

Callus Induction and Synthetic Seed Development in *Draceana sanderiana* Sanderex Mast: Lucky Bamboo

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Authors' contributions

This work was carried out in collaboration between both authors. Author MA designed the study, managed the literature searches, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author AM managed the analyses of the study. Both authors read and approved the final manuscript.

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ABSTRACT

The study was carried out for callus induction and synthetic seed development from the shoot tips of *Draceana sanderiana* sander ex Mast. The shoot tips were subjected to different concentrations (0.25, 0.5 & 1.0 mg/l) of 2,4-D on MS medium. The research findings revealed that the 2,4-D at concentrations of 0.25 mg/l was more suited for the profuse callus formation. The friable and light yellow callus was induced within 2 weeks of culture at 0.25 mg/l of 2,4-D on MS medium as compared to the other two concentrations of 2,4-D i.e.; 0.5 and 1.0 mg/l. Similarly the effect of sodium alginate and calcium chloride percentage on synthetic seed formation was observed, it was found that somatic embryos formed from shoot tips via callus kept in 2.5% sodium alginate and 100 milli molar CaCl₂ produced synthetic seeds with firm spherical beads. The study leads to the formation of synthetic seeds of *Draceana sanderiana* which can be used for the conservation of germplasm through cryopreservation and the micro propagation of the said plant species.

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1. INTRODUCTION

The vegetative propagated plants are prone to several bacterial, fungal, viral and mycoplasmal diseases, as a result their productivity declines [1]. Even mass propagation through seeds has many limitations. In living plants, callus cells are those cells that cover a plant wound. It is a growing mass of unorganized plant parenchyma cells. In biological research and biotechnology callus formation is induced from plant tissue samples (explants). Callus has a particular use in micro propagation where it can be used to grow genetically identical copies of plants with desirable characteristics. The plants with limited success through vegetative propagation can be propagated through plant tissue culture by using the callus. *Draceana sanderiana sander* ex Mast is one of the important indoor ornamental plant belonging to the family *Agavaceae*. It is also known as Ribbon dracaena, lucky bamboo, Belgian evergreen or ribbon plant. It is native to Cameroon in tropical West Africa. The species was named after the German English gardener Henry Frederick Conreal sander. *Dracena sanderiana* has long been associated with the eastern practice of Feng shui. *Dracena* species possess number of medicinal properties. An inactive saponin-spiroconazole-A which is obtained from *Dracena mannini* and *Dracena arborea* possess fungicidal, molluscidal, anti-malarial and anti leishmanial activities [2]. There are several steroidal saponins which are obtained from *Draceana draco* showed cytostatic activity on Leukemia HL 60 cells [3,4]. The extract obtained from *Draceana cochinesis* is reported to improve the clotting process in mice significantly [5]. The *Draceana draco* produces red resin (Dragons blood) has been frequently used as a herbal remedy in traditional medicine [6]. It was found that the extract obtained from the *D. loureiri* inhibits the estrogen effect by binding with the estrogen receptors [7]. Despite number of medicinal, ornamental and cultural importance, not much work has been done in *Draceana species* in *in vitro* conditions [8] and mostly relies on vegetative limitations like seed dormancy, low rate of germination [9]. The major barriers in the commercial adoption of true seed are high level of heterozygosity and heterogeneity of the crop. To overcome these problems and fulfill the required demand, it is a demand of the time to restore the productivity of plants by the use of plant tissue culture [10]. Synthetic seed production is an applied

technology which allows rapid multiplication of the elite plant species. The artificial seed development acts as an alternate to organogenesis for regeneration & germplasm preservation of the plants. The plant materials like shoot tips, axillary buds and somatic embryos are used to develop synthetic seeds. There are some other benefits of synthetic seeds like –low production cost, short and long time storability, facilitation of germplasm exchange between laboratories, easy handling, transportation of propagules to distant places for subsequent propagation [11]. In the present study, investigations was made to produce the callus and synthetic seeds in *Dracaena sanderiana* that can be used in future to enrich the ornamental industry. The aim of this study was to determine the optimum concentration of sodium alginate and CaCl_2 (encapsulation matrix) to optimize the size, shape and texture of alginate beads for maximum germination.

2. MATERIALS AND METHODS

Plant material: Healthy plants of *Draceana sanderiana Sander* ex Mast were collected from the Jamia Hamdard (Hamdard University campus, New Delhi India) herbal garden and was identified by Dr. M P. Sharma, Plant Taxonomist, Department of Botany, Hamdard University, New Delhi India). The voucher specimen was deposited in the Herbarium of the same department. Young shoot tips were used as experimental material.

