CO_2 Incubator

qRT-PCR

Fermenter

Thermocycler

Gel Documentation Unit

Centre for Plant Tissue Culture

-20°C Deep Freezer

Centre for Plant Tissue Culture

SOUVENIR

POPULAR LECTURE SERIES in BIOTECHNOLOGY

19th September 2015

Sponsored by

सत्यमेव जयते

Department of Biotechnology
Govt. of India
New Delhi

Organized by

UKA TARSADIA university

Imparting Knowledge. Awakening Wisdom. Transforming Lives.

C. G. BHAKTA INSTITUTE OF BIOTECHNOLOGY

Maliba Campus, Bardoli, Dist. Surat, Gujarat, Pin : 394350

POPULAR LECTURE SERIES in BIOTECHNOLOGY
Programme Schedule

Lecture 1: Biodiversity and biotechnological applications of cyanobacteria
Date:19/09/2015

Dr. N. Thajuddin
Professor & Head
Department of Microbiology
Bharathidasan University
Tiruchirappalli-23, Tamil Nadu

Lecture 2: Arbuscular Mycorrhizal fungi: Application in plant production system & monitoring its quality parameters
Date: 01/10/2015

Dr. Mahaveer P. Sharma
Principal Scientist
Directorate of Soybean Research (ICAR)
Indore, Madhya Pradesh

Lecture 3: Plant Tissue Culture methods with special reference to cultivation of medicinal plants
Date: 12/10/2015

Dr. R. Krishnamurthy
Director
C.G. Bhakta Institute of Biotechnology
Uka Tarsadia University
Bardoli, Surat, Gujarat

Research Facilities at CGBIBT


Green House Facility


Electrophoresis Unit


Fluorescence Microscope


Inverted Microscope


Net House Facility

Thajuddin, N. and Subramanian, G. (1995) Additions to the new reports of marine cyanobacteria from the east coast of India. Phykos 34: 33-37.

Thajuddin, N. and Subramanian, G. (2002) The enigmatic bloom forming marine cyanobacterium *Trichodesmium*. In : Advances in Marine and Antarctic Sciences. (Eds.) D. Sahoo and P.C. Pandey. APH Publishing Co. New Delhi. Chapter 4: 57-89.

Thajuddin, N. and Subramanian, G.(1994) Marine cyanobacterial flora of south India. In: Current Researchers in Plant Sciences. (Eds.) T.A.Sharma, S.S.Saini, M.L.Trivedi and M.Sharma, Bisen Singh & Mahendra Pal Singh, Dehra Dun. pp. 1 - 16.

Thajuddin, N., Nagasathya, A., Chelladevi, R. and Saravanan, P., (2002) Biodiversity of cyanobacteria in different saltpans of Pudukkottai District, Tamil Nadu. Seaweed Res. Utiln., 2002, 24, 1-11.

Thajuddin, N., Sandra A. Nierzwicki-Bauer and G. Subramanian (2007) Cyanobacterial Biodiversity and recent trends in their taxonomy. BDU J. Sci. & Tech. 1 : 1 14 (in Tamil), 15-28.(in English).

Uma, L. and Subramanian, G., (1990) Effective use of cyanobacteria in effluent treatment. In Proceedings of the National Symposium on Cyanobacterial N2 Fixation, IARI, New Delhi, pp. 437-444.

Uma, L., Palanisamy, M., Murugauyan, P., Akbarsha, M.A., and Subramanian, G. (1998) Novel cyanobacterial feed for Frog tadpoles. In: Cyanobacterial Biotechnology. (Eds.) G. Subramanian, B.D.Kaushik and G.S. Venkataraman. Oxford & IBH Publishing Co. Pvt.Ltd. New Delhi. 311-314.

Venkataraman, G. S.(1981) Bluegreen algae for rice production. FAO Soil Bull., 16: 33-42.

Venkataraman, L. V., (1983) In A Monograph on *Spirulina platensis* Biotechnology and Application, DST, New Delhi.

