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In vitro Propagation of Anther Culture of *Datura* (*Datura stramonium*. L) Using MS Media

Jamilu Danjuma Koko[a], Kasimu Shehu[a], Adamu Aliyu Aliero[b], Muhammad Danladi Abubakar Bunza[a], and Ja'afar Umar[a]
[a]Federal University Birnin Kebbi. Nigeria P.M.B. 1157 Birnin Kebbi and [b]Usmanu Danfodiyo University Sokoto, Nigeria P.M.B. 2346 Sokoto.

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***Corresponding Author Email:** danjumajamilu2@gmail.com

Abstract

Datura stramonium L. is a medicinal plant that makes tropane alkaloids and generates secondary metabolites during *in-vitro* culture. The study aimed at using standardizes explant (anther) surface sterilization to generate a virus free plant. A different culture of *Datura stramonium* was *in-vitro* propagated in MS basal medium with the addition of 30 g/L sucrose, 8 g/L agar, various mixtures of 2, 4, and Dichlophenoxyacetic acid (at three levels, 0, 1, and 2 mg/L), and kinetin (0, 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 NAA (at four levels, 0, 0.02, 0.2, and 1 mg/L). The newly grown shoots were planted in soil with IBA at three different concentrations (0.5, 1, and 1.5 mg/l). The best media for inducing callus from anthers as explants were discovered to contain 2 mg/L 2, 4-dihydroxyacetic acid + 0.5 mg/L kinetin and 2 mg/L of 2, 4-D alone. Furthermore, the best hormonal treatments for shoot regeneration from calli of anther explants were discovered to be 3mg/L BAP+ 1mg/L NAA media and 2mg/L BAP+ 1mg/L NAA media. Additionally, the best method for rooting regenerated stems was found to be 0.5 mg/L IBA. Further research on leaves for generating a virus free plant should be conducted.

Keywords
Datura stramonium, Callus, Regeneration, 1-Naphthaleneacetic, 6- Benzyl aminopurine

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INTRODUCTION:

A member of the Solanaceae family, Datura (*Datura stramonium* L.) is a medicinal plant that makes tropane alkaloids and other secondary compounds. It was discovered that under carefully controlled culture conditions, in-vitro culture can allow plants to generate secondary metabolites. Additionally, the development of medical plant cell cultures may have enormous promise as a substitute for the production of novel secondary metabolites in the future [1]. Additionally, tissue regeneration of transformed tissues requires the use of tissue culture methods. There are not many studies on Datura tissue culture. As a result, effective tissue culture methods for this plant are required given its significance in the pharmaceutical industry. Numerous plants, including Tylophora indica, have used anther bud explants to produce calluses. [2]. On the other hand, it was discovered that immature anther buds are suitable explants for various tissue culture approaches, such as callus induction or direct shoot regeneration in soybean (Barwal et al., 1996), somatic embryogenesis in Quercus acutissima. Melia azadirachta. Acacia, etc. In some plants, like *Allium*

cepa, mature anther buds have been found to be appropriate explants for callus production and other methods [3]. for determining the best hormonal treatments for each variety of explant, callus induction, and plant regeneration from callus. A tuberous-rooted subshrub with a usual height range of 0.6 to 1.5 meters is called Datura stramonium. The entire plant has a grayish appearance due to the short, soft hairs that coat its stems and leaves. It has elliptic leaves with straight edges and pinnate venation. Although most people find the fragrance of the flowers quite pleasant when they bloom at night, all parts of the plant emanate an unpleasant odor comparable to rancid peanut butter when crushed or bruised. The flowers are 12–19 centimeters (4.5–7.5 inches) long, white, and trumpet-shaped. They develop upright at first, then slant downhill [4].

From early summer to late autumn, it blooms. The fruit is a 5-cm-diameter egg-shaped, spiny shell. When ripe, its seeds break open irregularly, just like those of other species in the genus Datura's Section Dutra. Another method of fruit dispersal involves the spiny fruit becoming tangled in an animal's fur, which causes the animal to transport the fruit far from the mother plant. The seeds have a lengthy lifespan and can slumber in the soil for many years. The seeds, as well as the entire plant, have potent delirious effects and a high risk of overdose; the effects take time to manifest, which can lead one to mistakenly believe that the amount they took was ineffective [5].

