

In vitro propagation of *Cleome gynandra* L. through direct organogenesis

Karthikeyan A. V. P.^{*1}, Shanmuganathan T.², Karthi C.³

Department of Botany, Government Arts College (Autonomous), Karur 639005, Tamilnadu, India

Abstract: An efficient protocol was developed for *in vitro* regeneration of *Cleome gynandra* L. is a herb belonging to the family Cleomaceae. In the present study, shoot tip explants of *Cleome gynandra* were cultured on MS medium supplemented with different concentrations of BAP and Kin (5-25 μ M/l) alone or in combination with IAA (2 – 10 μ M/l) for multiple shoot bud induction. The highest frequency (100%) of shoot bud induction and maximum number of shoot buds (26.4 ± 0.48) was noticed on 10 μ M BAP with mean shoot length of 8.2 cm. The shoot proliferation was further improved by combination of BAP+IAA. The maximum number of shoots (35.8 ± 1.42) was developed on MS media containing 10 μ M/l BAP in combination with 2 μ M/l IAA. The regenerative shoot buds were transferred to half strength MS medium fortified with different concentrations of IBA and NAA (2-10 μ M/l) for root induction. The maximum numbers of roots (17.2 ± 1.95) were obtained from 4 μ M NAA with mean root length of 3.6 cm. *In vitro* regenerative plantlets were successfully acclimatized in red soil, farmyard manure and sand (2:1:1). Subsequently, the plants were successfully established in the field.

Keywords: *Cleome gynandra*, shoot tip explants, BAP, NAA, MS medium.

1. Introduction

Cleome gynandra L. is a member of Cleomaceae formerly capparaceae and it can be found in all over world. It grows as a weed in paddy fields and also in road sides and in open grass lands. In India it is never cultivated but grows spontaneously everywhere. Different species of *Cleome* can be found in all states of India. The medicinal application of this plant is also described in Ayurvedic pharmacopoeia of India and also in other ancient medical texts [1]. In Ayurvedic medicine it is a chief constituent in Narayana Churna. In Ayurveda it is used as an Antihelmentic, in ear diseases, pruritis and several other diseases like gastrointestinal disorders and gastrointestinal infections etc [2]-[4].

In several communities, boiled spider plant leaves are traditionally given to mothers before and after delivery of a child, and in other situations where blood has been lost, e.g. to warriors. Similarly, an infusion of the leaves is used to treat anemia. The leaves and seeds are used medicinally as rubefacient and vesicant, and to treat rheumatism, externally as well as internally. The whole plant is also used in the treatment of malaria, piles, rheumatism and also it shows antidiabetic and anticancer activity [5]-[7]. The methanol extract of *Cleome gynandra* possess very good antioxidant property [8]. The plant *Cleome gynandra* also possesses anti-inflammatory and lysosomal stability actions in adjuvant induced arthritic rats. An infusion of the roots is used as a medicine for chest pain, the leaves to treat diarrhoea. The seeds are used to feed birds. The seed contains edible polyunsaturated oil, which is extracted by simple pressing and does not need refining. The seed cake can be used as animal food [9].

During the last few years, many plants used in phytopharmaceutical preparations are being overexploited, and several species are disappearing at an alarming rate. In addition, medicinal plants can be associated with a broad variety of microbial contaminants and the main prerequisite

for the development of high quality phytopharmaceutical products is a consistent source of high-quality plant material. Hence, advanced biotechnological methods for culturing plant cells and tissues should provide alternatives for propagating valuable and endangered medicinal plants and their metabolites [10]-[12]. In fact, these strategies contribute to the conservation and the sustainable use of biodiversity. Tissue culture technologies have been intensively used in afforestation and plantation programs, but also for plant improvement and *in vitro* production of metabolites. Considering the medicinal plants, the possibility of exploring bioactive compounds without commercial harvesting based on wild populations, represent the most efficient strategy to maintain the natural resources [13]-[16].

However, very few studies have been taken up on *Cleome gynandra* clonal propagation [17]. The present study was undertaken to develop a more efficient protocol for rapid *in vitro* multiplication of *Cleome gynandra* using shoot tip explants.

