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Effects of plant growth regulators on callus induction from leaf explants of *Cleome viscosa*

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ABSTRACT

The aim of this study was to establish an effective protocol for callus induction from the leaf explants of the *Cleome viscosa*. *Cleome viscosa* Linn. (Capparidaceae) is a widely distributed herb with yellow flowers and long slender pods containing seeds. This is the important medicinal herb. Tender leaves, stems, pods and flowers are used as vegetables with high nutrition source like beta-carotene, vitamin-C and moderate levels of calcium, magnesium and iron. It has tremendous medicinal values against diphtheria, vomiting, headache, pneumonia, septic ears, stomach ailments and also used to increase the blood formation and antimicrobial activities. The surface sterilization of explants (leaf) was observed from 3% hydrogen peroxide, 0.1%mercuric chloride and 70% ethanol. Callus formation was observed from five different culture media (B5, WPM, MS, SH, and Y3) devoid of plant growth regulators. Among those five culture media the MS medium was maximum of 59% callus response with an average of 84.1±0.55mg fresh mass was observed from leaf explants cultured on MS medium than others. Callus induction - Maximum regenerative callus biomass in the leaf explants was observed from IAA (2mg/l). An efficient callus formation protocol was developed for medicinal plant *Cleome viscosa* by *in vitro* culture of leaf part of mature plant.

Keywords: Capparidaceae, Callus induction, IAA, Surface sterilization and medicinal plants.

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INTRODUCTION

In India *Cleome viscosa* Linn. (Capparidaceae), commonly known as "wild or dog mustard," is an annual, sticky herb found as a common weed all over the plains of India and throughout the tropics [9]. *Cleome viscosa* originate from Ethiopia, Somalia, and through Eastern and Central Africa. Tender leaves, stems, pods, and flowers are consumed as vegetables. It is known to contain high level of beta carotene, vitamin C, and moderate levels of calcium, magnesium and iron Leaves and young shoots - cooked as a vegetable. A sharp mustard like flavour. The pungent seed can be pickled or used as a mustard substitute in curries. The seedpods are made into pickles. The juice of the plant is used as a condiment. Oil obtained from the seeds is used for cooking [15]. *Cleome viscosa*, an annual herb locally known as jakhiya, grows naturally from seed in rain fed agricultural land and abandoned crop fields at altitudes ranging from 500 to 1500 m in scattered pockets of the Garhwal Himalaya. The seeds are mostly used as condiment. This species is a good substitute of cumin (*Cuminum cyminum*). Traditionally it is also used to cure a variety of diseases [8]. Medicinal plants, herbs and fruits with a high content of bioactive compounds and related antioxidant capacity are inversely associated with morbidity and mortality from atherosclerosis [4]. Many higher plants are major sources of natural products used as pharmaceuticals, agrochemicals, flavours, and fragrances ingredients, food additives, and pesticides. It has been mentioned that natural habitats for medicinal plants are disappearing fast and together with environmental and geopolitical instabilities [2]. Fresh leaves of this plant are used very effectively for the treatment of jaundice in the folk medicines of the Bundelkhand region of India. The hepatoprotective activity of the ethanolic extract of leaves was investigated against thioacetamide induced hepatotoxicity in rats [3]. Leaves and flowers of *Cleome viscosa* used for antimicrobial and antifungal activity against member of pathogenic bacterial and fungal organisms. According to ethno-pharmacological surveys, spider plant has a number of medicinal uses. In Uganda, the plant is used to induce labor during childbirth. After giving birth some women consume spider plant to increase blood formation. Spider plant remedies are used to alleviate migraine, vomiting, diphtheria, vertigo, headache, pneumonia, septic ears, and stomach ailments; the plant also used as eyewash and fed to boys after circumcision and also it has insecticidal and insect repellent properties. Spraying an aqueous extract of spider plant can considerably reduce aphid and thrip populations. Intercropping spider plant with cabbage also reduces diamondback moth as well as thrip attacks [13]. Biotechnology is one of the important tool for select, multiply and conserve the critical genotypes of Medicinal plants. *In vitro* regeneration holds tremendous potential for the production of high-quality plant-based medicine. *In vitro* production of secondary metabolites in plant cell suspension cultures has been reported from various Medicinal plants [15]. Plant tissue culture is the aseptic (free from microorganism) culture of any plant part *in vitro*. Tissue culture is utilized in the field of Biotechnology. Plant tissue culture has the potential to introduce genetic variability in genotypes through somaclonal variants, somatic hybrids or transgenic plants. However a prerequisite to applied plant biotechnology is the development of a suitable and reproducible plant regeneration system under least cost [12]. The induction of callus growth and subsequent differentiation and organogenesis is accomplished by the differential application of growth regulators and the control conditions in