The numerous human events are caused by the widespread distribution, high toxicity, and likelihood of occurrence in foods. The Datura genus is found in both tropical and mild temperate climates. There are about ten varieties of Datura, the two most significant drug plants being D. innoxia and D. stramonium. Datura is well-known throughout the globe as a medicinal plant and as a plant hallucinogen. The indigenous people of the Indian subcontinent were known to use Datura in ceremonial and medicinal practices during prehistoric times. Alkaloids, tannins, saponins, and cardiac glycosides are a few examples of these compounds whose existence confers therapeutic benefits on most plants. Saponins, tannins, steroids, alkaloids, flavonoids, phenols, and glycosides were found by the phytochemical analysis. As competing antagonists of muscarinic cholinergic receptors, atropine and scopolamine depress the central nervous system. Although the entire plant is poisonous, the mature seeds contain the most alkaloids. When these plants were unintentionally eaten, D. stramonium poisoning has been responsible for numerous instances of accidental poisoning [6].

Plants have always played a major role in the treatment of human traumas and diseases worldwide. The demand for medicinal plants is increasing in both developed and developing countries due to the growing recognition of natural products. Herbal medicine is an important component of both traditional and modern medical systems [7]. ***Datura stramonium*** is a widespread annual plant from the Solanaceae family. It is one of the best-known folklore medicinal herbs. It is a wild-growing flowering plant that 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 civilizations on, it was traditionally used for religious visionary purposes throughout the world and by witchcraft in medieval Europe. The god Shiva was known to smoke cannabis and datura. The small thorn apple is still used as an offering in Shiva icons at temples during festivals and special days. An extract made from the leaves is taken orally for the treatment of asthma and sinus infections, and strips of bark are applied externally to treat swellings, burns, and ulcers.

The purpose of this study is to use MS media to propagate an anther culture of *Datura stramonium L in vitro.* The goals are to: standardize explant surface sterilization, initiate direct haploid embryo and callus formation from anther, multiply direct haploid embryo and shoot, germinate direct haploid embryo and root multiple shoots, and produce haploid plants on a large scale [8].

MATERIALS AND METHODS:

In-vitro propagation of anthers from ***Datura stramonium*** buds requires different strategies like planting layout, site for planting, storage, subculture, etc. Now we will discuss some of the methods and materials required for the in vitro production of plants from Datura stramonium anther buds. Temperature, pH, and soil all play a role in the production of new plants from anther buds.

Planting Materials

Initially, we require the explants for production. An aseptic culture of an anther bud was initiated from a healthy anther bud of ***Datura stramonium*** procured from Birnin Kebbi. The plants were collected from their natural habitat, before flowering. The anthers were inoculated in a test tube and a culture tube, respectively.

Sterilization Methods

The Datura anthers were thoroughly washed under running tap water for 20 minutes, soaked in liquid detergent (klin) for 5 minutes, rinsed 5 times with distilled water, and then soaked in 70% ethanol for 40 seconds. After two rinses with distilled water, the anther was disinfected with a 0.1% aqueous solution of $HgCl_2$ for 5 minutes and rinsed 5 times in sterile distilled water. ***Datura*** anthers are free from contamination by bacteria or fungi. Bavistin powder (a fungicide) is used for surface treatment.

The MS media formulation was investigated to determine its effect on various parameters:

Media Formulation

Solidified media and liquid basal media were used in all experiments. Basal medium contained Murashige and Skoog's (MS) 1962 mineral salt supplemented with vitamins such as 100 mg/l myo-inositol, 2 mg/l glycine, 0.5 mg/l nicotinic acid, 0.5 mg/l pyridoxine HCL, 0.1 mg/l thiamine HCl, 30 g/l sucrose, 8 g/l agar, and various concentrations of plant growth regulators like 0.1 mg/l NAA (Naphthalene Acetic Acid) and 0.2 mg/l BAP (Benzyl Amino purine).