Callus induction: The young shoot tips were used for callus induction and were rinsed three times with sterilized double distilled water and surface disinfected for 10 minute in H_2O_2 (1%) (Hydrogen peroxide) solution. After the completion of rinsing process, the explants were placed on the sterilized blotting paper and finally placed on callus induction medium, the MS medium [12]. The explants were incubated on a basal medium (pH= 5.7) and were grown under 16 h photoperiod provided by 40 W cool-white tubes ($100 \mu\text{mol}^{-2} \text{sec}^{-1}$). The 2, 4-D hormone was used individually in different concentrations for callus induction. The inoculation was done under sterile, aseptic conditions inside the laminar flow cabinet. In the present study, young shoot tips were used for the induction of callus and mature cotyledonary somatic embryos were used for the generation of artificial seeds. The somatic embryos were formed from the callus.

Encapsulation: The Somatic embryos formed from shoot tips via callus of *Dracaena sanderiana* were encapsulated in different concentrations of sodium alginate (CDH, New Delhi, India; 1.5, 2.5, 3.5 and 3.5%) & $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ (75, 100 and 125 m M) solution (Merck, Mumbai, India). Alginate solution was prepared by adding 3% sucrose and different concentrations of sodium alginate (w/v) in double distilled water and later sterilized at 121°C . Embryos were mixed with sodium alginate solution for few seconds, picked up by the pipette and placed in sterile aqueous solution of $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ for up to 15 minutes for hardening, with occasional agitation on a rotary shaker, it resulted in bead formation. Beads were taken out by decanting off the CaCl_2 solution and washed with the sterilized water. Freshly prepared beads were transferred to MS medium fortified with different concentrations of plant growth regulators. The whole procedure was carried out under strict aseptic conditions.

3. RESULTS AND DISCUSSION

The term “callus” originates from the Latin word *callum*, which means hard, and in in medicine it

refers to the thickening of the dermal tissue. “Callus” in the early days of plant biology referred to the massive growth of cells and accumulation of callose associated with wounding. Today the same word is used broadly and disorganized cell masses are collectively called callus. Callus can be produced from a single differentiated cell and many callus cells are totipotent, being able to regenerate the whole plant body [13]. Under certain conditions, callus cells also undergo somatic embryogenesis, a process in which embryo are generated from adult somatic cells [14]. The somatic embryos are the most commonly used plant material in plant tissue culture for synthetic seed formation, it ensures easy root and shoot development [15]. In *Dracaena* the growth regulators and explant are the key factors for the somatic embryogenesis. Generally in plants the rates of embryo induction is very low [16]. Different shape and texture of beads were observed and they were classified as irregular, slightly round, round and fragile, soft, firm, hard respectively. In this study somatic embryos were used for the generation of synthetic seeds Fig. 2. Somatic embryos kept in 2.5% sodium alginate and 100 mm CaCl_2 produced synthetic seeds with firm coat, suitable

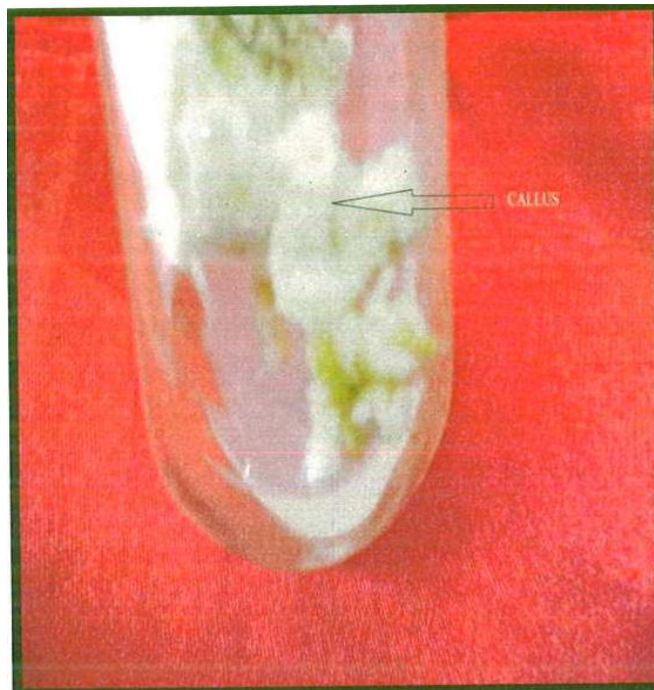


Fig. 1. The photograph showing friable callus formation of *Dracaena sanderiana* after two weeks on MS medium fortified with 0.25 mg 2, 4-D