the culture medium. With the stimulus of endogenous growth substances or by addition of exogenous growth regulators to the nutrient medium, cell division, cell growth and tissue differentiation are induced. There are many reports on the regeneration of various medicinal plants via callus culture and also organogenesis is one of an efficient method for mass multiplication of medicinal plants [15]. Due to increasing commercial demand of *Cleome viscosa*. Based on the above reason, and the vast usage of the *Cleome viscosa* and medicinal importance, the present study was undertaken to develop an efficient protocol for rapid callus induction of *Cleome viscosa* using leaf explants as an initial plant material. *In vitro* culture of *C.viscosa* has not, to our knowledge, been previously reported. The importance of this plant from medicinal point of view and its exploitation will leads to decline in its population. Therefore the present investigation, effects have been made for the development of its *in vitro* regeneration protocol.

MATERIALS AND METHODS

Plant materials collection

Cleome viscosa Linn. (Capparidaceae) an important medicinal herb was used in the present study. The plants were collected from in and around Virudhunagar district, Tamilnadu. Small young leaves were collected from healthy plants and field grown mature plants of *Cleome viscosa* L defoliated and sectioned into many segments as an explants.

Sterilization of glassware

Success of tissue culture is determined by the sterilization procedure adopted. All the glassware's such as, beaker, conical flask, measuring cylinders, Petridish and culture tubes were washed thoroughly with detergent (Teepol) in tap water and rinsed with double distilled water twice and the glassware need to be autoclaved before washing to remove agar medium and to destroy the various bacterial and other pathogenic organisms and their spores and dried in oven at 30°C. The distilled water and other accessories such as, forceps, blade holder, cotton plugs etc., were autoclaved at 121°C for 15 – 20 minutes. After autoclaving, they were kept in an oven until use.

Aseptic transfer of tissue was done in a laminar air – flow hood. The interior was swabbed with 95% ethanol before inoculation. The autoclaved instruments were flame sterilized 3 times before using them for tissue transfer using 95% ethanol taken coupling jar.

Surface sterilization of explants (leaf)

The leaves of *Cleome viscosa* were collected. The collected explants were washed with running water for 3 times. Followed by explants were treated with 0.1% mercury chloride for 1.5 minutes and washed with sterilized double distilled water for 3 times. The small washed explants were treated with 3% hydrogen peroxide for 1.5 minutes and then washed with

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sterilized double distilled water for 3 times. At final the explants were treated with 70% ethanol for 0.5 minutes and then washed with sterilized double distilled water for 3 times.

Preparation of medium

The medium comprised of macro and micro elements according to Murashige and Skoog (1962) with myoinositol (100 mg/l), thiamine, HCl (0.1 mg/l), Pyridoxine (0.5 mg/l), nicotinic acid (0.5 mg/l) and sucrose (30 g/l), solidified with 0.8% agar. The plant growth regulators used were Indole–3butyric acid (IBA), Indole–3acetic acid (IAA) and 2–Napthalene acetic acid (NAA). All experiments were carried out in culture tubes (25×150mm) containing 10ml of culture medium. The pH of the medium was adjusted to 5.8 prior to autoclaving at 121ºC for 20 min. Cultures were incubated under 16 hrs/8 hrs light/dark cycles.

Effect of media on callus induction

In vitro sterilized leaf explants were cultured on test tube containing 10ml of different media such B5, WPM, MS, SH and Y3. All the cultures were incubated at 25±2 ºC under continuous irradiation with a white fluorescent tube (30µmol $m^{-2}s^{-1}$) for a photoperiod of 16h light per day. A total of 40 explants was used for the experiment and was repeated 5 times. The culture conditions remained the same for all experiments unless otherwise specified. Data on percent of response with the amount of callus formation per explants was recorded after 30 days of culture.

Callus induction

The small segments of sterilized leaf explants were cultured on MS medium fortified with different auxins such as, IAA, NAA and IBA ranging from 0.5 – 4.0mg/l for callus initiation. Explants derived calli from leaf were subculture after two weeks of time intervals. A total of 40 explants were taken for each experiments and were repeated 5 times. Data on percent of callusing response with the amount of callus formation per explants was observed after 30 days of culture.

Statistical analysis

The experimental design was completely randomized design and factoral with auxins as independent variable. All the experiment was repeated 5 times. The data pertaining to frequency of callus induction per culture was subjected to standard deviation and mean separation was carried out using computer software.

