as before.

The nodal explants started growing after about one week of culture on MS medium with BA (0.75 and 1.0 mg/l), producing green shoot buds. Maximum number of shoots were obtained in the medium supplemented with 1.0 mg/l BA after 30 days of culture. The shoots were subcultured after 30 days. After 4 weeks of subculture, the shoots were cultured on MS medium supplemented with IAA. Adventitious roots developed from the base of the shoots after 21 days of culture and maximum number of roots developed in the presence of 1.0 mg/l IAA (Figure 1a). The rooted plants were allowed to grow on the MS medium containing IAA up to 30 days and then transferred to liquid MS medium without hormone for 10 days (Figure 1b). Thereafter, the plantlets were transferred into pots containing a sterilized mixture of garden soil and vermiculite (2:1). The plantlets were kept covered with a polythene bag for 10 days to check excessive transpiration. The plantlets were then successfully established in field condition and in big earthen pots (Figure 1c). The acclimatized plantlets showed 95% survival.

The protocol standardized through this study demonstrates the possibility of developing an efficient in vitro propagation system for successful mass propagation of *H. cordata*.

Figure 1. a, Initiation of rooting of *H. cordata* Thunb. in in vitro condition; b, In vitro growth of plantlet; c, Fully grown plant after 4 months of transfer.

Carbonaceous megaremains from the Neoproterozoic Owk Shales Formation of the Kurnool Group, Andhra Pradesh, India

Mukund Sharma and Manoj Shukla
Birbal Sahni Institute of Palaeobotany, 53, University Road, Lucknow 226 007, India

An assemblage of carbonaceous compression and impressions recorded from the Owk Shales Formation (OSF) of the Kurnool Group include, Chuarid (Chuaria circularis), Tawuid (Tawuiia sp.), Ellyposphysid, Moranid and Beltinid remains. The presence of Chuaria-Tawuiia assemblage provides strong evidence of correlation with the assemblage of Rewa and Bhandar groups of Vindhyan Supergroup and to some extent with the Halkal Formation of the Bhima Group. Chuaria-Tawuiia assemblage is being considered as a potential biostratigraphic marker. In this communication we report varied groups of carbonaceous compression and impressions from the OSF of the Kurnool Group. On the basis of the present fossil assemblage, the OSF is considered to be of the Neoproterozoic age.

MEGASCOPIC remains are well recorded from the various 1.1–0.7 Ga-old sedimentary successions of the world\(^1\).\(^2\). In India, carbonaceous megascopic compression and impressions have previously been recorded from the Vindhyan succession of central India\(^3\)–\(^5\) and Bhima Basin\(^6\)–\(^14\) of south India. A sketchy report has also been made from the Kurnool Basin\(^15\). We report here a very well preserved and diversified megascopic assemblage from the Owk Shales Formation (OSF) of the Kurnool Group, and discuss its biostratigraphic significance in the light of other discoveries of carbonaceous megaremains.

The OSF is part of the Kurnool Group of the Cuddapah Supergroup exposed in the Kurnool District, Andhra Pradesh. It conformably overlies the Narji Limestone Formation and grades upwards into massive Panum Quartzite Formation\(^16\)–\(^18\) (Figure 1). OSF is a well-laminated, thin-bedded unit with shales, siltstones, and silty-clay stones. The shales are noncalcareous; light grey, buff, and khaki in colour. These shales intercalate with thin-bedded silty claystone in the lower part of the OSF and siltstone in the upper part of the OSF. The carbonaceous compression and impressions reported here are found in abundance on bedding planes at the shale-siltstone, and shale-claystone junctions of the OSF.

The first reference of the occurrence of carbonaceous remains (presently considered biogenic) was made by King\(^16\) who reported, 'these are somewhat like cycloid shales of fish, but no organic structure has been recognized in them'. This observation suggests that although King noted these dark-spotted features on the bedding surface of the shale, he did not consider these as biogenic in origin. Later, Rajurkar\(^16\), in a very brief communication, restudied these and compared the circular structures with Fornoria—a genus presently being considered as a junior synonym of the Chuaria Walcott. He did not however mention the other forms present in the OSF. We have noted several additional types of carbonaceous remains. The morphotype diversity of the carbonaceous remains, present in Precambrian sediments, has been divided into several groups\(^5\). On the same lines the present occurrence has been described as well.

The collection of fossil assemblage described here has been made from the eastern part of the hilllock 1445, situated in the village Ankireddipalle (15°07'78"03') of the Kurnool district of Andhra Pradesh, south India (Figure 2). The exposed thickness of OSF at this place is about 15 m. While the lower 5 m part is dominated by claystone beds, the rest of the 10 m of succession is comprised of shalesiltstone alteration (Figure 3). The figures specimens are deposited in the repository of Birbal Sahni Institute of Palaeobotany, and catalogued under the BSIP Museum Numbers (38078–38082, slide number 12055), as cited in the individual figure captions. The present study of the fresh collection shows that the carbonaceous megafossil assemblage recorded from the OSF belongs to the following 5 groups.

Chuarid remains: The specimens are smooth-walled spheroids preserved as carbonaceous discs, ranging in size from 1–5 mm (E = 3.11; N = 261) (Figures 4 c−f). Occasionally a few forms show short, rounded extension at one place. Ford and Breed\(^19\) placed the size range of the Chuaria from 0.5 to 5 mm. Vidal\(^20\) from the study of assemblage in Visings Formation extended the lower extent of the size parameters to 0.09–0.2 mm and the upper limit up to 3 mm. Later, Vidal and Ford\(^21\) included specimens as small as 70 μm in diameter in the category of Chuaria circularis. This indicates that there is still no unanimity about the lower size limit of Chuaria. However, the upper size limit is generally agreed to be 5 mm.