2. Materials and Methods

2.1. Plant material

Shoot tip segments from young and healthy plants of *Cleome gynandra* L. (Cleomaceae) were collected from in and around Thanthonimalai village, Karur district, Tamilnadu. They were washed with tap water for 30 minutes followed by treatment with 5 % teepol for 5 minutes, After repeated washes in double distilled water to eliminate the microbial contamination. They were then rinsed with 70% (v/v) ethanol for 30-45 seconds and subsequently surface sterilized with 0.1% (w/v) HgCl₂ solution for 3-5 minutes under aseptic condition. After rinsing five times with sterile distilled water, the explants (approximately 2.0 cm in length) consisting of a lateral shoot tips were used for culture initiation.

2.2. Culture media and conditions

Basal medium used in this study was the MS medium containing 3% sucrose as a carbon source [18]. The pH of the

medium was adjusted to 5.8 before gelling with 0.8% agar and autoclaving at 121°C for 20 min. Cultures were maintained at 25±2°C with 16-h of light at an intensity of 2,000-3,000 lux.

2.3. Shoot induction and multiplication

For multiple shoot induction, the shoot tip explants were placed on MS medium supplemented with different concentrations of benzylaminopurine (BAP: 5-25 µM) or kinetin (Kin: 5-25 µM) alone or in combination with indole 3 acetic acid (IAA: 2-10 µM).

2.4. Rooting and hardening

In vitro raised shoots were excised from shoot cluster and transferred to half strength MS medium supplemented with various concentrations of indole butyric acid (IBA: 2-10 µM) or α naphthalene acetic acid (NAA: 2-10 µM). The root number and length were measured in each culture medium.

The complete plantlets were carefully removed from the medium and washed gently with sterilized doubled distilled water to remove any traces of medium on roots. Then they were transplanted to the paper cup containing a mixture of autoclaved red soil, farm yard manure and sand in the ratio of 2:1:1 mixture for two weeks before transplanted into soil under field conditions.

2.5. Statistical analysis

All experiments were performed using a randomized completely block design and each experiment consisted of ten replicates and each experiment was repeated five times. The analysis of variance (ANOVA) appropriate for the design was carried out to detect the significance of differences among the treatment means were compared using Duncan's Multiple Range Test (DMRT) at a 5% level of significance [19].

3. Results and Discussion

3.1. Shoot induction and multiplication

The present study reports direct shoot multiplication of *Cleome gynandra* L. from shoot tip on MS medium supplemented with different concentrations of BAP or Kin alone or in combination with IAA (Fig.1). The shoot tips were initially grown on MS medium supplemented with BAP and Kin separately in different concentrations ranging from 5 - 25 µM. The Maximum number of shoots was achieved in 30-45 days. Among these, the maximum number of shoots (26.4 ± 0.48) was developed on MS media fortified with 10 µM /l BAP, maximum shoot length was observed as 8.2± 1.38 cm with 100% shoot sprouting frequency on a medium supplemented with 10 µM BAP followed by 5 µM BAP with 94% shoot induction frequency. The data in respect of shoot induction frequency, number of shoots and length of shoots on different concentrations of each hormone on shoot tip were presented in Table 1.

The basal medium containing different concentrations of Kin induced more or less similar number of shoots like BAP. Maximum number of 21.3 shoots per explants was proliferated on MS medium supplemented with 15 µM Kin and mean shoot length 7.5 cm with 96% shoot induction

frequency. Among these two cytokinins tested BAP was found to induce more number of shoots when compared to Kin. Similar to our findings, many researchers showed that BAP induced multiple shoot formation [20]-[23]. The shoot induction and proliferation depend on plant growth regulators and types of explants [24]-[26].

Hence it is suggested that the optimum concentration of BAP promote multiple shoot induction. However the shoot implanted on MS medium supplemented with optimal level of BAP(10 µM) in combination with IAA (2-10 µM) showed emergence of multiple shoot buds after two weeks of inoculation. Presence of higher concentration of cytokinins and low concentrations of auxins positively influenced the induction of shoots. Among the various combination and concentration of hormone tested, the highest number of shoot was recorded on MS medium containing BAP (10 µM) + IAA(2 µM). The highest frequency of shoot regeneration (100 %) and maximum number of shoots (35.8±1.42) per explant were observed on MS medium fortified with 10 µM BAP + 2 µM IAA. The number of shoots induce was decreased when the combination of IAA is increased. Similar results were also reported in many plants [27]-[29]. In many plants, multiple shoots were obtained from the shoot tips or axillary buds by administering BAP or Kin [30] - [33].