Vijayakumar, S., N. Thajuddin and C. Manoharan (2007a) Biodiversity of Cyanobacteria in industrial effluents. Acta Botanica Malacitana 32: 27-34.

Vijayakumar, S., N. Thajuddin and C. Manoharan (2007b) Influence of dye effluent on the amino acid and fatty acid content of cyanobacteria. Asian Jr. Microbiol. Biotech. Env. Sc. 9 (3) : 525-528.

Vijaykumar, S., Thajuddin, N. and Manoharan, C. (2005). Treatment of dye industry effluent using cyanobacteria. Pollution Research 24(1): 69 - 74.

Watanabe, I. and Cholitkul, W., (1979) Field studies on nitrogen fixation in paddy soils. In : Nitrogen and Rice, IRRI, Los Banos, Philippines, pp. 223.

Whitton, B. A. and Potts, M. (2000) Introduction to the cyanobacteria. In: The ecology of cyanobacteria: Their diversity in time and space. Whitton, B. A. and Potts, M. (eds), Kluwer Academic Publishers, Dordrecht, pp. 1-11.

POPULAR LECTURE SERIES in BIOTECHNOLOGY

Title

Biodiversity and Biotechnological Applications of Cyanobacteria

Speaker

Dr. N. Thajuddin
Professor & Head, Department of Microbiology
Dean, Faculty of Science, Engineering & Technology
School of Life Sciences
Bharathidasan University
Tiruchirappalli 620 024. Tamilnadu

Sponsored by

सत्यमेव जयते

Department of Biotechnology
Govt. of India
New Delhi

Organized by

UKA TARSADIA university
Imparting Knowledge. Awakening Wisdom. Transforming Lives.

C. G. BHAKTA INSTITUTE OF BIOTECHNOLOGY
Maliba Campus, Bardoli, Dist. Surat, Gujarat, Pin : 394350

19th September 2015

marine cyanobacterium *Phormidium valderianum* BDU 30501. J. Ind. Microbiol. Biotechnol., 19: 130-133.

Singh, S. M., P. Singh and N. Thajuddin (2008) Biodiversity and distribution of Cyanobacteria at Dronning Maud Land, East Antarctica. Acta Botanica Malacitana 33: 17-28.

Stolz, P. and B. Obermayer. (2005) Manufacturing microalgae for skin care. Cosmetics Toiletries, 120: 99-106.

Subramanian, G. (1998). Marine cyanobacteria for feed, fine chemicals and pharmaceuticals. In: Cyanobacterial Biotechnology. (Eds.) G. Subramanian, B.D.Kaushik and G.S. Venkataraman. Oxford & IBH Publishing Co. Pvt.Ltd. New Delhi. 281-286.

Subramanian, G. and Thajuddin, N. (1995) Ecobiology of Marine Cyanobacteria. In: Advances in Ecology and Environmental Sciences. (Eds.) P.C.Mishra, B.K.Senapathi and B.C.Guru, Ashish Publishing House, New Delhi. pp. 189-210.

Subramaninan, G. and Uma, L., (1996) Cyanobacteria in pollution control. J. Sci. Ind. Res., 55: 685-692.

Sudha, S. S., Panneerselvam, A and N. Thajuddin (2007) Seasonal variation of cyanobacteria at Muthupet mangrove environs, Tamilnadu, South India. Seaween Res. Utiln. 29 (1 &2): 263-271.

Sundararaman, M., Subramanian, G., Averal, H. I. and Akbharsha, M. A. (1996) Evaluation of the bioactivity of marine cyanobacteria on some biochemical parameters of rat serum. Phytotherapy Res., 10: 9-12.

Thajuddin, N and Muralitharan, G (2008) Applications of PCR based fingerprinting in the phylogeny of marine cyanobacteria. Indian Hydrobiology 11(1):25 - 41.

Thajuddin, N. (2010) Potentials of Cyanobacteria and their industrial applications. In: Industrial Exploitation of Microorganisms. (Eds.) Maheshwari, D.K., Dubey, R.C. and Saravanamuthu, R.. I.K. International Publishing House. New Delhi. 70 - 85.

