IN VITRO SEED GERMINATION OF CANNONBALL TREE (COUROUPITA GUIANENSIS AUBL.):- AN ENDANGERED PLANT SPECIES OF JHARKHAND

¹Umakant Singh and ²Ashok Kumar Choudhary  
¹Ph.D Research Scholar, ²University Professor of Botany Department  
¹Tissue Culture Laboratory, University Department of Botany, Ranchi University, Ranchi-834008, Jharkhand (India)

ABSTRACT

Couroupita guianensis Aubl. commonly known as cannonball tree belongs to the Lecythidaceae family are in few numbers in the land of Jharkhand. It is endangered species in many parts of the country. The present study was carried to mitigate the problem by standardizing an efficient protocol for in vitro seed germination. Mature and immature seeds from cannonball like fruits as explants were collected from the site and were soaked in water for one to two days then total and partial decoating and scarification methods employed, sterilized and inoculated to Murashige and Skoog Medium (MS 1962) without any phytohormonal supplementation. The seeds were categorised under six groups based on the age of seeds used for inoculation. The seed groups S₃, S₄ and S₅ showed the sign of germination. The seedlings attained the height of 6 to 7cm.

KEYWORDS: Couroupita guianensis, cannonball, endangered species, in vitro.

INTRODUCTION

Couroupita guianensis Aubl. (Cannonball tree) belongs to the Lecythidaceae family is an endangered medicinal tree present on the campus of Ramkrishna Mission T.B Sanotorium, Tupudana, Ranchi; Ramkrishna Mission, Morabadi, Ranchi and in Indian Institute of Natural Resins and Gum, Namkum, Ranchi. The tree Couroupita guianensis was named by French botanist Jean Baptiste Christopher Fusee Aublet in 1775 (http:en.m.wikipedia). The common name of it are “cannonball tree” (because of cannonball like fruit structure), “Kailashpati” in Hindi (Asadujjaman et al., 2013 and Pandurangan et al., 2018), “Mallikarjuna” in Telgu, “Nagalinga pushpam” in Tamil because it looks like shivalingam shape at the centre of the flower and snake hood like pollen structure (Pandurangan et al., 2018), “Nagkeshar” in Bengali, “Nagakeshar” in Odiya (http.en.m.wikipedia). Couroupita guianensis is a threatened species worldwide( Mitre 2012; Rai 2014; Shekhawat et al., 2016). It is large deciduous, tropical tree grows up to 20m to 35 meters in height. It is indigenous to the Amazon rainforest. The clustered leaves vary in length generally from 8 to 31 centimeters. Some trees flower profusely until the entire trunk is adorned with reddish and pink flower, the inflorescence is racemose arising from the trunk. They are strongly scented at night (Prance et al., 2013) and in
the early morning (Senaratne, 2007). The fruits are huge rusty resembles cannonball (Shekhawat et al., 2016) hanging in clusters on string (Shete et al., 2013; Arokiamary et al., 2018). The fruits are 20 to 25 centimetres in diameter and weighing around 1 to 2 kilograms. The fruit contains 80 to 300 small seeds (Sai et al., 2011; Sundarajjan et al., 2014; Arokiamary et al., 2018) in a white pulpy mesocarp. The seed coat is covered by exotestal hairs (Arokiamary et al., 2018). It gains traditional importance as tree parts are used to treat hypertension, tumours, pain, inflammation, cold, stomach ache, skin diseases, malaria, wounds and toothache (Umachighi et al., 2007; Sanz-Biset et al., 2009; Arokiamary et al., 2018). These plants are endowed with many important biological properties like antibiotic, antifungal, antiseptic (Khan et al., 2003; Kavitha et al., 2011), antifertility (Geetha et al., 2005), immunomodulatory (Pradhan et al., 2009; Shekhawat et al., 2016), antihelminthic (Rajamanickam et al., 2009; Velliangiri and Subban, 2012), antinociceptive (Pinheiro et al., 2010), antibacterial (Azimi et al., 2012), antitumour (Premnathan et al., 2012), antipyretic (Usman et al., 2012), larvicidal, insecticidal, pesticidal (Basker and Ignacimuthu, 2012), cytotoxic, anticancer (Velliangiri and Subban, 2012; Gupta et al., 2014), antiallergic, anti-inflammatory, anti-diabetic (Swapnalatha and Rajeswari, 2014), antioxidant (Stalin G et al., 2012; Gupta et al., 2014), neuromorphological activities (Gupta et al., 2014) and many more. The fruit pulp, bark and flowers are used as ingredients which cure gastritis, scabies, bleeding piles, dysentery and scorpion poison (http://www.adaacademy.org/dagrdens_cannon1.html; Shete et al., 2013 and Rai Y. 2014). The flowers cure intestinal gas formation (Elumalai et al., 2012). The leaves have herbal hand wash formulation (Minakashi G Joshi et al., 2008), leaves juice to cure skin diseases, young leaves ease tooth pain and used as fodder for cattle and deer (Rai Y. 2014). The various part of this tree contain volatile oils (Rai Y. 2014), ketosteroids, glycosides, coumoptine (Pandurangan P. et al., 2018), isatin, indirubin, phenolics, stigmasterol, eugenol, linalool, fernesol, nerol, quercetin, saponins, tryptanthrine, indigo, linoleic acid, carotenoids, sterols, flavonoids and phenolic substances with medicinal properties (Jayashree et al., 2001; Rane et al., 2001; Ahire and Laddha 2002; Desal et al., 2003; Rajamanickam et al., 2009; Mariappan et al., 2012). Due to its immense biological properties are over exploited lead to dramatic reduction of its natural population and also environmental issues allows to serious threat of extinction. It is enlisted as a rare tree and flower in India (Shah et al., 2012; Shete et al., 2013; Sundararajan and Koduru, 2014). The Government of Puducherry (India) declared Couroupita guianensis flower as the Official State Flower to conserve valuable tree under its natural habitat in South India ((Deepa 2007; Shekhawat et al., 2016). The natural propagation of the tree through seeds are very slow (Arokiamary et al., 2018) due to its less viability, short life span because of relarlicant behaviour which does not allow seeds to dry well and to withstand low temperature (Gousia et al., 2013; Shekhawat et al., 2016). The studies were undertaken by Arokiamary et al., (2018) to optimize the condition for in vitro embryo germination and to investigate the behaviour of seed storage regulated by various factors like moisture content, germinability, dessication and storage temperature for replanting the tree into its natural habitats. The present investigation is to overcome the problems faced by
natural propagation through in vitro seed germination to conserve the rich genetic resource required in the land of Jharkhand rare and threatened worldwide.