Table 1: Effect of different concentrations of hormones on shoot induction from the Shoot tip explants of *C. gynandra* L

Concentration of hormone			Percentage of Response	Number of Shoots	Shoot Length in cm
Kin	BAP	IAA			
5µM	---	---	84	13.7±1.58 ^k	5.4±1.04 ^{ij}
10µM	---	---	90	17.5±1.66 ^{ij}	6.3±0.99 ^g
15µM	---	---	96	21.3±1.14 ^g _h	7.5±0.89 ^d _e
20µM	---	---	82	12.9±2.25 ^{kl}	4.3±0.68 ^l
25µM	---	---	68	6.6±0.96 ^{no}	2.8±0.34 ⁿ _o
---	5µM	---	94	22.7±0.80 ^{ef}	6.8±0.80 ^f
---	10µM	---	100	26.4±0.48 ^c _d	8.2±1.38 ^a _b
---	15µM	---	90	18.6±1.51 ⁱ	5.2±0.77 ^{jk}
---	20µM	---	82	12.7±1.79 ^l _m	4.3±0.50 ^l _m
---	25µM	---	76	8.3±0.83 ⁿ	3.2±0.41 ⁿ
---	10µM	2µM	100	35.8±1.42 ^a	8.5±0.87 ^a
---	10µM	4µM	100	32.3±1.98 ^b	8.0±0.71 ^b _c
---	10µM	6µM	96	27.0±1.83 ^c	6.2±0.30 ^g _h
---	10µM	8µM	90	24.4±1.29 ^d _e	7.8±1.08 ^c _d
---	10µM	10µM	88	22.5±1.98 ^{fg}	5.8±0.45 ^{hi}

Results are repeated in five replicates. Each replicates have 10 test tubes. Values in the last two columns are Mean \pm SE of Mean followed by the letters within the column indicating the level of significance at $P < 0.05$ by Duncan's Multiple Range Test (same letter within the column of the respective growth regulator indicates the absence of difference; different letters indicate the significant difference; and combination of letters indicate no significant difference).



Figure 1: *In vitro* propagation of *Cleome gynandra* L. a. Shoot tip explant growing on MS medium. b. Shoot multiplication. c. Shoot proliferation and elongation.

3.2. Root induction and multiplication

In vitro regenerated and elongated shoots (5 – 6 cm long) were excised and transferred on to the rooting medium containing half strength MS medium supplemented with different concentrations of IBA and NAA ranging from 2-10 μ M. Roots were initiated and well established in all the concentrations of the two auxins studied.

Table 2: Effect of different concentrations of auxins on rooting of isolated shoots of *C. gynandra* L

Concentration of hormone		Percentage of Response	Number of Roots	Root Length in cm
IBA	NAA			
2 μ M	---	84	7.8 \pm 0.64 ^{gh}	2.5 \pm 0.50 ^f
4 μ M	---	96	8.2 \pm 0.80 ^g	2.8 \pm 0.72 ^d
6 μ M	---	100	12.9 \pm 0.43 ^{cd}	3.1 \pm 0.71 ^c
8 μ M	---	78	5.2 \pm 0.73 ⁱ	1.8 \pm 0.26 ^{hi}
10 μ M	---	64	3.5 \pm 0.65 ^j	1.2 \pm 0.27 ^j
---	2 μ M	94	14.4 \pm 1.02 ^b	3.2 \pm 0.41 ^b
---	4 μ M	100	17.2 \pm 1.95 ^a	3.6 \pm 0.57 ^a
---	6 μ M	88	13.7 \pm 1.68 ^{bc}	2.8 \pm 0.60 ^{de}
---	8 μ M	80	11.3 \pm 1.26 ^e	2.4 \pm 0.59 ^{fg}
---	10 μ M	76	10.5 \pm 1.69 ^{ef}	1.8 \pm 0.31 ^h

Results are repeated in five replicates. Each replicates have 10 test tubes. Values in the last two columns are Mean \pm SE of Mean followed by the letters within the column indicating the level of significance at $P < 0.05$ by Duncan's Multiple Range Test (same letter within the column of the respective growth regulator indicates the absence of difference; different letters indicate the significant difference; and combination of letters indicate no significant difference).

Of the different concentrations of NAA, maximum number of 17.2 roots were induced on 4 μ M NAA with mean root length of 3.6 cm with 100% root induction frequency, followed by 2 μ M NAA with 14.4 roots, mean length of 3.2 cm with 94% root induction frequency (Fig.1d & e; Table 2).

