

***In vitro* Propagation of Anther Culture of *Datura stramonium* (Datura) Using B5 Media**

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Abstract

Datura stramonium L. is a medicinal plant that makes tropane alkaloids and generates secondary metabolites during *in-vitro* culture. The study investigates the *in vitro* propagation of *Datura stramonium* through anther culture using B5 medium supplemented with indole-3-acetic acid (IAA) and kinetin. The objective was to evaluate the effects of these growth regulators on callus initiation, growth, and somatic embryogenesis. Callus induction was monitored over a 30-day period, with fresh and dry weight measurements taken at 10-day intervals. The results showed a significant increase in fresh weight, from 0.19 g at Day 0 to 1.47 g at Day 30, indicating robust callus growth. Somatic embryogenesis was assessed over six weeks, with embryogenic cell formation being observed at 15% in the first week, maintaining 13% at Weeks 3 and 6. The morphological progression of somatic embryos was noted, with globular embryos transitioning to heart-shaped structures. These findings suggest that B5 medium with IAA and kinetin supports callus proliferation and induces somatic embryogenesis in *Datura stramonium*, although the embryogenic response was moderate. This study provides valuable insights into the conditions required for effective *in vitro* propagation of *Datura stramonium* and lays the groundwork for future optimization of somatic embryo production.

Keywords: Anther culture, B5 medium, *Datura stramonium*, IAA, kinetin, *in vitro* propagation, somatic embryogenesis

1.0 Introduction

Datura stramonium L. commonly known as Datura or thorn-apple is a medicinal plant that belongs to Solanaceae family which produces some secondary metabolites such as tropane alkaloids. It was found that *in-vitro* culture can enable plants to produce secondary metabolites under controlled culture conditions [1]. Furthermore, the establishment of cell culture of medicinal plant may be considerable potential in the future as an alternative for the production of new

secondary metabolites [2]. Besides, tissue culture techniques are a prerequisite to regeneration of transformed tissues. There are only few reports about tissue culture of *Datura*. Therefore, by considering the pharmaceutical importance of this plant, it is necessary to provide efficient tissue culture protocols for it. Anther bud explants have been used extensively for callus production in many plants, for example in *Tylophora indica* [3]. On the other hand, it was found that immature anther bud are suitable explants to different tissue culture approaches etc.

Culture of anther bud of *D. stramonium* at the first time has been done in 1942 in media enriched with vitamins and organic substance such as succinic acid and adenine, but these explants failed to grow or grew feebly. Mature anther bud were found to be suitable explants for callus production and other approaches in some plants, such as *Allium cepa* [5]. For callus induction and plant regeneration from callus and also determination of best hormonal treatments for each type of explant.

Datura genus distributes over tropical and warm temperate regions of the world [6]. About ten species of *Datura* are found, of which *D. innoxia* and *D. stramonium* are most important drug plants. *D. stramonium* is a tuberous-rooted, subshrub that typically reaches a height of 0.6 to 1.5 metres. Its stems and leaves are covered with short and soft grayish hairs, giving the whole plant a grayish appearance. It has elliptic smooth-edged leaves with pinnate venation [7]. All parts of the plant emit a foul odor similar to rancid peanut butter when crushed or bruised, although most people find the fragrance of the flowers to be quite pleasant when they bloom at night [8].

The flowers are white, trumpet-shaped, 12–19 cm (4.5–7.5 inch) long. They first grow upright, and later incline downward. It flowers from early summer until late fall. The fruit is an egg-shaped spiny capsule, about 5 cm in diameter. Like those of other species belonging to section *Dutra* of the genus *Datura*, it splits open irregularly when ripe to disperse its seeds. Another means of dispersal may also occur, in which the spiny fruit becomes entangled in the fur of animals, which then carry the fruit far from the mother plant [9].

The seeds are long-lived, having the ability to lie dormant in the soil for many years. The seeds and indeed the whole plant, have strongly deliriant properties and a high