MATERIALS AND METHODS

Plant material and explants sterilization:

The immature and mature seeds of Couroupita guianensis Aubl. were harvested from cannonball like fruits from the campus of Ramkrisha Mission T.B Sanatorium, Tupudana, Ranchi, Jharkhand. The seeds were separated from white pulpy mesocarp. The viability of seeds checked by dipping into the water. The five seeds in each were grouped under S1 (1 day old seeds), S2 (10 days old seeds), S3 (20 days old seeds), S4 (30 days old seeds), S5 (40 days old seeds) and S6 (50 days old seeds). The methods adopted for experiment were similar as employed by them (Singh et al., 2010; Aziza M. Taj Aldin., 2015; Singh et al., 2019).

The seeds were manually scarified with saw dust paper and peeling with hands to make water permeable. The seeds were soaked in water for one to two days. The method of total and partial decoating and scarification were employed to enables in vitro result for seed germination. The seeds were washed in running tap water for 10 minutes. Then washed with 1% (v/v) solution of Savlon for 5 minutes followed by Tween 20 detergent 2% (v/v) for 10 minutes and then soaked in 70% (v/v) ethanol for 30 seconds after each treatment the seeds were rinsed three times with distilled water. Further process of sterilization were performed under laminar air flow chamber by freshly prepared 0.1% (w/v) of mercuric chloride for 2-5 minutes and to remove the traces of it was washed three to four times with sterilized double distilled water (Murugan et al., 2018; Singh et al., 2019).

Culture Media:

The Murashige and Skoog basal medium containing 3% (w/v) sucrose, 0.8% (w/v) agar without any phytohormones used for in vitro seed germination. The pH was adjusted to 5.6 to 5.8 using hydrochloric acid and sodium hydroxide. It was heated until the solution becomes clear and transparent and then finally dispensed into pre sterilized culture tubes before autoclaving. The medium was sterilized in autoclave machine at 121°C for 30 minutes. After it the pre treated and surface sterilized explants were aspetically inoculated into culture tubes and kept under the light intensity of 100 lux, photoperiod 12 hours and relative humidity 70-75%.

RESULT AND DISCUSSION

Seed germination

In S1 and S2 groups the seeds did not show any sign of germination. The seeds found swelling but unable to grow and start decaying. In S6 group the seeds did not respond to basal medium. In S3, S4 and S5 groups altogether 80% seed germinated within duration of three weeks. All in vitro raised seedlings attained an average height of 5.6 centimetres. They showed distinct cotyledonary growth with hypocotyl formation. The apical leaves with clear veins and the root
hairs were observed. Later some of the seedlings were subcultured to MS medium with different grades of phytohormones for morphological responses and rest were acclimatized in green house.

Table 1:- Showing percentage of seed germination and height of seedling under different groups of seeds in MS culture medium

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Age of fruits (days)</th>
<th>Medium</th>
<th>Percentage of Seed germination</th>
<th>Height of Seedling (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>S1</td>
<td>MS medium</td>
<td>Nil</td>
<td>-</td>
</tr>
<tr>
<td>2.</td>
<td>S2</td>
<td>MS medium</td>
<td>Nil</td>
<td>-</td>
</tr>
<tr>
<td>3.</td>
<td>S3</td>
<td>MS medium</td>
<td>80%</td>
<td>5.5</td>
</tr>
<tr>
<td>4.</td>
<td>S4</td>
<td>MS medium</td>
<td>80%</td>
<td>6.0</td>
</tr>
<tr>
<td>5.</td>
<td>S5</td>
<td>MS medium</td>
<td>80%</td>
<td>5.3</td>
</tr>
<tr>
<td>6.</td>
<td>S6</td>
<td>MS medium</td>
<td>Nil</td>
<td>-</td>
</tr>
</tbody>
</table>

CONCLUSION:

It is concluded that in vitro raised seedlings and its acclimatized well could be the efficient protocol in conservation of the rare and endangered species of Couroupita guianensis with some utmost medicinal properties for the future generations.

ACKNOWLEDGEMENT

The authors wish to express sincere gratitude to the management of Ramkrishna Mission Ashrama, Divyayan Krishi Vigyan Kendra, Morabadi, Ranchi for providing all the facilities and encouragement for the study.

REFERENCES


13. http: en.m.wikipedia


33. Sanz, JB; Campos-de-la-Cruz, J; Epiquién-Rivera, MA; Canigueral, S (2009), “A first survey on the medicinal plants of the Chazuta valley (Peruvian Amazon)”, J Ethnopharmacol, 122, 333–362.