The MS basal medium with 6 μ M IBA showed 100% root induction frequency with mean number of 12.9 roots per shoot and the mean root length of 3.1 cm, followed by 4 μ M IBA showing 96% root induction frequency with average number of 8.2 roots per shoot and mean length of 2.8 cm.

In the present study of the two auxins tested, NAA was found to induce more number of roots than IBA. The number of roots and root length decreased when the concentration of IBA and NAA was increased. However NAA was proved to be best for rooting. Similar to our findings, many researchers showed that NAA induced rooting in many plants [34]-[38].



Figure 1: *In vitro* propagation of *Cleome gynandra* L. d. Root initiation. e. Root multiplication

3.3. Hardening and acclimatization

The rooted plantlets were carefully taken out from the culture tubes and washed thoroughly with distilled water to remove the culture medium. Then they were transplanted to the paper cup containing a mixture of autoclaved red soil, farm yard manure and sand in the ratio of 2:1:1 (Fig. 1f). The survival rate of these plants was 80% after acclimatization [39,40].



Figure 1: *In vitro* propagation of *Cleome gynandra* L. f. Hardening and acclimatization

Acknowledgements

The authors are grateful to **Dr. P.Velayutham**, Associate Professor and **Dr. K. Murugesan**, Head, P.G. and Research Department of Botany, Govt. Arts College, Karur for providing constant engorgement and support throughout the study.

References

- [1] K. Anbazhagi, G. Kadavul, Suguna and A.J.A. Petrus, "Studies on the pharmacognostical and *in vitro* antioxidant potential of *Cleome gynandra* Linn. Leaves", *Natural Product Radiance* 8(2), pp. 151-157, 2009.
- [2] S. S. Mishra, S.K. Moharana, and M.R. Dash, "Review on *Cleome gynandra*". *International Journal of research in pharmacy and chemistry*, 1(3), pp. 681-89, 2011.
- [3] R.T. Narendhirakannan, M. Kandaswamy and S.Subramanian, "Antiinflammatory activity of *Cleome gynandra* L. on hematological and cellular constituents in adjuvant induced arthritic rats", *Indian J. Med. Food*, 8(1), pp. 93-99, 2005.
- [4] T.Kalaivani, C. Rajasekaran and Lazar Mathew, "*In vitro* free radical scavenging potential of *Picrorhiza kurroa*", *Journal of Pharmacy Research*, 3(4), pp. 849-854, 2010.
- [5] S.N. Mule, R.V Ghadge, A.R. Chopade, B.A. Bagul, S.B. Patil, and N.S. Naikwade, "Evaluation of antinociceptive and anti-Inflammatory activity of leaves of *Gynandropsis pentaphylla*", *Journal of Herbal Medicine and Toxicology* 2(1), pp. 41- 44, 2008.
- [6] Asis Bala, Biswakanth Kar, K. Pallab Haldar, K. Upal Mazumder and SamitBera, "Evaluation of anticancer activity of *Cleome gynandra* on Ehrlich's Ascites Carcinoma treated mice", *Journal of Ethnopharmacology*, 129(1): pp 131-134. 2010.