Thajuddin, N. and G. Subramanian. (2004) Cyanobacterial biodiversity and potential applications in biotechnology. Current Science, 89: 47-57.

Thajuddin, N. and Subramanian, G. (1990) Cyanobacterial phytoplankton of the Gulf of Mannar region. In: Proc. Natl. Symp. on Cyanobacterial Nitrogen Fixation. IARI, New Delhi. pp. 457-463.

Thajuddin, N. and Subramanian, G. (1991) New reports of cyanobacteria from the southern east coast of India. Phykos 30: 19-23.

Thajuddin, N. and Subramanian, G. (1992) Survey of Cyanobacterial flora of the southern east coast of India. Botanica Marina. 35: 305-311.

hypersaline environment of the salt pans of southern east coast of India. Asian Journal of Plant Sciences 7(5): 473 -478.

Nagasathya, A. and Thajuddin, N. (2008a) Decolorization of paper mill effluent using hypersaline cyanobacteria. Research Journal of Environmental Sciences 2(5): 408 -414.

Nagasathya,A. and Thajuddin, N. (2008b) Antioxidant Property of Hypersaline Cyanobacteria, *Phormidium tenue* (KMD 33) International Journal of Pharmacology. 4(2): 125 -129.

Pandiaraj, D., Mubarak Ali, D., Praveen Kumar, R., Ravikumar, S. and N. Thajuddin (2012) Molecular characterization and phylogeny of Marine cyanobacteria from Palk Bay region of Tamil Nadu, India. Ecologia. 2(1): 23 - 30.

Patterson, G. M. L., (1996) Biotechnological applications of cyanobacteria. J. Sci. Ind. Res., 55: 669-684.

Prabhakaran, D. and Subramanian, G., (1995) Hydrogen photoproduction by marine cyanobacteria *Dicothrix bauriana* BDU 40481. Physiol. Mol. Biol. Plants, 1: 45-57.

Prabhakaran, D., Sumathi, M. and Subramanian, G., (1994) Ability to use amphicillin as nitrogen source by marine cyanobacterium *Phormidium valderianum* BDU 30501. Curr. Microbiol., 28: 315-320.

Praveenkumar, R., Leo Antony, M., Vijayan, D., Muthukumar, C. and N. Thajuddin (2007) Morphological diversity and phylogeny of cyanobionts from the coralloid roots of cycads BDU J. Sci. & Tech. 1(2) : 169 - 176 (in Tamil)& 177-184 (in English).

Rai, A. N. (1990) Cyanobacteria in symbiosis. In: Handbook of Symbiotic Cyanobacteria (ed. Rai, A. N.), CRC Press, Boca Raton.

Rajeshwari K., Kumar M S and Thajuddin N. (2012) Adsorption Hydrobiology 11(1):25 - 41.

Thajuddin, N. (1991) Marine cyanobacteria of the southern east coast of India survey and ecobiological studies. Ph D thesis, Bharathidasan University, Tiruchirappalli.

Thajuddin, N. (2010) Potentials of Cyanobacteria and their industrial changes associated with bioaccumulation of chromium by a fresh water cyanobacterium *Chroococcus* sp. Biotechnology Letters. 34: 247-251.

Satheeshkumar, M., Muralitharan, G. and N. Thajuddin. (2009) Screening of a hypersaline cyanobacterium *Phormidium tenue* for the degradation of aromatic hydorcarbons: naphthalene and anthracene. Biotechnology Letters 31:1863 -1866.