Table-1 Stock solution of Murashige and Skoog (1962) media

Stock	Chemical Compound	Stock Solution Conc. (Mg/L)	Stock Sol. (m/l)
A	NH_4NO_3	82.5g/l	20ml
B	KNO_3	95.0g/l	20ml
C	H_3BO_3	1.24g/l	5ml
	KH_2PO_4	34.0g/l	
	KI	0.166g/l	
D	Na_2MoO_4	0.05g/l	5ml
	$CaCL_2.2H_2O$	88.0g/l	
	$MgSO_4.7H_2O$	74.0g/l	
E	$MnSO_4.4H_2O$	4.46g/l	5ml
	$ZnSO_4.7H_2O$	1.72g/l	
	$CuSO_4.5H_2O$	0.005g/l	
F	Na_2EDTA	7.45g/l	5ml
	$FeSO_4.7H_2O$	5.50g/l	

VITAMINS	Mg/l
Inositol	100mg/l
Nicotinic acid	0.5mg/l
Pyridoxine HCl	0.5mg/l
Thiamine	0.1mg/l
Glycine	2.0mg/l

CARBON SOURCE	Mg/l
Sucrose	30000mg/l
Agar	8000mg/l
pH	5.8

MS medium is prepared by using stock solution. The stock MS solution comprises six different solutions, namely A, B, C, D, E, and F. The chemical composition and concentration of these different substances are given in Table 1 above. During MS Media preparation, 20 mL/L of each stock solution A and B are used. Then, in Table 1, stock solutions C, D, E, and F have the same concentration of 5ml/l and are used in MS preparation.

Use of Stock Solutions

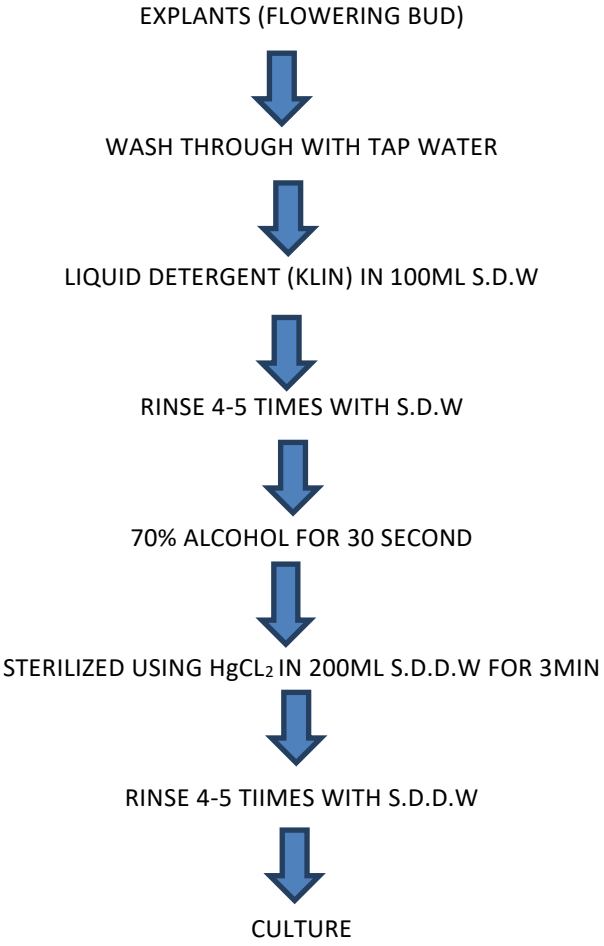
- For making a liter of MS medium, start with 700 to 800 ml of distilled water in a beaker.
- Place a stir bar in the beaker and stir while adding 30 g sucrose.
- Added inorganic and organic stock solutions.
- I brought the volume to near 1000 mL with distillate water.
- Adjust the pH to 5.8.
- Bring the volume of the medium to 1 liter using a graduated cylinder.
- Added 8 grams of agar.

- Place the beaker on the hotplate to melt the agar.
- While the medium is warmed (above 40^{O}C), pour the medium into each test tube.
- Autoclaved the test tubes for 15 minutes at 121^{0}C.

Anther Culture on MS Medium Supplemented with IAA and Kinetin

Flower buds were collected from Birnin Kebbi and surface sterilized. Anthers were then separated from the flower buds and maintained in MS media supplemented with growth regulators, i.e., IAA and kinetin, in test tubes with two- to three-week subculture intervals.

FLOW CHART FOR SURFACE STERILIZATION



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 re-differentiated shoots. Earlier researchers reported that plant regeneration occurs through calluses. Anthers are derived either from the inflorescent stalk, bud, cormel tips, cormel slices, or basal leaf region. [10].