Table 1. In-vitro response of young shoot tip of *Draceana sanderiana* when cultured on MS medium supplemented with various 2,4-D hormone concentrations. Data scored after three weeks of inoculation

2,4D(mg/dl)	Shoot tip
0.25	Swelling at cut end of shoot tip was found within a week, gradually friable callus was found to engulf the whole surface of the explants
0.5	Callusing rate was very rapid, light yellow friable callus was induced within two weeks of culture
1.0	Explant response was very slow initially, later on amount of callusing was more

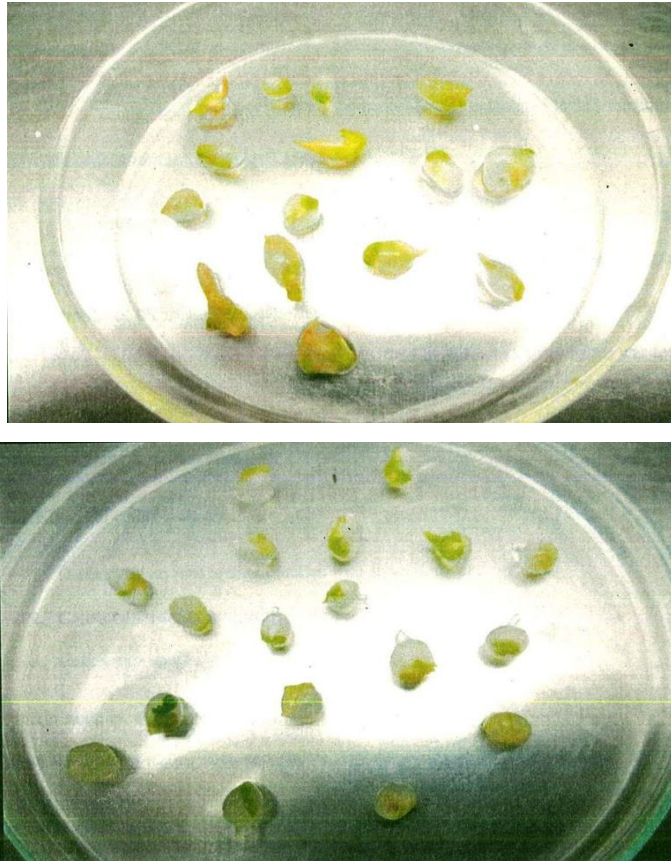


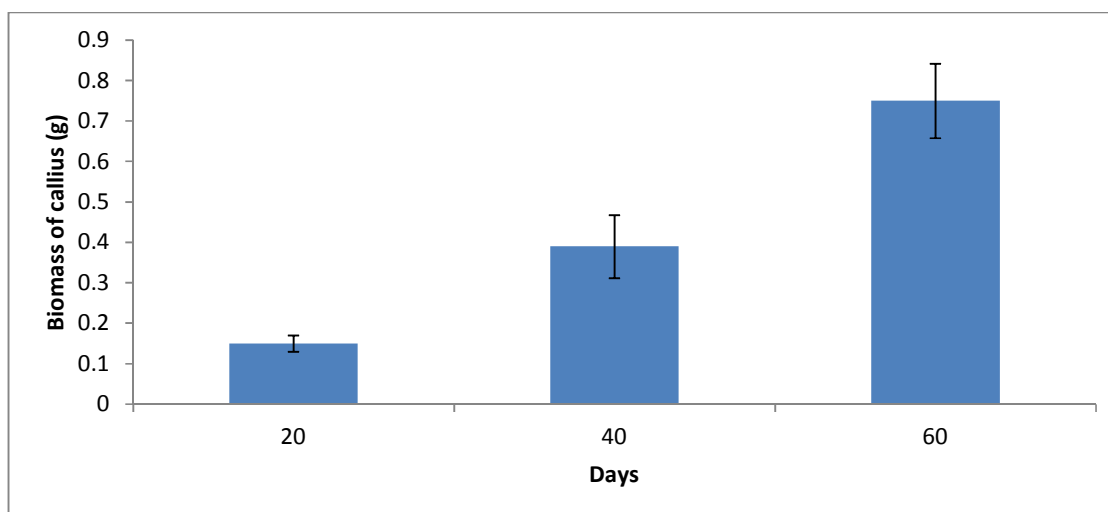
Fig. 2. Photographs showing developed synthetic seeds at 2.5% sodium alginate & 100 mm calcium with firm round and uniform beads in *Draceana sanderiana*

