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The present investigation deals with *in vitro* Callus induction and plant regeneration of *Abutilon indicum* (L.)

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Abstract
Abutilon indicum (L.) is an important medicinal plant which belongs to the family Malvaceae. It is widely used as folk medicine and treating for many diseases. The efficient *in vitro* regeneration of *Abutilon indicum* was achieved from leaf, node and internode explants on MS medium with B5 vitamins and different concentrations and combinations of PGRs like BAP, NAA, IAA, IBA, KIN, 2, 4-D and AdS were tested. The best response for callus induction was observed from leaf, node and Inter nodal explants in combination of BAP (8.88 µM) and NAA (0.53 µM). The high frequency of shoot regeneration was observed from leaf, node and Internodal explants on BAP (8.88 µM), 2, 4-D (0.53 µM) and Ads (0.578 µM). The regenerated shoots were transferred in to half strength MS medium fortified with IBA for shoot induction. Plantlets were successfully acclimatized and transfer in to the field conditions.

Keywords: *In vitro* regeneration; MS medium; BAP; IAA and NAA.

1. Introduction
Abutilon indicum (Indian Abutilon, Indian mallow; is a small shrub in the Malvaceae family, native to tropic and subtropical regions and sometimes cultivated as an ornamental [1]. This plant is often used as a medicinal plant and is considered invasive in certain tropical islands Description Medium sized, branched perennial shrub. Up to 2 meters in height. Plant covered with minute hairs. Leaves are alternate, cordate and acute. Flowers are yellowish, with 5 petals. Fruits have 15-20 chambers, arranged spirally. Seed colour is blackish brown. This plant is useful in gout, tuberculosis, ulcers, bleeding disorders, and worms. It cures burning sensation. Decoction used in toothache and tender gums. Leaves are locally applied to boils and ulcers. Roots are used in fever, chest affection and urethrities. (*Abutilon indicum*.) The species occurs in a number of tropical and subtropical zones. An example occurrence is within parts of the Great Barrier Reef islands of the Coral Sea C. Michael Hogan (2011). It is sweet, cooling, digestive, laxative, expectorant, diuretic, astringent, analgesic, anti-inflammatory, anthelmintic, demulcent and aphrodisiac. It is useful in gout, tuberculosis, ulcers, bleeding disorders, and worms. Decoction used in toothache and tender gums. Demulcents of leaves are locally applied to boils and ulcers. Roots are prescribed in fever, chest affection and urethrities. The plant is very much used in Siddha medicines. In fact, the root, bark, flowers, leaves and seeds are all used for medicinal purposes by Tamils. The leaves are used as adjunct to medicines used for pile complaints. The flowers are used to increase semen in men. (Dr. J. Raamachandran Book) [21]. A methanol extract of *A. indicum* had some Antimicrobial properties Jigna Parekh and Nehal Karathia (2006) [5]. A chemical compound, β-sitosterol, which has been identified as the active ingredient in many medicinal plants, is present in *A. indicum* and a petroleum ether extract provided larvicidal properties against the mosquito larvae *Culex quinquefasciatus* Abdul Rahuman, Geetha and Gopalakrishnan (2008) [4]. For this above medicinal purposes, this plant is highly focused in many countries and pharmaceutical industries. Tissue culture plays an important key role for medicinal plants in rapid propagation, conservation and enhanced the production of secondary metabolites. The secondary metabolites production can be possible through *in vitro* plant cell culture [6, 7]. In this present investigation was undertaken with an objective to develop an efficient *in vitro* regeneration protocol for important medicinal plant *Abutilon indicum*. Through leaf, nodal and internodal explants.

2. Materials and Methods

Abutilon indicum L. Plants were collected from Pachaimalai hills of Tiruchirappalli district, Tamilnadu. Live specimens were planted in the Botanical Garden, National College (Autonomous), Tiruchirappalli in green house conditions leaf, Nodal and internodal explants of *Abutilon indicum* L. were collected from two months old greenhouse grown plants.

2.1. Chemicals and Instrument

The sterilization of explants was done by dipping them in 70% ethanol for 10 seconds followed by continuous shaking. Then the explants were washed with detergent Tween-20 for 5 mines and after that explants were surface sterilized by 0.1% mercuric chloride ($HgCl_2$) for 1 min then finally rinsed for 3 times with sterilized distilled water. All the process of sterilization and transfer were carried out inside the laminar air flow with proper sterilization techniques. The leaf, nodal and internodal explants were inoculated to the MS medium [10] with B5 vitamins [11] and different concentrations and combinations of plant growth regulators like BAP (4.44-13.32 µM), KIN(2.32-13.92 µM), IAA (0.28-1.43 µM), IBA(0.24-1.71µM), NAA(0.26-1.34 µM), AdS (0.144-0.867 µM).The cultures were maintained at 25± 2 ^{0}C under a 16 hour photoperiod of 35 mol m-2 s-1 irradiance provided by cool white fluorescent light with 55-65% relative humidity. Observations were recorded after an interval of four weeks. The prepared medium was autoclaved and placed in dark condition up to inoculation. After the inoculation of explants callus induction was observed. During the process of callus induction, 12 – 13 days subculture was strictly followed. Otherwise browning of explants on medium was noticed. During each experiment 250 explants were taken for callus and multiple shoot induction and each experiment was repeated three times.