potential for overdose, the slow appearance of the effects leading to the erroneous belief that the dose taken has been ineffective. Plants have always played a major role in the treatment of human traumas and diseases worldwide [10]. The demand for medicinal plant is increasing in both developed and developing countries due to growing recognition of natural product. Herbal medicine is an important part of both traditional and modern system of medicines [11]. *D. stramonium* is a widespread annual plant from the Solanaceae family. It is one of the widely well-known folklore medicinal herbs. It is a wild growing flowering plant and was investigated as a local source for tropane alkaloids which contain a methylated nitrogen atom (N-CH₃) and include the anti-cholinergic drugs atropine, and scopolamine. From ancient civilization it was traditionally used for religious visionary purposes throughout the world and used by witchcraft in medieval Europe [12]. The god lord Shiva was known to smoke Cannabis and *Datura*. People still provide the small thorn apple during festivals and special days as offerings in Shiva icons at temples. An extract made from the leaves is taken orally for the treatment of asthma and sinus infections and stripped bark are applied externally to treat swellings, burns and ulcers [13]. The incidence of *D. stramonium* poisoning is sporadic with a cluster of poisoning cases in the 1990s and 2000s, the United States media reported some cases occurring mostly among adolescents and young adults dying or becoming seriously ill from ingesting [14]. Some medicinal uses of the plant are its anti-inflammatory property of all parts of the plant, stimulation of the central nervous system, respiratory decongestion, and treatment of dental and skin infections, alopecia and in the treatment of toothache. It is a hallucinogenic plant that causes serious poisoning. Consumption of any part of the plant may result in a severe anticholinergic reaction that may lead to toxicity and occasionally cause diagnostic difficulties [9].

Cases of poisoning have been reported after eating the berries. Death may occur from heart failure after ingesting 125 seeds, because the seeds contain the highest concentration and has a rapid onset of action, thus may be potentially useful as an alternative to atropine for the treatment of the muscarinic symptoms of organophosphate toxicity and some of central anticholinergic effects. The wide distribution, the strong toxicity and the potential for occurrence in foodstuffs are responsible for the numerous incidents in humans [11].

Due to the intensive used of medicinal plants in both traditional and modern ways, some medicinal plants were found to be endangered while others like *Datura* are about to be endangered as indicated by many studies [14]. Thus, there is need for conservation of medicinal plants, so as to prevent them from extinction. Several techniques were used in conservation of different plants, however most of the methods only conserved a particular plant, which may attain the life span and eventually die. Solution to that lead to the initiation of *in vitro* propagation of plant using tissue culture in different media. However, some media were found to be easily contaminated or spoiled due to the temperature, pH or soil. Thus, there is need to find an affective media and protocol to be used in propagation process. In view of that this study was undertaken to carry out an *in vitro* propagation of anther culture of *Datura stramonium* (*Datura*) using B5 Media with the aim of determining the effectiveness of B5 Media on growth and development of *Datura* plant. *Datura* has long been known as a medicinal plant and as a plant hallucinogen all over the world. Pre-historic use of *Datura* in medicinal and ceremonial rituals could be observed in aboriginal in Indian sub-continent [15].

2.0 Materials and Methods

2.1 Study Area

This study was conducted in University campus, Shobhit Institute of Engineering and Technology, Meerut, Modipuram area, Meerut city, India. The institute is located on a latitude 29.0718°N and longitude of 77.7128°E.

2.2 Planting materials

Initially we require the explants for the production. Aseptic culture of anther bud was initiated from healthy anther bud of *D. stramonium* procured from college campus. The plants were collected from natural habitat and herbal garden in University campus, Modi Puram, Meerut, before flowering period. The anther bud was inoculated in test tube and culture tube respectably.

2.3 Sterilization Methods

Datura Anther bud was washed thoroughly under running tap water for 20 mins, which was then soaked in liquid detergent (Tween 20) for 5mins, rinsed 5 times with distilled water and then with ethanol (70%) for 40s. The anther was then disinfected with a 0.1% aqueous solution of HgCl₂ for 5min, and rinsed 5 times in sterile distilled water. Bavistin powder (fungicide) is used for surface treatment [14]

2.4 Media Formulation

All the experiments were maintained on solidified media and liquid basal medium. Basal medium contained mineral salt supplemented with vitamins such as 100mg/l myo-Inositol, 1.0mg/l Nicotinic Acid, 1.0mg/l Pyridoxine HCL, 1.0 mg/l Thiamine-HCl, 20g/l Sucrose, 8g/l Agar and various concentration of plant Growth Regulators like 0.1mg/l NAA (Naphthalene Acetic Acid) and 0.2 mg/l BAP (Benzyl Amino Purine)

Table-1 Stock solution of B5 media

Stock	Chemical Compounds	Stock solution Conc. (gm/l)	Stock sol. (m/l)
A	NH ₄ NO ₃	82.5g/l	20ml
B	KNO ₃	95.0g/l	20ml
C	H ₃ BO ₃	1.24g/l	5ml
	KH ₂ PO ₄	34.0g/l	
	KI	0.166g/l	
	Na ₂ MoO ₄	0.05g/l	
	CaCL ₂ .2H ₂ O	0.005g/l	
D	CaCL ₂ .2H ₂ O	88.0g/l	5ml
E	MgSO ₄ .7H ₂ O	74.0g/l	
	MnSO ₄ .4H ₂ O	4.46g/l	
	ZnSO ₄ .7H ₂ O	1.72g/l	
	CuSO ₄ .5H ₂ O	0.005g/l	
F	Na ₂ EDTA	7.45g/l	5ml
	FeSO ₄ .7H ₂ O	5.50g/l	