for handling which also favored easy development of plantlets and was found most suitable for the formation of ideal beads. In contrast, at low concentrations of sodium alginate, uniform and sufficiently firm beads were not formed whereas at higher concentration the beads were uniform and round, but hard enough to cause considerable delay in sprouting and reduce germination (Table 4). Several authors [17,18] observed significant difference in the shape and texture of the beads when used low to high percentage of sodium alginate. Encapsulated embryos were also exposed to

different levels of CaCl_2 in order to optimize the right concentrations. The CaCl_2 at 100 mM proved too effective for spherical bead formation as compared to 125 mM. The study also revealed that the embryos kept in CaCl_2 for 15 minutes produced firm round and uniform beads. Embryo exposure below and above 15 minutes induced soft and harder beads respectively. The maximum encapsulation was seen by treating the somatic embryos formed from young shoot tips via callus with 2.5% sodium alginate and 100 mm calcium chloride.

Table 2. Callusing intensity and frequency, when young shoot tip of *Draceana sanderiana* were cultured (cultivated) on various 2, 4-D concentrations

Explant	2,4D(mg/l)	Intensity of callusing	% response
Young shoot tip	0.25	+++	100%
	0.5	++	
	1.0	+	

**Fig. 3. Growth rate of callus (biomass) of *Draceana sanderiana* on MS medium fortified with 0.5 mg/l of 2, 4-D**

Young shoot tips were used for callus formation Fig. 1, inoculated in MS medium with different concentrations of 2, 4-D (0.25, 0.5 and 1.0 mg/l). The three different concentrations of 2, 4-D (auxin) were tested for optimizing the growth rate of callus i.e.; 0.25, 0.5, 1.0 mg/l. The observations revealed that 2, 4-D at concentration of 0.25 mg/l was more suited for

the profuse callus formation (Table 1) and the rate is also shown in the form of a graph. Friable and light yellow callus was induced within 2 weeks of culture. Similarly callusing intensity and frequency with growth rate at 0.25, 0.5 and 1.0 mg/l of 2, 4-D concentrations is as shown in Table (1-3c) and graphical trends is shown in Figs. 3, 4 and 5 respectively.

Table 3 (a). Growth rate of callus (biomass) of *Draceana sanderiana* on MS medium fortified with 0.25 mg/l of 2, 4-D

Fresh weight of callus(g)	
Days	Young shoot tip
20	0.23±0.027
40	0.76±0.088
60	0.89±0.098

Table 3(b). Growth rate of callus (biomass) of *Draceana sanderiana* on MS medium fortified with 0.5 mg/l of 2, 4-D

Fresh weight of callus (g)	
Days	Young shoot tip
20	0.15±0.02
40	0.39±0.078
60	0.75±0.092

Table 3 (c). Growth rate of callus (biomass) of *Draceana sanderiana* on MS medium fortified with 1.0 mg/l of 2, 4-D

Fresh weight of callus	
Days	Young shot tip
20	0.10±0.015
40	0.25±0.028
60	0.66±0.057