RESULT AND DISCUSSION

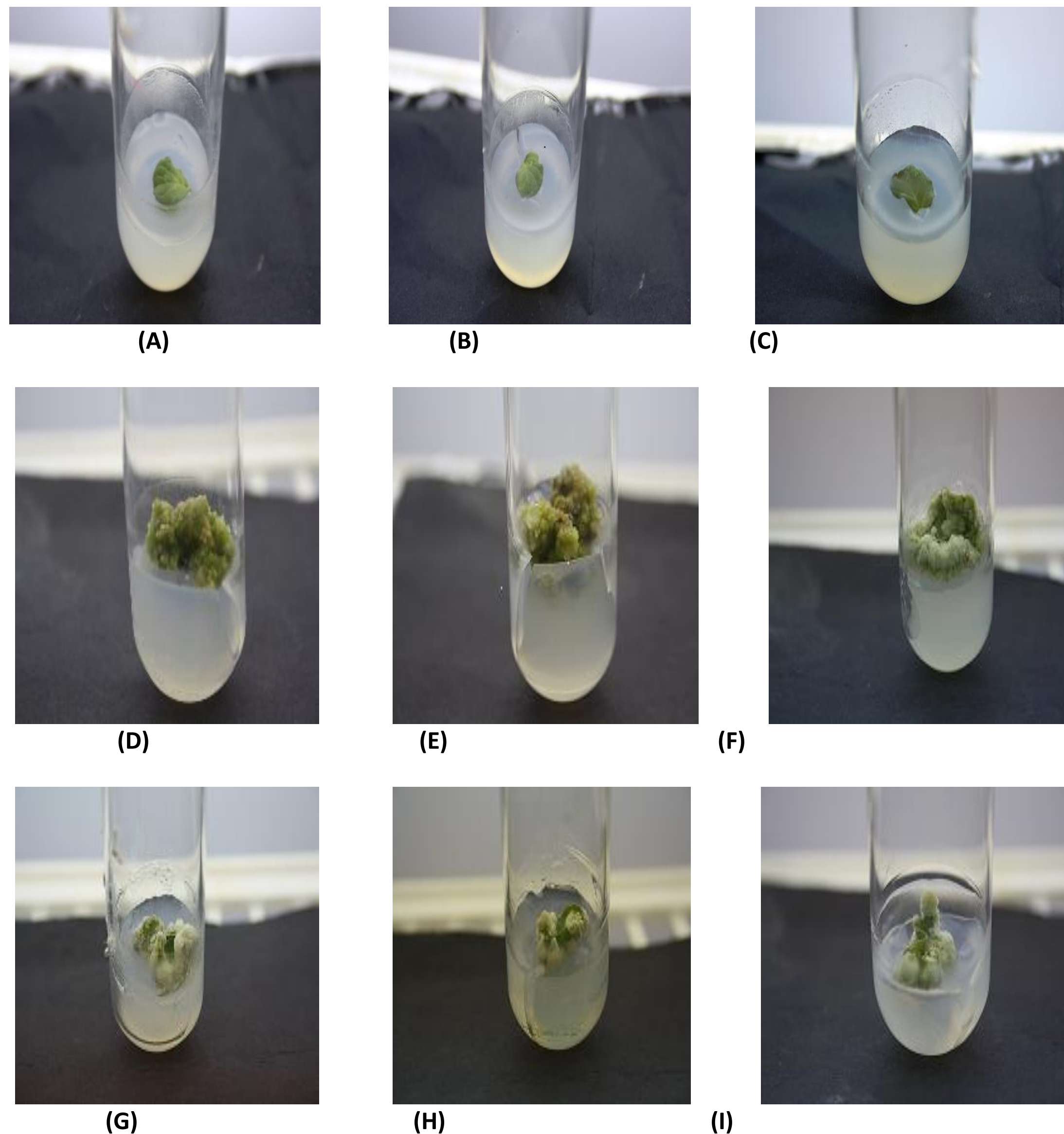

Figure 1. (A) Callus initiation in *Cleome viscosa* from leaf explants in MS media containing 2mg/l IAA. (B) Callus initiation in *Cleome viscosa* from leaf explants in MS media containing 2mg/l IBA. (B) Callus initiation in *Cleome viscosa* from leaf explants in MS media containing 2mg/l NAA. (D) Green nodular calli formation in *Cleome viscosa* from leaf explants in MS media containing 2mg/l IAA. (E) Green nodular calli formation in *Cleome viscosa* from leaf explants in MS media containing 3mg/l IAA. (F) White green compact calli formation in *Cleome viscosa* from leaf explants in MS media containing 2mg/l IBA. (G) White green compact calli formation in *Cleome viscosa* from leaf explants in MS media containing 3mg/l IBA. (H) White yellow friable calli formation in *Cleome viscosa* from leaf explants in MS media containing 3mg/l IBA. (I) White yellow friable calli formation in *Cleome viscosa* from leaf explants in MS media containing 3mg/l IBA.