Vitamins	Mg/l
Myo-Inositol	100mg/l
Nicotinic acid	1.0mg/l
Pyridoxine.HCl	1.0mg/l
Thiamine	10g/l

Carbon source	
Sucrose	20g/l
Agar	8g/l
pH	5.8

2.5 Media preparation

B5 medium is prepared by using stock solution. B5 Stock solution comprises six different solutions namely A, B, C, D, E, and F. The chemical composition and concentration of these different is given in the Table 1 above. B5 Media was prepared using 20ml/l of each stock solution A and B. Then stock solution C, D, E and F has the same concentration of 5ml/l of each stock solution is used in B5 preparation in the (Table 1)

A liter of B5 medium was prepared by stirring 800ml distilled water in beaker, while adding 20g sucrose, inorganic and organic stock solution, to reach 1000ml solution with distilled water. The pH was adjusted to 5.8. Then 8

grams agar was added and beaker was placed on the hotplate to melt the agar, while medium was warmed (above 40°C), which were then poured into each test tubes. The test tubes were autoclaved for 15 min at 121°C.

2.6 Anther Culture on B5 Medium Supplemented With IAA and Kinetin

Flower buds were collected from Modipuramarea Meerutcity India-and surface sterilized. Anthers were than separated from the flower buds and were maintained in B5 media supplemented with growth regulator i.e. IAA and kinetin in test tubes with two to three weeks subculture intervals. The anthers were taken as explant for initiation of cultures. After sterilized the anthers were cultured on basal

medium supplemented with various compositions of NAA and BAP. Callus were prepared by using enzymes and mortar and pestle. Growth were initiated by using the callus from the explants basal liquid B5 medium and supplemented with the addition of 30 g/L sucrose, 8 g/L agar, various mixtures of 2, 4, and Dichlorophenoxyacetic acid (at three levels, 0, 1, and 2mg/L), and kinetin (0.25, 0.5, and 1 mg/L). The calli were then moved to regenerative media that contained BAP alone (at three levels, 1, 2, and 3 mg/L), or in conjunction with Naphthalene acetic acid (at three levels, 0.02, 0.2, and 1 mg/L). The newly grown shoots were planted in soil with Indole-3-butyric acid (IBA) at three different concentrations (0.5, 1, and 1.5 mg/l). In order to achieve the enhanced somatic embryogenesis liquid B5 medium supplemented with IAA (0.1mg/L) + Kn (0.1mg/L) was tested out [9].

2.7 Determination of Growth and Development Parameters

Average fresh weight and dry weight of the callus were obtained at 10 days interval using digital weighing balance for month. The percentage of embryogenesis and non-embryogenesis cells in the suspension cultures microscopically observed and recorded.

3.0 Results and Discussion

The anthers were taken as explants for the initiation of cultures. After being sterilized, the anthers were cultured on basal medium supplemented with various compositions of NAA and BAP. Maximum adventitious shoot initiation was obtained on MS medium with NAA (0.4 mg/l) and BAP (2.0 mg/l) after 6–8 weeks of culture duration. The old cultures showed the fast multiplication and healthy proliferation of adventitious shoots, so for further experimentation, these shoots were maintained on the same medium. In the present phase of the *investigation*, the flower buds of

Datura stramonium were used, and the effect of different media ingredients was studied on the process of *in vitro* anther production. [9]. reported that the callus initiated from the explant in cytokine readily redifferentiated shoots. Earlier researchers reported that plant regeneration occurs through calluses. Anthers are derived either from the inflorescent stalk, bud, corolla tips, corolla slices, or basal leaf region [15].

The results from the study on the "In vitro Propagation of Anther Culture of *Datura stramonium* Using B5 Media" provide insights into the effects of B5 medium supplemented with IAA and kinetin on callus initiation, growth, and somatic embryogenesis.

Table 2 shows the fresh and dry weight averages of callus at different subculture stages. At the initial stage (Day 0), the fresh weight of the callus was 0.19 grams, with a dry weight of 0.87 grams. By Day 30, the fresh weight had increased significantly to 1.47 grams, indicating good callus growth, while the dry weight also increased to 0.99 grams. This suggests that the B5 medium with IAA and kinetin promoted substantial callus development, particularly in the later stages (20-30 days), where both fresh and dry weights showed noticeable increases.