Shashirekha, S., Uma, L. and Subramanian, G., (1997) Phenol degradation by the

Organizing Committee

Patrons

Dr. D. R. Shah Shri. Kiritbhai Patel Shri. Bhagubhai Patel

Chairman

Dr. R. Krishnamurthy

Co-ordinator

Dr. N. Amaresan

Discipline Committee

Dr. Meonis Pithawala Prof. Rajashekar I
Dr. Sankar Narayanan

Registration Committee

Dr. Parul S
Ms. Urjita Sheth
Dr. Vijayalakshmi G
Ms. Dhruti H. Mistry
Ms. Nilofer Sheikh
Ms. Amita Rathod

Stage Committee

Dr. Prasant Kumar
Dr. Anoop Markande
Dr. Mitesh K Dwivedi
Dr. Gopal Jee Gopal
Dr. Srivathsa N
Mr. Bhrugesh Joshi

Advertisement Committee

Ms. Nimisha Patel
Ms. Avani Kasture
Ms. Sonal Vashi
Ms. Vrutika Lad
Ms. Divya Patel
Ms. Shraddha Saha
Ms. Swetal Patel
Ms. Trusha Mistry
Ms. Avani Pandya
Mr. Harsh Desai
Ms. Shreya Desai
Ms. Jemisha Mistry
Ms. Sandipa Thesiya

Food Committee

Mr. Prittesh Patel Mr. Rushabh Shah

Mitsui, A., Murray, R., Entenmann, B., Miyazawa, K. and Polk, E. (1981) Utilization of marine bluegreen algae and macroalgae in warm water mariculture. In : Biosaline Research. A Look to the Future (ed. San Pietro, A.), Plenum Press, New York, pp. 215-225.

Moore, R.E., Blackman, A.J., Cheuk, C.E., Mynderse, J.S., Matsumoto, G.K, Clardy, J., Woodard, R.W., Craig, J.C. (1984) J. Org. Chem., 49: 2484-2489.

Mubarak Ali D. and Thajuddin, N. (2009) Biosynthesis of colloidal silver nanoparticles using fresh water cyanobacterium *Oscillatoria earlei* NTDM03.Seaweed Res. Utiln., 31 (1&2) 219 - 226.

Mubarak Ali D., Sasikala, M., Gunasekaran, M. and Thajuddin, N. (2011a) Biosynthesis and characterization of Silver nanoparticles using marine cyanobacterium *Oscillatoria willei* NTDM01. Digest Journal of Nanomaterials and Biostructures. 6(2): 415 - 420.

Mubarak Ali, D., A. Suresh., R. Praveen Kumar M. Gunasekaran and N. Thajuddin (2011b) Efficiency of Textile Dye Decolorization by Marine cyanobacterium, *Oscillatoria formosa* NTDM02. African Journal of Basic & Applied Sciences 3 (1): 9-13.

Mubarak Ali, D., Vinodh Kumar, T. and N. Thajuddin (2008) Screening of some selected hypersaline cyanobacterial isolates for biochemical and antibacterial activity. Indian Hydrobiology, 11(2): 241 -246.

MubarakAli, D., Arunkumar, J., Harish Nag, K., Sheik Syed Ishack, K.A., Baldev, E., Pandiaraj, D. and Thajuddin N. (2013) Gold nanoparticles from Pro and eukaryotic photosynthetic microorganisms- Comparative studies on synthesis and its application on biolabelling. Colloids and Surfaces B: Biointerfaces. 103: 166 -173.

Mubarakali, D., Arunkumar, J., Suriya, K. R., Ishack K. A. S. S. and N. Thajuddin (2012b) Molecular modeling and phylogenetic analysis of C-phycocyanin gene sequence from marine cyanobacterium *Phormidium tenue* NTDM05. Seaweed Res. Utiln. 34(1&2): 35 -44.

MubarakAli, D., Gopinath, V., Rameshbabu, N. and N. Thajuddin (2012a). Synthesis and characterization of CdS nanoparticles using c-phycocyanin from the marine cyanobacteria. Materials letters 74: 8 -11.

Muralitharan, G. and Thajuddin, N. (2008) Evidence on the presence of tRNAfmet group I introns in the marine cyanobacterium *Synechococcus elongatus*. J. Microbiol. Biotechnol. 18(1) : 23- 27.

