

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

<sup>1</sup>Umakant Singh and <sup>2</sup>Ashok Kumar Choudhary

<sup>1</sup>Ph.D Research Scholar, <sup>2</sup>University Professor of Botany Department

<sup>1</sup>Tissue 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 S<sub>3</sub>, S<sub>4</sub> and S<sub>5</sub> 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 Sanatorium, 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 pushpan” 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 1to 2 kilograms. The fruit contains 80 to 300 small seeds (Sai et al., 2011; Sundarajan 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( Umachigi 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), antihelmintic (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), antiulcer, anti-arthritic, anti-diarrheal (Elumalai et al., 2013), 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.da-academy.org/dagrdens\\_cannon1.html](http://www.da-academy.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, courouptine (Pandurangan P. et al., 2018), isatin, indirubin,phenolics, stigma- sterol, eugenol, linalool, fernesol, nerol, quercertin, 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 relacitrant 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 Ramkrishna 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 (1day 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 aseptically 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 S<sub>1</sub> and S<sub>2</sub> groups the seeds did not show any sign of germination. The seeds found swelling but unable to grow and start decaying. In S<sub>6</sub> group the seeds did not respond to basal medium. In S<sub>3</sub>, S<sub>4</sub> and S<sub>5</sub> 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

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



D. Fig 1.

*In vitro* seed germination of *Couroupita guianensis* : A: Seed coat removal and establishment on MS medium( without hormones). B: Seed germination with cotyledonary emergence. C: Shoot induction and root formation. D: *In vitro* regenerated seedling.

## 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.

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## REFERENCES

1. Ahire AE, Laddha KS (2002): Beta amyirin palmitate- isolation from *Couroupita guianensis* Aubl leaves. Indian Drugs. 39: 216-216.
2. Asadujjaman, Aslam- Hossain, Karmakar UK (2013): Assessment of DPPH free radical scavenging of some medicinal plants. Journal of Pharmacy and Biological Sciences. (1): 161-165.
3. Azimi H; Fallah-Tafti M; Khakshur AA; and Abdollahi M (2012): “A review of phytotherapy of acne vulgaris: perspective of new pharmacological treatments”, *Fitoterapia*, 83(8), 1306-1317.
4. Aziza M. Taj Aldin (2015): Breaking up dormancy of *Adansonia digitata* L. seeds and regeneration of plantlets from stem nodal segments in vitro. Al Azhar Buletin of Science 26(2), 47-52
5. Baskar K and Ignacimuthu S (2012): “Chemosphere antifeedant, larvicidal and growth inhibitory effects of ononitol monohydrate isolated from *Cassia tora* L. against *Helicoverpa armigera* (Hub.) and *Spodopteralitura* (Lepidoptera: Noctuidae)”. *Chemosphere*, 88(4), 384-388.
6. Deepa HR (2007). Puducherry comes out with the list of state symbols. The Hindu, Chennai, TN, India.
7. Desal T, Golatakar SG, Rane JB, Ambaye RY, Kamnath VR (2003): Larvicidal property of *Couroupita guianensis* Aubl. Indian Drugs, 40:180-191.
8. Elumalai, A; Naresh, V; Eswaraiah, MC; Narendar, P; and Kumar, R (2012), “Evaluation of antiulcer activity of *Couroupita guianensis* leaves”, *Asian J Pharm Tech*, 2(2), 64–66.
9. Elumalai, A; Eswaraiah, MC; Koppula Naresh, K; Kumar, R; Meruva, A; Vidhyulatha, C (2013), “Antidiarrhoeal activity of *Couroupita guianensis* leaves on castor oil induced diarrhoea in albino rats”, *Int J Pharmacol-Research Gate*, 3(2), 42-44.
10. Geetha, M; Shankar, MB; Mehta, RS; & Saluja, AK (2005), “Antifertility activity of *Artabotrys odoratissimus* Roxb. and *Couroupita guianensis*”, *J Nat Rem*, 5, 121–125.