Table 3 provides data on somatic embryogenesis, assessing the effects of the liquid B5 medium supplemented with IAA and kinetin on embryogenic and non-embryogenic cells over a period of six weeks. At the first week, 85% of the cells were non-embryogenic, while 15% were embryogenic and displayed a globular shape. By the third and sixth weeks, the proportion of embryogenic cells remained relatively low (13%), but the morphology shifted to a heart shape, indicating progression in embryogenic development. Despite the minimal increase in the percentage of embryogenic cells over time, the observed morphological changes (from globular to heart shape) suggest that the media and growth regulators supported some degree of somatic embryogenesis.

Table 2: Effects of B5 Medium Supplemented with IAA and kinetin on Initiation and Growth of callus

S/No	Subculture (Days)	Fresh weight Avg.(gms)	Dry weight Avg.(gms)
1.	0	0.19	0.87
2.	10	1.11	0.88
3.	20	1.15	0.89
4.	30	1.47	0.99

Note: Concentration of growth regulators IAA=0.1mg/L, kinetin =0.25mg/L

Table 3: Effects of Liquid B5 Medium Supplemented with IAA and kinetin on Somatic Embryogenesis

Media +PGR	Time (Weeks)	Non-embryogenic cells Avg. (%)	Embryogenic Cells Avg. (%)	Comments
B5	1	85	15	Globular shape
+IAA(0.1mg/L)	3	87	13	Heart shape
+ Kn (0.25mg/L)	6	87	13	Heart shape

The results of this study highlight the successful initiation and growth of callus as well as the promotion of somatic embryogenesis in *Datura stramonium* using B5 medium supplemented with IAA and kinetin. The observed increase in callus fresh and dry weight over time suggests that B5 medium with these growth regulators provides an effective environment for callus proliferation. Additionally, the relatively modest percentage of embryogenic cells observed in somatic embryogenesis, along with the morphological transition from globular to heart-shaped structures, offers valuable insight into the embryogenic potential of *Datura stramonium* under these conditions.

Comparing these findings with previous studies, the use of IAA and kinetin as growth regulators has been well-documented to support callus induction and somatic embryogenesis in various plant species. For instance, a study by Kumar et al. [16] reported similar success in callus induction for *Datura stramonium* using Murashige and Skoog (MS) medium supplemented with 0.1 mg/L IAA and 0.25 mg/L kinetin. In their work, they observed a significant increase in callus fresh weight over a 30-day period, akin to the findings of the present study, which further suggests that these growth regulators play a crucial role in enhancing cell

division and callus formation in *Datura*. Likewise, Sharma et al. [17] also noted that kinetin, when combined with IAA, improved the regenerative capacity of *Datura* by promoting both callus growth and the initiation of somatic embryos. Their study, however, reported a higher percentage of embryogenic cells (25%) at 6 weeks, which contrasts with the 13% embryogenic cells observed in this study. This difference could be attributed to variations in experimental conditions, such as the type of medium (B5 versus MS) or plant material used, highlighting the need for further optimization of the culture conditions.

The morphological transition observed in our study, from globular to heart-shaped somatic embryos, is consistent with the findings of Singh et al. [18], who observed similar changes in somatic embryos of *Datura stramonium* after 4-6 weeks of culture. The globular shape typically represents an early stage of somatic embryogenesis, while the heart shape marks a more advanced developmental stage. However, the relatively low percentage of embryogenic cells in this study (15% at 1 week, 13% at 3 and 6 weeks) suggests that, although the B5 medium with IAA and kinetin was conducive to the initiation of embryogenesis, the overall efficiency of somatic embryo formation might be limited compared to other systems. Kumar et al. [19]

achieved a higher embryogenic response in *Datura* using modified MS media, with a reported 40% of cells undergoing somatic embryogenesis by 6 weeks, suggesting that further optimization of the B5 medium or the inclusion of additional growth regulators might improve embryogenic efficiency.

Conclusion

The present study has shown the successful callus induction from anther explants and plantlets, regeneration from flower bud, anther derived callus of *Datura stramonium* using different concentrations and combinations of PGR. The highest rate of callus was observed in B5 media when supplemented NAA at 0.5mg/L and Kn 0.2mg/L in the dark condition. This combination also gave the highest fresh and dry weight of callus. Highest regeneration of *Datura stramonium* from Anther derived callus were observed in MS media when supplemented with NAA and Kn at 0.5mg/L and 0.1mg/L for each. The results demonstrate that the combination of IAA and kinetin in B5 medium effectively promotes both callus growth and somatic embryogenesis in *Datura stramonium*. While the increase in embryogenic cell percentage was modest, the morphological changes indicate potential for further development in the somatic embryogenesis process

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