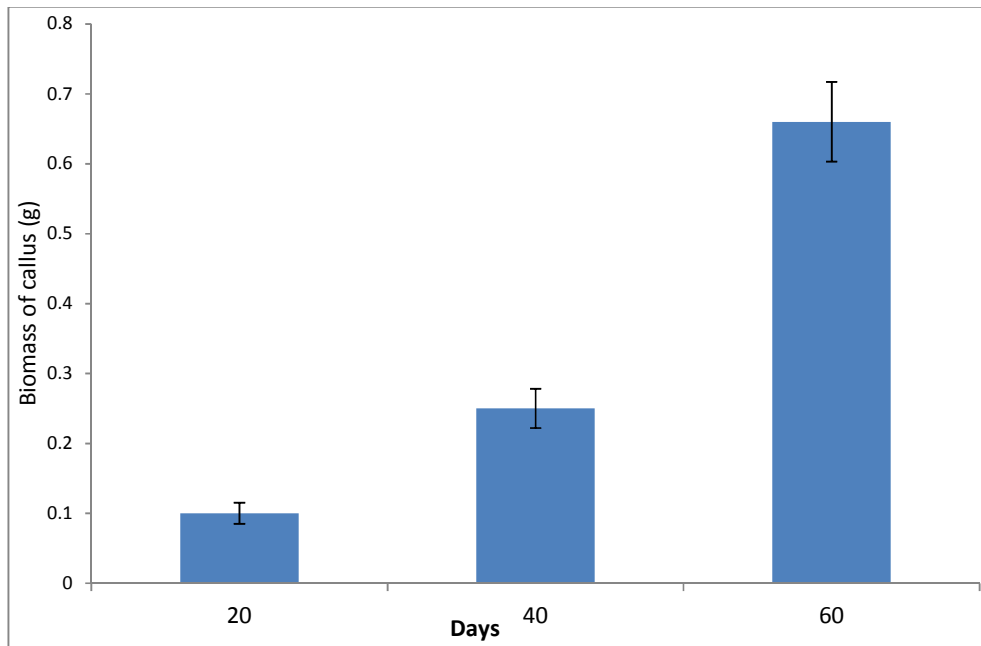


Fig. 4. Growth rate of callus (biomass) of *Draceana sanderiana* on MS medium fortified with 1.0 mg/l of 2, 4-D

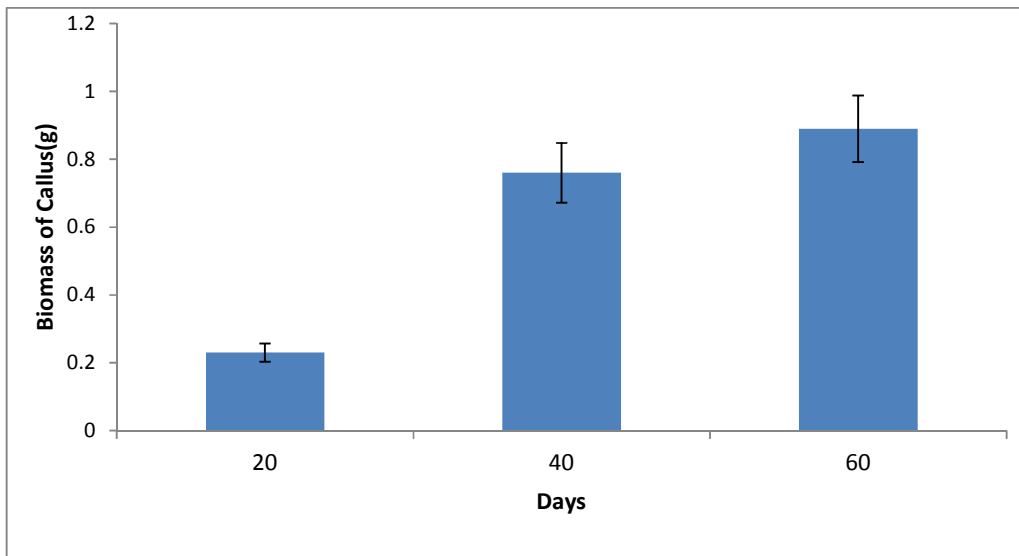


Fig. 5. Growth rate of callus (biomass) of *Draceana sanderiana* on MS medium fortified with 0.25 mg/l of 2, 4-D

Table 4. Physical characteristics of synthetic seed by encapsulation with different concentrations of sodium alginate and calcium chloride in *Dracaena sanderiana*

Sodium alginate (%)	Calcium chloride (mM)	Bead formation
1.5	75	Irregular, fragile Irregular, Soft Irregular,
2.5	100	Slightly round, Firm Round
3.5	125	Slightly round, Hard Round

4. CONCLUSION

Artificial seed development acts as an alternate to organogenesis for regeneration and germplasm preservation of plants. Different concentrations of sodium alginate and calcium chloride have been used for synthetic seed development. The regulatory action of auxin helped *in-vitro* de-differentiation of shoot tips into a friable callus. The same regulatory action of cell auxin over other explants was also reported. The media with 2,4-D hormone gave 100% callus formation irrespective of the explants as reported in *Lenis caulinaris* [13]. Among the three different concentrations of 2,4-D (i.e 0.25, 0.50, and 1.0 mg/l), the 0.25 mg/l concentration gave the most callus biomass production. The maximum encapsulation was seen by embryos with 2.5% sodium alginate and 100 mM CaCl₂. But in plants like mulberry [19] 3.0% sodium alginate along with the 70-80 mM calcium chloride were used for encapsulation and showed maximum conversion capacity.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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