11. Gupta S., Ghosal M., Choudhary, D., and Mandal, P. (2014). Dynamic Changes in Antioxidant Activity during Floral Development of *Couroupita guianensis*. *Journal of Pharmaceutical Research International*, 4(6), 676- 694.
12. Gousia SK, Ashok KK, Vinay KT, Naveen LL.(2013): Biological activities and medicinal properties of *Couroupita guianensis*. *Int. J. Pharm Pharmaceut Sci. Res.* 3(4): 140-143.
13. [http: en.m.wikipedia](http://en.m.wikipedia)
14. [http://www.da-academy.org/dagrdens\\_cannon1.html](http://www.da-academy.org/dagrdens_cannon1.html)
15. Jayshree BR, Severina JV, Suprabha GG, Ambaye RY, Khadse BG. (2001): Chemical examination of flowers of *Couroupita guianensis* Aubl. *Indian J Pharm Sci.* 63: 72-73.
16. Kavitha R, Kamalakannan P, Deepa T, Elamathi R, Sridhar S, Suresh KJ (2011): In vitro antimicrobial activity and phytochemical analysis of Indian medicinal plant *Couroupita guianensis* aubl. *J. Chem Pharm Res.* 3:115-121.
17. Khan MR, Kihara M, Omoloso AD (2003) Antibiotic activity of *Couroupita guianensis*. *J Herbs Spices Med Plant* 10:95–108.
18. Mariappan P, Srinivasan R, Kandasamy K (2012) Antioxidant and anticancer activities of isatin (1 H-indole-2,3-dione), isolated from the flowers of *Couroupita guianensis* aubl. *Indian J Med Res* 136:822–826.
19. Minakshi GJ, Kamat DV and Kamat SD (2008): Evaluation of herbal handwash formulation. *Natural Product Radiance.* 7(5), 413-415.
20. Mitre M 2012 *Couroupita guianensis*. In: IUCN Red List of Threatened Species. Version 2012.2. <http://www.iucnredlist.org>
21. Murashige T, Skoog FA (1962) A revised medium for rapid growth and bioassay with tobacco cultures. *Physiol Plant* 15:473–497.
22. Murugan, M. & Kamaraj, M. (2018), *In vitro propagation & conservation of useful ethnomedicinal plant of Hybanthus Enneaspermus (Linn.) F.Muell. belonging to the Violaceae Family*, *IJCRLS.* 7(7), 2493-2499.
23. Pandurangan P, Sahadeven M, Sunkar S, Dhana SKNM. (2018): Comparative analysis of biochemical compounds of leaf, flower and fruit of *Couroupita guianensis* and synthesis of silver nanoparticles. *Pharmacogn J.* 10(2): 315-323.
24. Pinheiro, MM; Fernandes, SB; Fingolo, CE; & Fernandes, D (2013): “Anti-inflammatory activity of ethanol extract and fractions from *Couroupita guianensis* Aublet leaves”, *J Ethnopharmacol*, 146, 324–330.
25. Pradhan, D; Panda, PK; and Tripathy, G (2009): “Evaluation of Immunomodulatory activity of the methanolic extract of *Couroupita guianensis* flowers in rats”, *Nat Prod Rad*, 8(1), 37-42.
26. Prance, GT; Mori, SA (1986): “*Annals of the Missouri Botanical Garden*”, 73, 99-101.
27. Premanathan, M; Radhakrishnan, S; and Kulangiappar, K (2012): “Antioxidant & anticancer activities of isatin (1H-indole-2, 3-Dione), isolated from the flowers of *Couroupita guianensis*”, *Indian J Med Res*, 136, 822–826.
28. Rai Y,(2014): Early seedling growth status of threatened medicinal tree species *Couroupita guianensis* Aubl. in district Meerut, U.P, India. *International Journal of Innovation and Scientific Research.* 8(2), 252-255.