Muthukumar, C., Vijayakumar, R., Muralitharan, G., Panneerselvam, A. and N. Thajuddin (2007) Cyanobacterial biodiversity from different freshwater ponds of Thanjavur, Tamilnadu, (India). Acta Botanica Malacitana 32: 17-25.

Nagasathya, A. and N. Thajuddin (2008) Cyanobacterial diversity in the

Jaki, B., Zerbe, O., Heilmann, J., Sticher, O. (2001) Two novel cyclic peptides with antifungal activity from the cyanobacterium *Tolypothrix byssoidea* (EAWAG 195). J. Nat. Prod., 64: 154-158.

Jeraci, J. and Vansoest, P., (1986) In:Handbook of Dietary Fiber in Human Nutrition (ed. Spiller, G.), CRC Press, Boca Raton, pp. 299-303.

Kalavathi, F. D., Uma, L. and Subramanian, G. (2001) Degradation and metabolization of the pigment melanoidin in distillery effluent by the marine cyanobacterium *Oscillatoria boryana* BDU 92181. Enz. Microb. Technol., 29, 249-251.

Kannaiyan, S., (1985) Algal Biofertilizers for Low Land Rice, TNAU, Coimbatore, pp. 14.

Kannan, K., Vijayan, D., Mubarak Ali, D., Praveenkumar, R., Parveez Ahamed, A and N. Thajuddin (2014). Evidence-based analysis of novel symbiotic and epiphytic cyanobacteria associated with *Azolla* by cyto and molecular taxonomy. Biotechnology:13(20): 46 -53.

Karna, R. R., Uma, L., Subramanian, G. and Mohan, P. M. (1999) Biosorption of toxic metal ions by alkali extracted biomass of a marine cyanobacterium *Phormidium valderianum* BDU 30501. World J. Microbiol. Biotechnol., 15: 729-732.

Kaushik, B. D. and Venkataraman, G. S., (1979) Effect of algal inoculation on the yield and vitamin C content of two varieties of tomato. Plant Soil, 52: 135-137.

Kulik, M.M. (1995) The potential for using cyanobacteria (blue-green algae) and algae in the biological control of plant pathogenic bacteria and fungi. European J. Plant Pathol. 101: 585-599.

Kumar M S, Praveenkumar R, Byong-Hun Jeon and N. Thajuddin (2014) Chlorpyrifos induced changes in the antioxidants and fatty acid compositions of *Chroococcus turgidus* NTMS12. Letters in Applied Microbiology. 59(5): 535-541.

Lewis Oscar, F., MubarakAli, D., Nithya, C., Priyanka, R., Gopinath,V., Alharbi, N.S. and N.Thajuddin. (2015) One pot synthesis and anti-biofilm potential of copper nanoparticles (CuNPs) against clinical strains of *Pseudomonas aeruginosa*. Biofouling 31 (4): 379-391.

Litchfield, J.H. (1983) Single cell proteins. Science, 219: 740-746.

Malliga, P., Uma, L. and Subramanian, G., (1996) Lignolytic activity of the Cyanobacterium *Anabaena azollae* ML 2 and the value of coir waste as a carrier for BGA biofertilizer. Microbios, 86: 175-183.

Misra, S. and Kaushik, B. D., (1989) Growth promoting substances of cyanobacteria. I. Vitamins and their influences on rice plants. Proc. Indian Natl. Sci. Acad., 55: 295-300.

About the Institutes

Uka Tarsadia University

The establishment of Uka Tarsadia University (UTU) in 2011 by the Bardoli Pradesh Kelavani Mandal (BPKM) is an effort towards meeting the growing demand for quality education. The University offers admission in a comprehensive array of academic programs across the disciplines of Pharmacy, Management, Architecture, Nursing, Physiotherapy, Computer Science, Engineering & Technology and Applied Science. The university provides educational opportunities to over 7500 students. More than 350 faculty members of the university represent an eclectic mix of professional and academic, national and international experiences. UTU has established MoUs with several Foreign Universities, like Cape Breton University (Canada), University of Ilorin (Nigeria) and University of Prince Edward Island (Canada).