- [7] Karimulla Shaik, Afsar Shaik, Deepak Kumar, Devi Kadirvel, "Evaluation of preliminary phytochemical properties and hypoglycemic activity of *cleome gynandra* L." *Int.J. of Pharm. and Pharm. Sci.*, Vol 5(3), pp. 824-828, 2013.
- [8] M. Muchuweti, C.Mupure, C. Ndhlala, T. Murenje, and M.A.N. Benhura, "Screening of antioxidant and radical scavenging activity of *Vigna unguiculata*, *idens pilosa* and *Cleome gynandra*". *American Journal of Food Technology*, 10, pp. 161-168, 2007.
- [9] C.K. Katiyar, N.B. Brindavanam, P. Tiwari, and D.B.A. Narayana, *Immunomodulation* (Eds.). Narosa Publishing House, New Delhi. pp: 163-187, 1997.
- [10] S.J. Murch and P.K. Saxena, "Molecular fate of thidiazuron and its effects on auxin transport in hypocotyls tissues of *Elargonuim xhortorum* Bailey", *Plant Growth Regul.*, 35, pp. 269-275, 2001.
- [11] W. Kneifel, E. Czech and B. Kopp, "Microbial Contamination of Medicinal Plants - A Review", *Planta Med.*, 68: pp. 5-15, 2002.
- [12] S.M. Nalawade and H.S.Tsay, "*In vitro* propagation of some important Chinese medicinal plants and their sustainable usage", *In vitro Cell. Dev. Biol. Plant*, 40, pp. 143-154, 2004.
- [13] A. Paunescu, "Biotechnology for endangered plant conservation: A critical overview", *Rom. Biotechnol. Lett.*, 14, pp. 4095-4103, 2009.
- [14] S. Sharma, S.K. Chattopadhyay, T. Priyanka, and D.U. Bawankule, "Synthesis and anti-inflammatory activity of derivatives of coumarinolignoid, cleomiscosin A and its methyl ether", *Eur. J. Med. Chem.*, 45, pp. 5150-5156, 2010a.
- [15] S. Sharma, N. Rathi, B. Kamal, D. Pundir, B. Kaur, and S. Arya, "Conservation of biodiversity of highly important medicinal plants of India through tissue culture technology - A review", *Agric. Biol. J. N. Am.*, 1, pp. 827-833, 2010b.
- [16] K. Sharma and S. Dubey, "Biotechnology and conservation of medicinal plants", *J. Exp. Sci.*, 2, pp. 60-61, 2011.
- [17] Nitika Singh Rathore, Nisha Rathore and N.S. Shekhawat, " *In vitro* propagation and micro morphological studies of *Cleome gynandra*; a c4 model plant closely related to *Arabidopsis thaliana*", *Acta Physiol. Plant.* 35, pp 2691-2698, 2013.
- [18] T. Murashige and F. Skoog, "A revised medium for rapid growth and bioassay with tobacco tissue culture", *Physiologia Plant.*, 15, pp. 473-497, 1962.
- [19] K. A. Gomez and K. A. Gomez, "Statistical procedures for agricultural research with emphasis on Rice", *Philippines International Rice Research Institute, Los Bans.* Grieve, A Modern Herbal. Penguin, 1976.
- [20] Jeyaraj Anburaj, Chinnappan Ravinder Singh, Shenbagamoorthy Sundarraj and Soundarapandian Kannan, "*In vitro* regeneration of *Cleome viscosa* – an important medicinal herb", *Journal of Cell and Molecular Biology*, 9(1), pp. 37-44, 2011.
- [21] Priyanka Pandey, Rakesh Mehta and Ravi Upadhyay, "*In vitro* propagation of an endangered medicinal plant *Psoralea corylifolia* linn", *Asian J. Pharm. Clin. Res.*, 6(3), pp. 115-118, 2013.