29. Rajamanickam V, Rajasekaran A, Darlin quine S, JesupillaiM, Sabitha R (2009) Anthelmintic activity of the flower extract of *Couroupita guianensis*. *Int J Alt Med* 8:107–111.
30. Ramalakshmi, C; Ranjitsingh, AJA; Kalirajan, K; Kalirajan, A; Athinarayanan, G and Mariselvam R (2013), “A preliminary screening of the medicinal plant *Couroupita guianensis* for its antimicrobial potential against clinical and fish-borne pathogens”, *Elixir Appl Biol*, 57, 14055–14057.
31. Rane JB, Vahanwala SJ, Golatkar SG, Ambaye RY, Khadse BG (2001) Chemical examination of the flowers of *Couroupita guianensis* aubl. *Indian J Pharm Sci* 63:72–73.
32. Sai KC, Gaddala N, Vanamala S, Naresh V, Elumalai A (2011) A short review on therapeutic uses of *Couroupita guianensis* aubl. *Int Res J Pharm Appl Sci* 1:105–108.
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.
34. Senaratne, LB. (2007): “ Spreading the splendour of Sal. The Sunday Times, Colombo, Sri Lanka 42(10).
35. Shah, GN; Shete, SA; Patil, VS; Patil, KD and Killedar, SG (2012), “Standardization and antibacterial activity of *Couroupita guianensis* fruit pulp extract”, *Int J Pharmacog Photochem Res*, 4(4), 1-5.
36. Shekhawat MS and Manokari M. (2016): In vitro propagation, micromorphological studies and ex vitro rooting of cannon ball tree (*Couroupita guianensis* Aubl.): a multipurpose threatened species. *Physiol Mol Biol Plants*. 22(1),131–142.
37. Shete SA, Shah GN, Walke SS, Patil VS, Patil KD, Killedar SG (2013) Standardization and anti bacterial activity of *Couroupita guianensis* fruit shell extract. *Int J Bio* 2:360–364.
38. Singh, S., Rai, S & Khan, S. (2010): *In vitro* seed germination of *Adansonia digitata* L.: An endangered medicinal tree. *Nanobiotechnica Universales*. 1(2), 107-112.
39. Singh U, Choudhary AK, (2019): In vitro seed germination of an endangered plant species of Jharkhand:- *Adansonia digitata* Aubl., *Journal of emerging technology and innovative research*. 6(6),717-722.
40. Stalin G, Vishnuvardhan T, Sanyamounika K, Arun Chand Roby K, Lakshmi Prasanna T. (2012): Phytochemical screening and antioxidant activity of flowers and leaves of *Couroupita guianensis* Aubl. *Int. J. Phytopharmacy Research*. 3(1), 20-23.
41. Sundararajan R. and Koduru R. (2014): “A complete profile on *Couroupita guianensis* – traditional uses, pharmacological activities and phytoconstituents”. *Pharmacophore* 5(1), 147-159.
42. Swapnalatha S., Rajeshwari V.D. (2014): antidiabetic activity of *Couroupita guianensis*. *Int J Pharm Biol Sci*. 9(3), 41-
43. Umachigi, SP; Jayaveera, KN; Ashok, CK; Kumar, GS, (2007), “Antimicrobial, wound healing and antioxidant potential of *Couroupita guianensis* in rats”, *Pharmacology online*, 281(10), 269–281.

44. Usman, MRM; Somani, RP; Mohammed, A; Mohammed, U (2012), "Evaluation of antipyretic activity of *Anthocephalus cadamba* roxb. leaves extracts", Res J Pharm Biol Chem Sci, 3(1), 825–834.
45. Velliangiri P, Subban R (2012): "Quantification of quercetin and stigmasterol of *Couroupita guianensis* aubl by HPTLC method and in-vitro cytotoxic activity by mtt assay of the methanol extract against Hela, nih 3 t3 and hepg2 cancer cell lines. Int J Pharm Pharmac Sci 4:126–130