C. G. Bhakta Institute of Biotechnology

CGBIBT of Uka Tarsadia University (UTU) is ably managed by Bardoli Pradesh Kelavni Mandal (BPKM) which needs no introduction about its significant contribution in providing collegiate education over the last 60 years. CGBIBT established in 2005 for initiating exclusive education programs in Biotechnology and Basic Science. Since its establishment, CGBIBT has been striving to emerge as a center of excellence in imparting career based knowledge to students to realize their professional ambition. In addition, CGBIBT has also taken up a mission to support the local farming community by supplying quality planting materials. For this purpose, a sophisticated plant tissue culture laboratory and climate controlled Green House have been established. The institute is offering five years Integrated M.Sc Biotechnology / Microbiology; two years M.Sc Biotechnology / M.Sc Microbiology as well as Ph.D program in Biotechnology / Microbiology. The institute is equipped with basic as well as advanced instrumentation facilities. As a part of academic exchange programme and research collaborations, CGBIBT, UTU has signed an MoU with University of Ilorin (Unilorin) Nigeria & IITA, Nigeria and many private industrial organizations.

Biography

Dr. N. Thajuddin
Professor & Head
Department of Microbiology
Dean, Faculty of Science, Engineering and Technology
Bharathidasan University
Tiruchirapalli-620 024
Syndicate Member, (March 2009 January 2010)
Senate Member, (May 2007 - till date)
President, Alumni Association (2007 - 2011)

Dr. N. Thajuddin, Professor & Head, Department of Microbiology, Bharathidasan University, Tiruchirappalli is a cyanobacteriologist with vast experience in cyanobacterial and microalgal taxonomy, cultivation, extraction of valuable products and most importantly expertise in employing molecular tools in the identification and phylogeny of various microorganisms and bioremediation of effluents using hypersaline cyanobacteria. His preliminary work on the survey of marine cyanobacteria has resulted in the establishment of marine cyanobacterial germplasm of 350 strains at National Facility for Marine Cyanobacteria, from which three technologies were developed and transferred to an industry. He has also developed germplasm of cyanobacteria, microalgae, bacteria, actinobacteria and fungi in his laboratory. He had one year post- doctoral training on molecular taxonomy and phylogeny of cyanobacteria at the Department of Biology, Rensselaer Polytechnic Institute, Troy, New York, USA through Dept of Biotechnology (Govt of India) long term overseas award. He deposited 500 ribosomal RNA of cyanobacteria, bacteria, actinobacteria, microalgae, fungi, zooplankton, ITS, Phycocyanin and Group I Intron genes in GenBank and developed barcodes for 6 indigenous fungi using ITS region. He identified and characterized several cyanobacterial genera and species symbiotically associated in the coralloid roots of Cycads, *Azolla* and Cyanolichens. He published 219 research and review articles, a book on Microbiology & Immunology at Post graduate level in Tamil and 2 edited books. He received major research projects from the Government funding agencies namely DBT, DST, UGC and MoES at the tune of Rs. 4.41 crores. He is a life member of various academic bodies and member in editorial boards in national and international journals. He is also a member of Research Advisory Committee, project evaluation committee, chairman of project monitoring committee for

Cyanobacteria have long been considered either as organisms of academic curiosity or as organisms of nuisance value. Pioneering work in India and subsequently other countries have raised the status of these microbes to the level of biotechnologically most useful ones. Therefore, in tropical countries like India, it is essential not only to understand and preserve the biodiversity of cyanobacteria found in all habitats but also to gainfully exploit them for different biotechnological applications including pollution abatement.

References

Adams, D. G. (2000) Cyanobacterial phylogeny and development: Questions and challenges. In: Prokaryotic Development (eds Brun, Y. V. and Shimkets, L. J.), ASM Press, Washington DC, pp. 51-81.

Becker, E. W., (1994) Micoalgae Biotechnology and Microbiology, Cambridge Univ. Press, UK.

