Seed germination studies in *Caesalpinia bonduc* (L) Roxb.

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Abstract: *Caesalpinia bonduc* (L) Roxb. is mainly grown for its immense medicinal value. This plant has an important role in the human health care system. Seeds *C. bonduc* are used to treat cancer, eyesores, tuberculosis, asthma, toothaches, and fever. The seed extract of this plant has antioxidant, anti-microbial, anti-diabetic, anti-bacterial and antipyretic properties. However, the number of this medicinally important plant has significantly become low due to the poor seed germination rate, longer duration of seed dormancy, over-utilization as medicinal plant and destruction of natural habitats. In the present investigation, efforts have been made to employ the *in vivo* and *in vitro* methods to increase the seed germination percentage. It has been recorded that the seed germination percentage in the in vivo methods is very low as compared to *In vitro* seed germination method. The seeds of *C.bonduc* (L)Roxb. shown 100% germination and rapid growth of root as well shoot in the semisolid agar medium (without any addition of nutrients) using cotyledonary *explants*. The seeds grown by *In vivo* technique shown slower growth and low germination percentage (10%) even after breaking seed coat. In the present investigation, the protocol developed is valuable for the conservation of *C.bonduc* (L)Roxb. and it is also useful for commercial ventures for in mass production of herbal medicines in Pharmaceutical industry.

Key words- *Caesalpinia bonduc* (L) Roxb. Human health care system, *in vitro*, *in vivo*, seed germination.

I. INTRODUCTION

Medicinal plants serve as a potential source of therapeutic products. According to the estimates of World Health Organization (WHO) approximately 80% of the people in the developing countries depend on the traditional medicines for 'Primary Health Care' needs [1]. Medicinal plants, if judiciously utilized, can provide rational healthcare and it has potential to contribute significantly to economic growth and development [2]. In the recent years, the usage of the plant-derived drug has increased. This revival of interest in plant origin drugs is mainly due to the concept of 'green medicine'. This "green medicine" is a safe, less costly with no adverse side effects and having a broad spectrum of biological activities as compared to synthetic drugs [3]. By a conservative estimate, the annual world trade in medicinal plants is estimated to touch US $ 5 trillion by the year 2050 [4].

Caesalpinia bonduc (L) Roxb (syn.) or *Caesalpinia bonducella* (L) fleming, syn *Caesalpinia. crista* (Linn) is a member of Caesalpiniaceae. It is an annual shrub with yellow prickles. "Bonducella," the name of the species, is derived from the Arabic word "Bonduce," meaning a "little ball" that indicates the globular shape of the seed [5]. This plant is known as 'Sagargota' or 'Fevernut' or 'Nata Karanja' in India. In Sanskrit, it is known as 'Kuberakshi.' *C. bonduc* is widely distributed all over the world mainly found in Indian tropical regions. The seeds of *C. bonduc* are thick, with the brittle shell, grey coloured and resembled eyeballs. The Sanskrit name 'kuberakshi,' meaning eyes of Kubera (Hindu god of wealth). It has also been called 'vajrabijaka' that implies to its seed coat-diamond like hard. All the parts of this plant have therapeutic value, and it is being used in the traditional system of medicine since ancient times. This plant is used in Ayurveda. The seeds of possessing ayurvedic properties of seed is such as Rasa: Tikta (bitter), kashaya (astringent), Guna: Laghu (light), ruksha (dry), tikshna (sharp), Veerya: Ushna(hot)Vipaka: Katu (pungent) Dosha: Pacifies tridosha.

C. bonduc (L) Roxb possesses a variety of chemicals viz. alkaloids, flavonoids, glycosides, saponins, tannins, triterpenoids, phytosterinin, β-sitosterol, flavonoids, bonducellin, aspartic acid, arginine, citrulline and β-carotene [6]. The seed kernels contain bonducellin (Isoflavones), aminoacids (Aspartic acid, arginine) and citrulline. In Ayurveda, the embryo (after removing its hard-shiny seed coat) is traditionally used for it is medicinal properties. This plant has valuable medicinal properties such as anti-diabetic [7]-[9], antitumor [10], antimicrobial [11], antioxidant [12], diuretic [13] and antifertility [14]. This plant also shows an antifungal property. The Leaf infusion, root powder and powdered seed used as medicine. The sprouts of seed are used to cure tumours and paralysis. The seeds, after roasting with castor oil, is applied externally to inflammatory swellings (inflamed piles and hydrocoele). The seeds of plants also have ethnoveterinary importance. *C. bonduc* (L) Roxb. is used for the treatment of tachycardia, bradycardia, tuberculosis, tympanites, pain in the abdomen, fever, cold and cough, and liver fluke in ruminants. The seeds are considered tonic, febrifuge, anthelmintic and antiblennorrhagic. Children unable to digest mother's milk were given the extract of the kernel or its powder along with ginger, salt, and honey to get a good stomachic effect.