EFFECTS OF LIQUID MS MEDIUM SUPPLEMENTED WITH IAA AND KINETIN ON SOMATIC EMBRYOGENESIS

Somatic embryogenesis was successfully achieved on liquid MS medium supplemented with IAA (0.1 mg/L) and Kn (0.1 mg/L). The percentage of embryogenic and non-embryogenic cells in the suspension cultures was microscopically observed within two weeks of culturing. The cultures were routinely sub cultured on the same medium every three weeks. The data was recorded for up to 16 weeks of culturing. The further development of advanced heart-stage embryos will be seen in the next couple of weeks.

TABLE2: **Effects of Liquid MS Medium Supplemented With IAA And Kinetin On Somatic Embryogenesis.**

Media +PGR	Time(Days)	Non-embryogenenic cells Avg.(%)	Embryogenic Cells Avg.(%)	Comments
MS + IAA(0.1mg/L) + Kn (0.1mg/L)	8	68	33	Early globular stage To Advanced globular stage found
	12	78	23	Advanced globular stage To Early heart shape stage found
	16	73	28	Early heart shape stage To Advanced heart shape stage found

EFFECTS OF LIQUID MS MEDIUM SUPPLEMENTED WITH IAA AND KINETIN ON SOMATIC EMBRYOGENESIS

Liquid MS medium supplemented with IAA (0.1 mg/L) and Kn (0.25 mg/L) was not found satisfactory as compared to IAA (0.1 mg/L) and Kn (0.01 mg/L) for the induction of somatic embryogenesis. With increases in the concentration of kinetin, somatic embryogenesis was found to be diminished. After [16] weeks of culturing and sub-culturing the embryogenesis cells, they were seen to have achieved the globular stage only. (Table 2)

TABLE3: **Effects of Liquid MS Medium Supplemented With IAA And Kinetin On Somatic Embryogenesis.**

Media +PGR	Time (Days)	Non-embryogenenic cells Avg. (%)	Embryogenic Cells Avg. (%)	Comments
MS + IAA(0.1mg/L) + Kn (0.25mg/L)	8	95	5	No stage found
	12	96	4	Globular stage found
	16	96	4	Globular stage found

PLATE-1

Fig.1 Anther explants of *Datura stramonium*

PLATE-2

Fig.2 Maturation and Multiplication of direct somatic embryos from anther culture after 6-8 weeks

PLATE-3

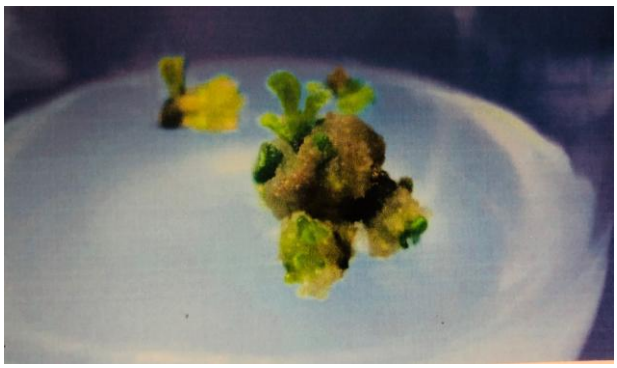


Fig.3 Organogenesis from callus of *Datura stramonium*

PLATE-4

Fig.4 Multiplication of Shoot on Agar Media

PLATE-5

Fig.5 Multiplication of shoots of haploid culture of Datura *Stramonium*

PLATE-6

Fig-6 Multiplication of shoots of haploid culture of Datura *Stramonium*

Fig.7 –Rooted Haploid Plantlet of *D*atura *stramonium*

CONCLUSION:

The present study has shown successful callus induction from anther explants and plantlets, regeneration from flower buds, and anther-derived callus formation in ***Datura stramonium*** using different concentrations and combinations of PGR. The highest rate of callus formation was observed in MS media when supplemented with NAA at 0.5 mg/L and Kn at 0.2 mg/L in the dark condition. This combination also gave the highest fresh and dry weight of callus. Highest regeneration of ***Datura stramonium*** from anthers-derived callus was observed in MS media when supplemented with NAA and Kn at 0.5 mg/L and 0.1 mg/L for each.

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