Becker, W. (2004) Microalgae in human and animal nutrition, in Richmond, A (ed), Handbook of microalgal culture, Blackwell, Oxford, 312-351.

Bender, J., S. Rodriguez-eaton, U.N. Ekanemesang, P. Philippes. (1994) Characterization of metal binding bioflocculants produced by the cyanobacterial components of mixed microbial mats. Appl. Environ. Microbiol., 7: 2311-2315.

Carmichael, W.W. (1992) Cyanobacteria secondary metabolites the cyanotoxins. J. Appl. Bacteriol. 72: 445-459.

Castenholz, R. W. (2001) Oxygenic photosynthetic bacteria. In: Bergey's manual of systematic bacteriology. Boone, D. R. and Castenholz, R. W. (eds), 2nd edn. Springer-Verlag, New York, USA, pp. 474-599.

Dadhich, K. S., Verma, A. K. and Venkataraman, G. S. (1969) Effect of *Calothrix* inoculation on vegetable crops. Plant Soil, 31: 377-379.

De, P. K., (1939) The role of bluegreen algae in nitrogen fixation in rice fields. Proc. R. Soc. B., 127: 121-139.

Desikachary, T. V. (1959) Cyanophyta. Indian Council Agricultural Research, New Delhi. pp 686.Elhai, J. and Wolk, C. P. (1988) Conjugal transfer of DNA to cyanobacteria. Methods Enzymol., 167: 747.

Emodi, A. (1978) Carotenoids: Properties and Applications. Food Technol., 32: 38-42.

Gustafson, K.R., Cardellina, J.H., Fuller, R.W., Ewislow, O.S., Kiser, R.F., Snader, K.M., Patterson, G.M.L. and Boyd, M.R. (1989) J. Natl. Cancer Inst., 81: 1254-1258.

Herdman, M., Janvier, M., Rippka, R. and Stanier, R. Y. (1979) Genome size of cyanobacteria. J. Gen. Microbiol., 111: 73-85.

Kaushik and Venkataraman, 1979). The importance of *Azolla* as an organic fertilizer in rice cultivation is well appreciated and practiced in several countries (Kulasooriya, 1998). The cyanobacterial symbiont *Anabaena azollae* found within a center cavity which fixes atmospheric nitrogen estimated between 120 -312 kg N_2 per hectare. *Azolla* supplies substantial amounts of green manure between 150 -300 tons per hectare per year, which supports the growth of soil microorganisms including heterotrophic N_2 fixers (Kannaiyan, 1985).

The use of algae and cyanobacteria in waste treatment could prove beneficial in different ways since they will bring about oxygenation and mineralization, in addition to serving as food source for aquatic species. Like many genera of eubacteria, they synthesize polyhydroxyalkanoates (PHAs), a thermoplastic class of biodegradable polyesters that includes polyhydroxybutyrate (PHB). PHAs are carbon- and energy-storage compounds that are deposited in the cytoplasm as inclusions. Using the marine cyanobacteria *Oscillatoria* sp. BDU 10742 and *Aphanocapsa* sp. BDU 16 and a halophilic bacterium *Halobacterium* US 101, (Uma and Subramanian 1990) could treat ossein factory effluent and reduce calcium and chloride level significantly at field levels to enable 100% survival and multiplication of Tilopia fish with only the cyanobacteria as the feed source. Shashireka et al. (1997) found that another marine cyanobacterium *Phormidium valderianum* BDU 30501 was able to tolerate and grow at a phenol concentration of 50 mg/l and remove 38 mg/l with in a retention period of 7 days. This result opens the possibility of treating a variety of phenol containing effluents. The same organism was used to study the condition and reagents optimal sorption/desorption of heavy metal ions (Cd^{2+}, Co^{2+}) (Karna et al. 1999). Another marine cyanobacterium *Oscillatoria boryana* BDU 92181 was found to effectively degrade and metabolize melanoidin pigment which is abundant in distillery effluents (Kalavathi et al. 2001). Subramanian and Uma (1996) have identified suitable cyanobacteria for treating a number of noxious effluents containing organophosphorus pesticides, detergents, antibiotics etc. and even degradation of solid wastes like coir pith by the lignolytic action of certain cyanobacteria (Malliga et al. 1996). Satheesh kumar et al. (2009) have demonstrated the ability of hypersaline cyanobacterium *Phormidium tenue* in the bioconversion of anthracene to 8 hydroxy- anthracene 1,2 dione & 10 hydroxy- anthracene 1,2 dione, whereas napthalene is converted into 1,2 napthoquinone & napthalene 1,2 diol. The application of cyanobacteria in the removal of CO_2 from the flue gases of coal-fired power stations and the development of biofuels from the biomass. They present multiple possibilities for fuel end-products biodiesel, ethanol, methane, jet fuel, biocrude and more via a wide range of process routes.