It has been proved that the surface sterilization of explants is very essential for establishment, as well as for optimum induction of callus induction in *in vitro* condition [16]. In the present study 3% hydrogen peroxide, 0.1% mercuric chloride and 70% ethanol was used to

sterilize the leaves **(Table-1)**. Leaves were surface sterilized with 0.1%mercuric chloride for 1.5minutes, 3% hydrogen peroxide for 1.5minutes and 70% ethanol for 0.5minutes resulted the maximum growth without contamination. Similarly, [11] reported that the cultured explants showed more than 80% contamination free cultures when treated with 0.1%$HgCl_2$ for 2minutes for surface sterilization.

Table 1. Surface sterilization of leaf explants

S.No	Disinfectant	Concentration (%)	Exposure (min)
1	Mercuric chloride	0.1	1.5
2	Hydrogen peroxide	3	1.5
3	Ethyl alcohol	70	0.5

Table 2. Effect of different media on callus induction from leaf of *Cleome viscosa*

S.No	Media	Leaf	
		% of response	Callus growth (mg fresh mass)
1	B5	42	37.4±0.55
2	WPM	36	31±0.71
3	MS	59	84.1±0.55
4	SH	00	0.0±0.0
5	Y3	00	0.0±0.0

In the present investigation, *In vitro* callus were directly initiated from leaf explants of *Cleome viscosa* cultured on different culture media (B5, WPM, SH and Y3) devoid of plant growth regulators. Different authors have used different explants; some reports indicate that leaf explants are the best for callus induction [7]. The results indicated that about 42 and 36% callus response with minimum amount of callus formation was observed in leaf explants cultured on B5 and WPM media respectively. However, maximum of 59% callus response with an average of 84.1±0.55mg fresh mass was observed from leaf explants cultured on MS basal medium **(Table-2)**. Babu *et al*., (2003) reported that WPM medium was the best for inducing callus for producing multiple shoot from leaf explants of *Cinnanomum camphora.* However, the MS medium found to be the best for inducing callus from leaf explants. Earlier studies also confirmed that MS medium was highly preferred for plant regeneration of many tree species [5]. Khan *et al*., (2008) reported that an important attempt has been made to analyze the response of two different media; Murashige and Skoog and Woody plant medium for callus induction. Among these two media MS medium showed maximum response of callus induction.

The MS basal medium was the most effective for callusing of leaf explants. The explants cultured on MS basal medium supplemented with different auxins of IBA, NAA and IAA showed varied response for callusing. Among the three types of auxins IBA and IAA found to be very effective (Singh *et al*., 2009). However in the present investigation calli were initiated from leaf explants of *Cleome viscosa* after two weeks of culture on MS medium fortified with different auxins IBA (2mg/l), NAA (2mg/l) and IAA (2mg/l). About 81% callusing response with maximum of 178mg fresh mass was observed in leaf explants cultured on MS basal medium

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supplemented with IAA (2mg/l). The optimum concentration of IBA (2mg/l) showed 70% callusing response in leaf explants. Low callusing response in leaf explants cultured on NAA (2mg/l) fortified medium **(Table-3)**.

Table 3. Effect of different concentration of auxins on callus induction from leaf of *Cleome viscosa*

Growth regulators (mg/l)	% of response	Callus growth (mg fresh mass)	Callus morphology
IAA 0.5	60	129±0.83	Green nodular calli
1	65	147±0.45	Green nodular calli
2	81	178±0.71	Green nodular calli
3	80	156±0.55	Green nodular calli
4	75	140±0.89	Green nodular calli
IBA 0.5	65	122±0.54	White green compact calli
1	60	109±0.71	White green compact calli
2	70	110±0.57	White green compact calli
3	60	114±0.84	White green compact calli
4	60	80±0.99	White yellow compact calli
NAA 0.5	45	84±0.55	White yellow friable calli
1	50	103±0.83	White yellow friable calli
2	55	107±0.57	White yellow friable calli
3	40	100±0.45	White yellow friable calli
4	15	39±0.89	White yellow friable calli

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