3. Results and Discussion

Leaf, Node and internode explants were inoculated on MS basal medium with B5 vitamins supplemented with various concentrations and combination of BAP (4.44-13.32 µM), KIN (2.32-13.92 µM), IAA (0.28-1.43 µM), IBA (0.24-1.71 µM), NAA (0.26-1.34 µM), AdS (0.144-0.867 µM) were used for culture initiation and multiplication of shoots. After 12 days of inoculation multiple shoot induction was observed from the explants. The mean number of multiple shoots was recorded on after 4 weeks of inoculation. In leaf, nodal and internodal explants were showed better response for callus indication on BAP (13.32 µM) + KIN (13.92 µM) + IAA (1.43 µM) + IBA (1.71 µM) + NAA (1.34 µM) + AdS (0.867 µM) and obtained the mean value 100.0 is the best response (Table. 1) (Fig. 1. A), (Table. 2) (Fig. 2. B). The similar results have also suggested by Ramar *et al*., (2014) [12] on *Solanum americanum* in BAP 3.0 mg/l+2, 4-D 0.5 mg/l+GA3 2.0 mg/l through nodal explants. These findings are in agreement with who observed in other plant species *Aegle marmelos* (L) (Ajithkumar and Seeni, 1998). The best response foe callus induction was recorded from leaf explants on different concentration and combination of plant growth regulators like BAP (8.88 µM) + 2, 4-D (o.53 µM) showed the mean value 99.4 is the best response (Table. 3) (Fig. 3 C).The similar results were obtained for *Solanum americanum* where BAP (2.0 mg/l) + 2, 4- D (1.0 mg/l) + IAA (1.0 mg/l) stimulated a number of multiple shoots through leaf explants (Ramar *et al*., 2014) [12]. Results described by (Monokesh Kumer Sen *et al.,* 2014) [14] also in agreement with our result for using this synthetic plant growth regulator in the culture medium for *Achyranthes aspera* L. After 4 weeks the elongated shoots from leaf, nodal and internodal explants were transferred to the callus induction medium containing half strength MS basal medium with IBA (1.71 µM). The *in vitro* regeneration of medicinal plant *Abutilon indicum* revealed that the tissue culture showed a good response in proliferation of multiple shoots in MS medium by supplementing with BAP, GA3 KIN, IAA, IBA, NAA 2, 4-D. & AdS. These present study was to establish reliable regeneration protocol for *Abutilon indicum*, which can be used for easier cultivation, propagation and plant genetic studies. In this present investigation has also opened new researchers for genetic manipulation of *Abutilon indicum* for disease, pest Resistance or enhancing secondary metabolites, using a rapid regeneration protocol.

[A] Leaf Callus of *Abutilon indicum* L.

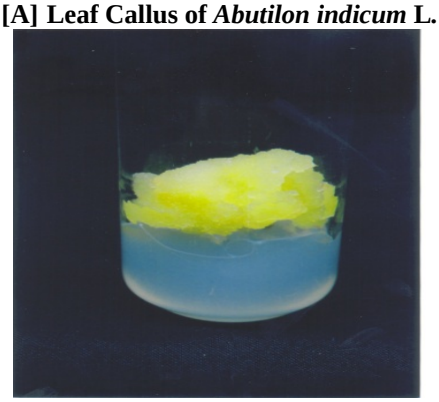

Fig 1 A: *In vitro* callus induction**:** development stages from leaf explants

[B] Nodal Callus of *Abutilon indicum* L.

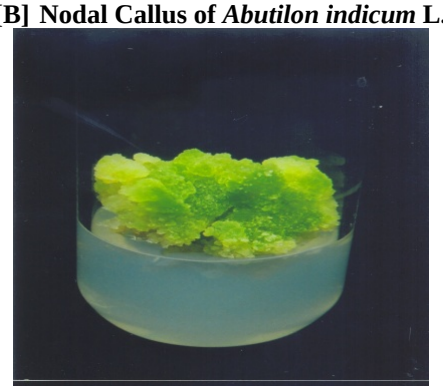

Fig 2 B: *In vitro* callus induction**:** development stages from nodal explants

[C] Internodal Callus of *Abutilon indicum* L.

