In vitro studies on seed germination and callus induction on *Cassia alata* Linn.

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Abstract
Cassia alata Linn is a significant traditional medicinal plant which is used to treat skin diseases, asthma and rheumatic pains. Plant tissue culture has been used as an alternative method for the large scale production of plantlets. This study reports the in vitro seed germination and callus induction of in vitro grown Cassia alata leaf, stem and root explants on Murashige and Skoog's (MS) medium supplemented with various concentrations of plant growth hormones.

In seed germination, the maximum germination response was found in BA (1.0 mg /l), both scarified and non-scarified seeds showed 100% germination and the maximum callusing response (91%) was found at BA 0.05 mg/l with IAA 3.0 mg/l in stem explants. Thus, it is evident that in the present study, the seed germination method can be used for easy germination of C. alata seeds and the protocols can be used for conservation of this medicinal plant.

Keywords: *In vitro,* Seed germination, Callus induction, Medicinal plant and Conservation.

Introduction

Plants are the producers and play important liability to the millions of the people to whom traditional medicine serves as the only chance for health care but also to those who use plant commodities for various purposes in their daily lives and also as a basis for new pharmaceuticals[11]. Plants still remain the most efficient and cheapest alternative sources of drugs. In recent years, pharmaceutical companies are developing drugs from natural products[8]. Nowadays, plants are used as a sources of direct therapeutic agents, as a model for new synthetic compounds and as a taxonomic marker for the expansion of more complex semi-synthetic chemical compounds[2].

Cassia alata Linn is an endangered tropical plant belonging to the family of Caesalpiniaceae and also known as "Dadmardan" or "Dadmari"[9]. Different parts of this plant contain various kinds of alkaloids and anthroquinone derivatives which exhibit a variety of biological activities like antimicrobial, antifungal, antitumor, antioxidant, cytotoxin and hypoglycaemic property[7, 15].

The leaves of this plant are regarded as an excellent source of medicine for ringworm disease, itching, cough, asthma, sanke-bites, eczema, herpes and skin diseases[8].

Seed dormancy is the most restrictive factor for plant propagation. Still, the blocking of water access into the seed is the most common cause of delay in seed germination [13]. Cassia sp. suffers from dormancy due to the presence of water impermeable thick seed coat and germination-inhibitor chemical compounds and they require specific treatments for breaking dormancy[1,15]. It is therefore necessary to develop an important protocol for *in vitro* propagation of medicinally important taxon from further depletion. Regeneration through callus induction of *Cassia alata* Linn offers an enormous prospective for large scale multiplication of such useful species and consequent exploitation[5].

Material and Methods

Collection of Plant material: The seeds of *Cassia alata* Linn were collected from various places of Tiruchirappalli district, Tamil Nadu, India. Disease free mature seeds were chosen for the study and then allowed to dry for several days.

***In vitro* Seed germination**

Surface sterilization of seeds: The air dried mature seeds of *Cassia alata* were rinsed with running tap water (few minutes) to remove the dust particles and are treated with 0.1% bavistin for 15 minutes, then they were again washed thoroughly with sterile distilled water and taken to the laminar air flow chamber. The seeds were surface sterilized with 0.1% $HgCl_2$ for 3 minutes and washed using sterile distilled water for 3 to 4 times.