- [22] A.Benniamin, V.S. Manickam, M, Johnson and L. H. Joseph, "Micropropagation of *Crataeva Magna* (Lour) DC. – A medicinal plant", Ind. J. Biotech. 3 pp. 136-158, 2004.
- [23] Jitendra Mehta, Monika Sain, Banwari Lal Mathuriya, Ritu Naruka, Ambika Kavia and Dev Ratan Sharma, "Rapid micropropagation and callus induction of *Terminalia bellerica* Roxb. - An endangered plant" Asian Journal of Plant Science and Research, 2(3), pp.364-368, 2012.
- [24] T.W. Yang, Y.I. Hsing and W.C. Cheang, "Clonal propagation of *Stevia rebusiana* through axillary shoot proliferation *in vitro*", Bot. Bull. Academic Sin, 22: pp. 57-62, 1981.
- [25] J. Patnaik and P.K. Chand, "Micropropagation of *Hemidesmus indicus* (L.) R. Br. through axillary bud culture", Plant Cell Rep., 15, pp. 427-430, 1996.
- [26] S.V. Mohamed, M. Jawahar, M. Thiruvengadam, M. Jeyakumar and N. Jayabalan, "Effect of cytokinins on the proliferation of multiple shoots in horse gram (*Macrotyloma uniflorum* (L.) Verdc.)", Jour. Plant Biotech., 1, pp. 79-83, 1999.
- [27] M.M. Rahman U. R. Ankhi and A. Biswas, "Micropropagation of *Mentha viridis* L.: An aromatic medicinal plant", Int. J. of Pharm. & Life Sci. 4(9), PP. 2926-2930, 2013.
- [28] Chandra Gurnani, Vikram Kumar, Shinam Mukhija, Abha Dhingra, Sohan Rajpurohit and Poonam Narula, "In vitro regeneration of brahmi (*Bacopa monneiri* (L.) Penn.) - A threatened medicinal plant", Kathmandu University J. of Sci., Engg. and Technol., 8(1), pp 97- 99, 2012.
- [29] C.M. Ganesan and S. Paulsamy, Mass propagation of an economically important medical plant, *Lobelia nicotianaefolia* Heyne using *in vitro* culture technique, Nat. prod. Int. J., 8 pp. 35-40, 2102
- [30] M. Tatari Vernosefadrani, N. Askari Raberi and S.Z. Nosrati, "Optimization of *in vitro* culture for *Gerbera* cv. Tropic Blend", J. Sapling Seed. 2(25). pp. 389-401, 2009.
- [31] Afshin Ahmadi Hesar, Behzad Kaviani, Alireza Tarang and Sahar Bohlooli Zanjani, "Effect of different concentrations of kinetin on regeneration of ten weeks (*Matthiola incana*)", Plant Omics Journal, 4(5), pp. 236-238, 2011.
- [32] M.A. Bhat, A. Mujib, A. Junaid and M. Mohamooduafar, "In vitro regeneration of *Solanum nigrum* with enhanced solasodine production", Biologia Plantarum 54(4). pp. 757-760, 2010.
- [33] H. Padmapriya, A.V.P. Karthikeyan, G. Jahir Hussain, C. Karthi and P. Velayutham, "An efficient protocol for *in vitro* propagation of *Solanum nigrum* L. from nodal explants", Journal of Agricultural Technology, 7(4), pp. 1063-1073, 2011.
- [34] H.E. Lee-Epinosa, J. Murguia-Gonzalez, B. Garcia-Rosas, A.L. Cordova-Contreras and C. Laguna, "In vitro clonal propagation of vanilla (*Vanilla planifolia* Andrews)", Hort. Sci., 43, pp. 454-458, 2008.
- [35] F.T.Z. Jabeen, R.B. Venugopal, G. Kiran, C.P. Kaviraj and S. Rao, "Plant regeneration and *in vitro* flowering from leaf and nodal explants of *Solanum nigrum* (L.) - An important medicinal plant", Plant Cell Biotechnology and Molecular Biology, 6(1&2), pp. 17-22, 2005.
- [36] A.A. Jahan and M. Anis, "In vitro rapid multiplication and propagation of *cardiospermum halicacabum* L. through axillary bud culture", Acta Physiol. Plant, 31, pp. 133-138, 2009.
- [37] P.Nalini and P.Velayutham, "In vitro Mass Propagation of *Enicostemma littorale* Blume from Shoot Tip Explants", Journal of Biology Agriculture and Healthcare, 3(5), pp. 72-79. 2013.
- [38] P.Velayutham and B.D. Ranjitha Kumari, "Direct shoot regeneration from leaf explants of chicory (*Cichorium intybus* L.)", Plant Cell Biotech Mol. Biol., 4(3&4): 125-130, 2003.
- [39] Asha Bagadekar and M. Jayaraj, "In vitro Rhizogenesis from leaf and stem callus of *Heliotropium indicum* L. - Medicinal Herb". International Journal of Plant, Animal and Environmental Sciences, 2(2), pp. 1-5, 2011.
- [40] K. Satyavani, V. Dheepak, S. Gurudeeban and T. Ramanathan, "Direct Organogenesis of Seaside Heliotrope (*Heliotropium crassavicum*) Using Stem Explants", Pakistan Journal of Biological Sciences, 16, pp. 1216-1219, 2013.

Author Profile



Dr. A.V.P. Karthikeyan, Plant Biotechnologist has specialized in Plant Tissue Culture. He did his Ph. D. in Botany (2001) from Bharathidasan University, Tiruchirappalli and M. Sc. in Botany (1996) from St. Joseph's College, Tiruchirappalli, Tamil Nadu, India. Currently he is working as an Assistant Professor in P.G. & Research Department of Botany, Government College, Karur, Tamil Nadu, India from 2007 until now.



T. Shanmuganathan, a part time research scholar, and working as BT Assistant in Science at P.U.M. School, Kattur, Karur Dist. He received academic proficiency medal for his UG & PG courses at St. Joseph's college, Tiruchirappalli.



C. Karthi, a full time research Scholar, P.G. & Research Department of Botany, Government College, Karur. Working as a JRF in UGC – Major Research Project.