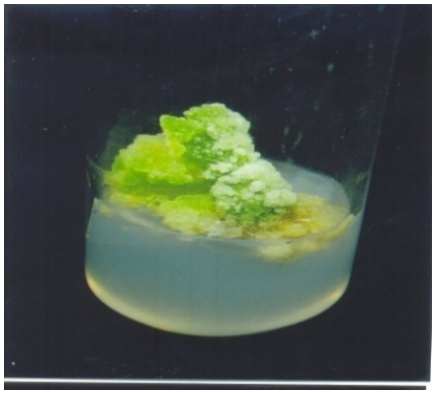

Fig 3 C: *In vitro* callus induction**:** development stages from internodal explants

Table 1: Effect of bap with different concentrations of auxins on callus induction from leaf explants cultured on ms medium With B5 vitamins.

Concentration of growth regulators (µM)	Percentage of response	Basal callus formation
BAP		
4.44	94.2	-
6.66	95.7	-
8.88	96.1	-
11.1	97.4	-
13.32	86.2	+
KIN		
2.32	88.2	-
4.64	89.9	-
6.96	87.3	-
9.28	86.6	-
13.92	86.2	-
BAP+IAA		
8.88+0.28	78.9	-
8.88+0.57	79.5	-
8.88+0.85	80.2	-
8.88+1.15	79.2	+
8.88+1.43	78.7	+
BAP+IBA		
8.88+0.24	77.4	-
8.88+0.49	77.6	-
8.88+0.74	78.3	-
8.88+1.14	78.8	-
8.88+1.39	77.7	+
8.88+1.71	76.9	+
BAP+NAA		
8.88+0.28	97.6	-
8.88+0.57	98.8	-
8.88+0.85	98.7	-
8.88+1.15	97.4	-
8.88+1.43	97.0	+
BAP+NAA+AdS		
8.88+0.53+0.144	98.6	-
8.88+0.53+0.289	99.0	-
8.88+0.53+0.433	99.4	-
8.88+0.53+0.578	100.0	-
8.88+0.53+0.722	98.4	+
8.88+0.53+0.867	98.1	+

Number of explants tested-25

Table 2: Effect of BAP with different concentrations of auxins on callus induction from nodal explants cultured on MS medium With B5 vitamins

Concentration of growth regulators (µM)	Percentage of response	Basal callus formation
BAP		
4.44	90.2	-
6.66	95.7	-
8.88	93.1	-
11.1	97.4	-
13.32	91.2	+
KIN		
2.32	86.2	-
4.64	89.9	-
6.96	81.3	+
9.28	86.6	-
13.92	86.2	-
BAP+IAA		
8.88+0.28	70.9	-
8.88+0.57	79.5	-
8.88+0.85	80.2	-
8.88+1.15	79.2	+
8.88+1.43	71.7	+
BAP+IBA		
8.88+0.24	77.4	-
8.88+0.49	77.6	+
8.88+0.74	78.3	-
8.88+1.14	78.8	-
8.88+1.39	77.7	+
8.88+1.71	76.9	+
BAP+NAA		
8.88+0.26	97.6	-
8.88+0.53	98.8	-
8.88+0.80	98.7	-
8.88+1.07	97.4	-
8.88+1.34	97.0	+
BAP+NAA+AdS		
8.88+0.53+0.144	91.6	-
8.88+0.53+0.289	99.0	+
8.88+0.53+0.433	99.4	-
8.88+0.53+0.578	80.0	-
8.88+0.53+0.722	95.4	+
8.88+0.53+0.867	98.1	+

Number of explants tested -25

Table 3: Effect of BAP with different concentrations of auxins on callus induction from internode explants cultured on MS medium With B5 vitamins

Concentrations of growth regulators (µM)	Percentage of response	Basal callus formation
BAP		
4.44	91.2	-
6.66	95.7	-
8.88	92.1	+
11.1	93.4	-
13.32	96.2	-
KIN		
2.32	81.2	-
4.64	89.9	-
6.96	81.3	-
9.28	85.6	+
13.92	86.2	-
BAP + IAA		
8.88 + 0.28	73.9	-
8.88 + 0.57	79.5	-
8.88 + 0.85	81.2	-
8.88 + 1.15	79.2	+
8.88 + 1.43	78.7	-

BAP + IBA		
8.88 + 0.24	76.4	-
8.88 + 0.49	75.6	+
8.88 + 0.74	78.3	-
8.88 + 1.14	78.8	-
8.88 + 1.39	77.7	+
8.88 + 1.71	76.9	+
BAP + NAA		
8.88 + 0.26	93.6	-
8.88 + 0.53	98.8	-
8.88 + 0.80	98.7	-
8.88 + 1.07	97.4	-
8.88 + 1.34	97.0	+
BAP + NAA + AdS		
8.88 + 0.53 + 0.144	96.6	+
8.88 + 0.53 + 0.289	99.0	-
8.88 + 0.53 + 0.433	99.4	-
8.88 + 0.53 + 0.578	100.0	-
8.88 + 0.53 + 0.722	98.4	+
8.88 + 0.53 + 0.867	98.1	+

Number of explants tested-25

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