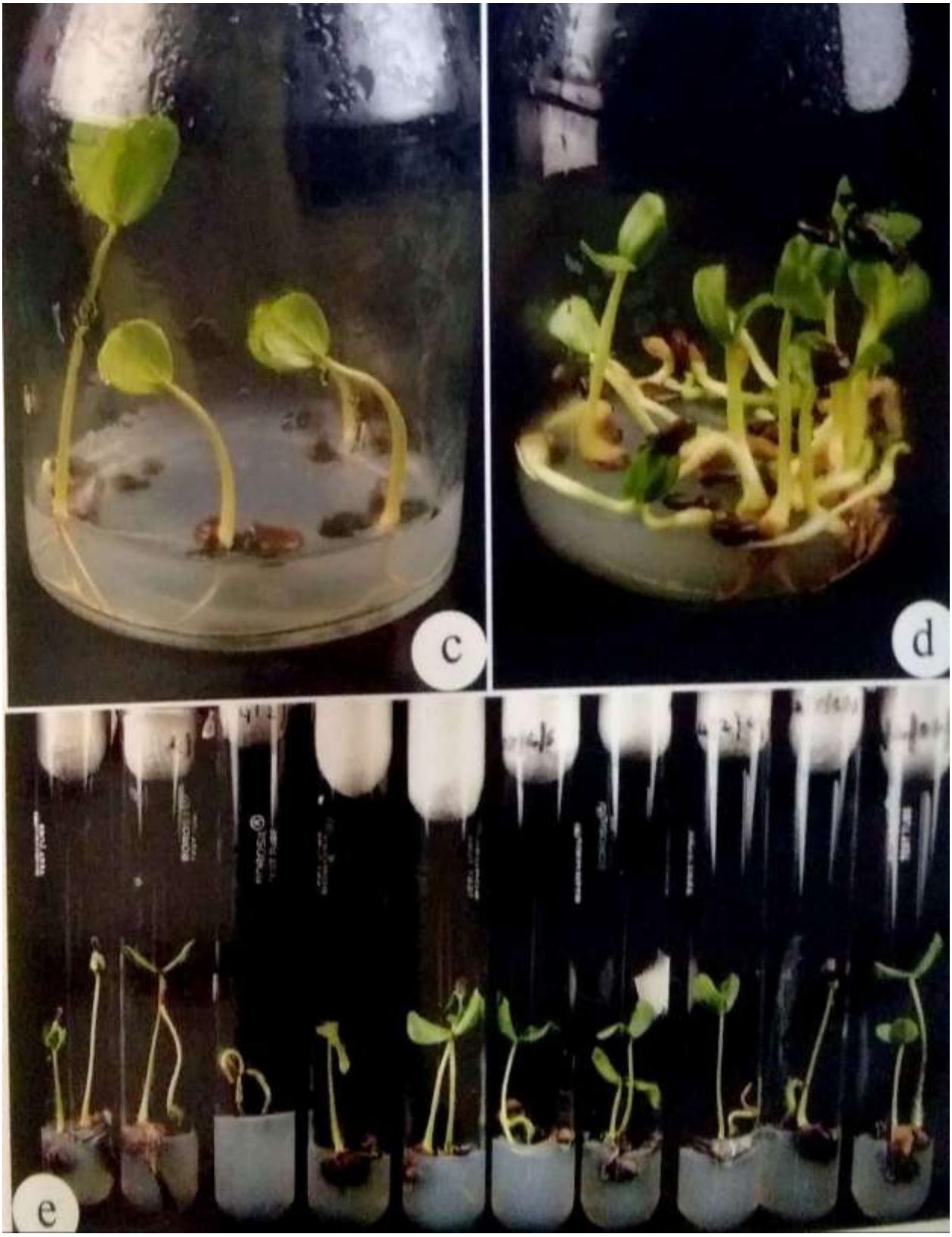
Medium for Seed germination: Murashigs and Skoog (MS) medium was used for seed germination, the basal medium added with (3%) sucrose, hormones (GA_3 - 0.5, 1.0, 1.5, 2.0 and 3.0 mg/l and BA -1.0, 2.0, 3.0 and 4.0 mg/l) and agar (0.8%) the solidifying agent. The pH was adjusted to 5.7 before autoclaving at 121ºC for 15 minutes. The inoculated cultures were maintained at 25±2ºC under 18 hrs light and 6 hrs. dark cycles. Healthy seedlings were selected and used as source of explants for callus induction.

Medium for Callus induction: For callus induction, the MS medium was supplemented with different concentrations of 2,4-D (1.0, 2.0, 3.0, 4.0 and 5.0 mg/l) and IAA (1.0, 2.0, 3.0, 4.0, 5.0, 6.0 and 7.0 mg/l) along with BA (0.05mg/l).

Results and Discussion

Seed Germination of *Cassia alata*: The scarified and non-scarified seeds of *C. alata* were inoculated on MS medium with GA_3 and BA at different concentrations. Of the two types of seeds, the scarified seeds showed maximum percentage of response (100%) response compared to non-

scarified seeds. Of the different concentration of hormones used, the scarified and non-scarified seeds showed maximum of 100% response at 1.0 mg/l BA (Fig. 1). It was followed by BA 1.5 mg/1, in which the scarified seeds showed 99% response and non-scarified seeds showed 92% response (Plate 1a and 1b). In BA containing medium, the non-scarified seeds were germinated after 3 days of inoculation whereas the scarified seeds were germinated the very next day of inoculation (Table 1).



a. Germination of scarified seeds on GA3 b. and c. Germination of scarified seeds on BA
Plate 1: *In vitro* seed germination

In GA_3 containing medium, the non-scarified were germinated after 6 days of inoculation (Plate 1c) and scarified seeds started to germinate after 4 days of inoculation. From this study, it is concluded that the seeds require scarification for maximum germination response. Similar finding was observed in morphologically dormant seeds of *Polygonum macrophyllum*[12]. The explants leaf, stem and root were collected from the *in vitro* grown seedlings and used for inoculation.