C. bonduc shows seed coat dormancy [15]. The seeds of this plant germinate very slowly due to hard seed coats. The literature survey shown that very scanty work has been done on this plant related to *in vivo* and *in vitro* seed germination. The main aim of the present investigation is to study the germination of the embryo *in vitro*, to standardize the medium for *in vitro* germination and to compare germination percentage with *in vivo* method. The mass propagation of *C. bonduc* through *in vitro* culture has commercial applications of the herbal industry.

II. MATERIALS AND METHODS

Plant material: The healthy plant specimens for the study were collected from Pune, India 17 º 54' and 19 º 24' N latitude and between 73 º19' and 75 º10' E longitude. The plant has been identified by following the standard methods. Seeds of *C. bonducella* were collected and shade dried. The hard seed coat of *C. bonduc* cracked with special technique and embryo was used for in *vivo*

and *in vitro* studies. *In vivo* studies were carried by following standard methods. Soil medium and Petridish methods were used. 5 seeds were placed on 90 mm Petri dishes on Whatman No. 2 filter paper moistened with 15 ml distilled water. Seeds were kept in germinator at 25 ºC in darkness. Germination was counted in 24 h intervals and continued until fixed state. 100 seeds were grown on soil medium while 200 seeds were tested in petridish. The embryo was grown on agar medium (without nutrients) and MS medium 5mg/IBA was used. The seeds were surface sterilized with laboline and 2% $HgCl_2$. The inoculated cultures were kept at 23°C ± 2°C, 12hr photoperiod, and light intensity of 1000 ~ 3000 lux. The root and shoot length of *C. bonduc* was recorded over the period for both *in vitro* and *in vivo* cultures.

III. RESULTS AND DISCUSSION

Table no. 1. Length of root and shoot in the *in vivo* germinated *C. bonduc* (L) embryos.

Medium Used	Total no. of seeds	Germination (days)	Root length (mm)	Shoot length (cm)	% seed germination
Soil	100	5	2.0±0.20	10.0±0.30	2.0
		10	10.0±0.50	15.0±0.40	4.0
		15	15.0±0.75	20.0±0.50	10.0
		20	17.0±0.60	22.0±0.80	10.0
		25	20.0±0.52	25.0±1.0	10.0

Table no. 2. Length of root and shoot in *vivo* germinated *C. bonduc* (L) embryos.

Medium Used	Total no. of seeds	Germination (days)	Root length (mm)	Shoot length (mm)	% seed germination
Paper media in petri dish	200	5	3.0±0.50	8.0±0.10	2.0
		10	12.0±0.75	15.0±0.60	4.0
		15	18.0±0.95	20.0±0.50	10.0
		20	19.0±1.0	21.0±0.60	10.0
		25	22.0±0.80	23.0±0.90	10.0

Table no. 3. Length of root and shoot in *vitro* germinated *C. bonduc* (L) embryos

Medium Used	No. of inoculated bottles with embryo culture	No. of days (after inoculation)	Root Length (mm)	Shoot length (mm)	% of successful inoculations
Semi-solid agar medium	100	5	5.0±0.30	10.0±0.50	100
		10	16.0±0.50	25.0±0.60	100
		15	20.0±0.70	30.0±0.75	100
		20	30.0±1.20	35.0±0.80	100
		25	32.0±1.0	40.0±0.90	100

Table no. 4. Length of root and shoot in *vitro* germinated *C. bonduc* (L) embryos

Medium Used	No. of inoculated bottles with embryo culture	No. of days (after inoculation)	Root Length (mm)	Shoot length (mm)	% of successful inoculations
MS medium with 5mg/lit	100	5	5.0±0.25	10.0±0.50	100
		10	13.0±0.5	23.0±0.75	100
		15	20.0±1.0	25.0±0.80	100
		20	28.0±1.2	32.0±0.50	100
		25	30.0±075	35.0±0.75	100

Fig. 1

Fig. 2

Fig.1. – Very few seeds shown seed germination.

Fig. 2. On 30th day germination of *C. bonduc* L. seeds *in vitro*

IV. CONCLUSION: Seeds of *C.bonduc* (L)Roxb. shown 100% germination and rapid growth of root as well shoot in the semisolid medium (without any addition of nutrients). *In vivo* germination seeds shown slower growth and low germination percentage (10%) after breaking seed coat after 40-42 days. Kumar *et al.* 2012 carried out the shoot initiation using cotyledonary *explants* by employing MS medium with 5mg/IBA and 2mg/l TDZ [16]. In the present investigation, *in vitro* germination (especially in agar semisolid medium without nutrients showed 100%. So the current protocol developed is valuable for the conservation of *C.bonduc* (L)Roxb. as well for the Pharmaceutical industry.

V. ACKNOWLEDGMENT

The author is grateful to experts to Principal of Sir Parashurambhau College, Pune for providing all the facilities required to carry out the present work.

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