Callus induction from *in vitro* grown leaf explants: The leaf explants were collected from *in vitro* grown seedlings and inoculated on callus induction medium. The explants started to swell after 2 days of inoculation. The initiation of callus was observed after 5 days of inoculation along with the cut margins of explants. Maximum response (59%) was observed at IAA (5.0 mg/l) and BA (0.05 mg/l) combination. The calli were green and hard in appearance (Plate – 2a). Similar findings were reported in *Solanum nigrum* in which the compact nodular green calli were reported[3].

In the present study, the combination of BA and 2,4 – D produced friable and creamy, pale yellow colour calli. Poor response was found with decreasing concentrations of IAA and 2,4-D. The results were presented in table 2.

Leaf Callus

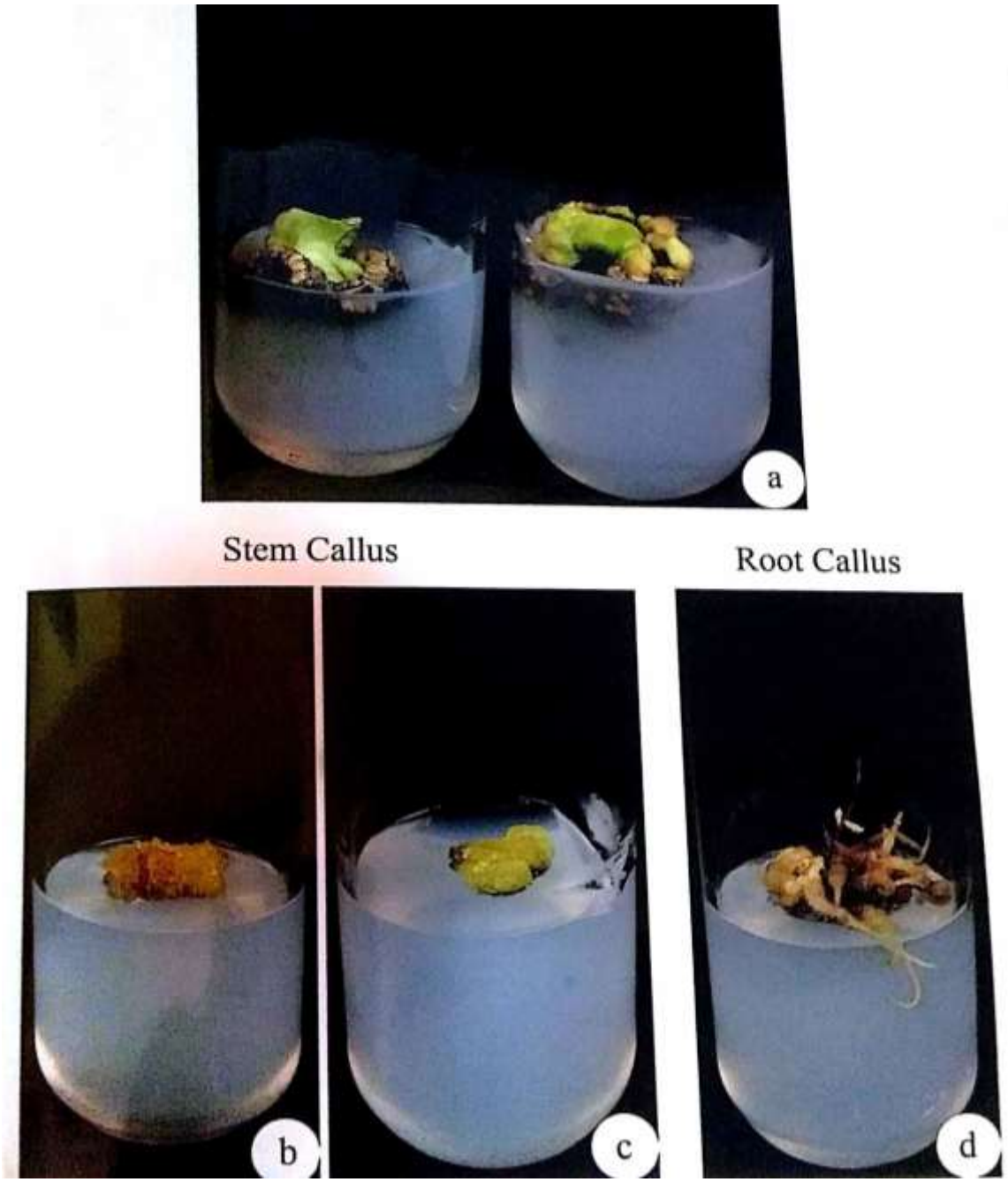


Plate 2: Callus induction from in vitro grown leaf, stem and root explants of *C. Alata*

Table 1
***In vitro* Seed Germination of *Cassia alata* L.**

Seeds	Hormones in mg/l	Percentage of seed germination (%)	No. of days for seed germination
Non – scarified seeds	**GA3**		
	1.0	50	6 day
	2.0	57	6 day
	3.0	60	4 day
	4.0	46	6 day
Scarified seeds	1.0	48	6 day
	2.0	53	6 day
	3.0	62	4 day
	4.0	50	6 day
Non – scarified seeds	**BA**		
	0.5	80	4 day
	1.0	100	3 day
	1.5	92	3 day
	2.0	81	4 day
	3.0	71	5 day
Scarified seeds	0.5	97	1 day
	1.0	100	1 day
	1.5	99	1 day
	2.0	78	1 day
	3.0	73	1 day

Table 2
Callus Induction from Leaf explants of *Cassia alata* L.

Hormones (mg/l)		Percentage of callus	Morphology of calli
BA	**2,4 - D**		
0.05	1.0	30	Pale brown, friable calli
0.05	2.0	35	Pale brown, friable calli
0.05	3.0	37	Pale brown, friable calli
0.05	4.0	39	Pale brown, creamish calli
0.05	5.0	27	Pale brown, creamish calli
BA	**IAA**		
0.05	1.0	25	Hard green calli
0.05	2.0	31	Hard green calli
0.05	3.0	32	Hard green calli
0.05	4.0	47	Hard green calli
0.05	5.0	59	Hard green calli
0.05	6.0	31	Hard green calli

Callus induction from *in vitro* grown stem explants: The stem explants of *C. alata* started to swell after 3 days of inoculation followed by the callus formation from cut end of the explants. The maximum of 91% of callusing was observed at 0.05 mg/l BA and 3.0 mg/l IAA combination. The calli were green and compact in nature. Similar findings were observed in *Plumbago zeylanica*[4,14]. The combination of BA and 2,4-D produced green, compact and hard calli (Plates 2b and 2c). The results obtained were tabulated in table 3.

Callus induction from *in vitro* grown root explants: The root explants started to swell after 7 days of inoculation. Callus initiation was observed after 10 days of culture. Maximum response (33%) was found at 0.05 mg/l BA and 6.0 mg/l IAA combination (Plate – 2d). The calli were pale green in colour and the explants did not show any response with decreasing concentrations of IAA. The results were interpreted in table 4.

Conclusion

Cassia alata Linn. is one of the most important medicinal plants used for skin diseases, asthma and rheumatic pains etc. The plants raised through i*n vitro* seed propagation was found to be most effective method for production of more number of plantlets. Thus, this method will be valuable for commercial cultivator for the mass production of these plantlets. The callus induction protocol could be used for regeneration of shoots in large scale.

Table 3
Callus Induction from Stem explants of *Cassia alata* L.

Hormones (mg/l)		Percentage of callus	Morphology of calli
BA	**2,4 - D**		
0.05	1.0	31	Green, compact hard calli
0.05	2.0	53	Green, compact hard calli
0.05	3.0	42	Green, compact hard calli
0.05	4.0	33	Green, compact hard calli
0.05	5.0	27	Green, compact hard calli
BA	**IAA**		
0.05	1.0	58	Green, compact hard calli
0.05	2.0	75	Green, compact hard calli
0.05	3.0	91	Green, compact hard calli
0.05	4.0	67	Green, compact hard calli
0.05	5.0	55	Green, compact hard calli

Table 4
Callus Induction from Root explants of *Cassia alata* L.

Hormones (mg/l)		Percentage of callus	Morphology of calli
BA	IAA		
0.05	1.0	-	-
0.05	2.0	-	-
0.05	3.0	-	-
0.05	4.0	18	Light green colour
0.05	5.0	21	Light green colour
0.05	6.0	33	Light green colour
0.05	7.0	19	Light green colour

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