government funded projects. Dr. Thajuddin guided 27 Ph.D candidates, acted as doctoral committee member for 142 Ph.D. candidates and currently guiding 4 post doctoral and 8 doctoral candidates on various aspects of microbiology. He organized several national level symposia, workshops, refresher courses and DST-INSPIRE Programs. Dr. Thajuddin with his research team have participated 25 international and 79 national level symposia, conferences and seminars, presented around 296 papers, of which 35 papers received best paper awards. Recently Department of Biotechnology (Govt of India) sanctioned to a major grant to Prof. N. Thajuddin for the Establishment of National Repository for Freshwater Microalgae & Cyanobacteria. He received Dr. G. S. Venkataraman Memorial NABS-Best Scientist Award of National Academy of Biological Sciences for the year 2014**.** He visited United States of America, United Kingdom, Kingdom of Saudi Arabia, Republic of Korea, Malaysia, Honk Kong, Singapore and Germany to disseminate his expertise and to keep himself abreast of the advanced techniques in the field of Microbiology.

Biodiversity and Biotechnological Applications of Cyanobacteria

Dr. N. Thajuddin

Cyanobacteria are one of the largest sub-groups of Gram-negative photosynthetic, especially oxygenic, autotrophic prokaryotes (Adams, 2000). Fossil evidence points to their presence in geographically diverse regions during the Precambrian (2 to more than 3.5 billion years ago). Members of this group possess chlorophyll *a* and phycobiliproteins such as phycocyanin and phycoerythrin, which are responsible for the blue-green pigmentation often evident in this group. As a result of their pigmentation, cyanobacteria were traditionally referred to as blue-green algae. Several names are commonly used for these organisms including cyanophyceae, cyanoprokaryotes, cyanophytes and myxophyceae. The oxygenic photoautotrophic nature of cyanobacteria requires only light, CO_2, and H_2O as the sources of energy, carbon and electron donor, respectively. They can grow well in all aquatic habitats (as plankton) such as rivers, ponds (Muthukumar et al. 2007), lakes, water tanks, paddy fields (Rout et al. 2012) all types of marine environments including sea water, hypersaline salt pans (Nagasathya and Thajuddin 2008), brackish waters (Sudha et al. 2007), soda lakes, all types of soils, deserts, cave walls, hot springs, polar regions (Singh et al. 2008), on tree barks, on leaf surfaces, rocks, sewage and industrial effluents (Vijayakumar et al .2007a) and other extreme environments.

Cyanobacteria are also known as symbionts in a variety of other organisms (example - the marine diatom *Rhizosolenia*, leaves of *Azolla* and the roots of *Cycas)* (Rai, 1990; Thajuddin et al. 2007; Praveenkumar et al. 2007; Kannan et al. 2014). Above all, recent reports showed the possibility of cyanobacterial existence in Mars. In addition to their widespread geographic distribution, cyanobacteria have probably displayed a major role throughout the biological history of the earth. Cyanobacteria have proven to be useful tool in examine endosymbiotic origin of eukaryotic chloroplasts (Castenholz, 2001). The genome size of cyanobacteria, representative of all major taxonomic groups, lie in the range of 1.6×10^9 to 8.6×10^9 daltons. The majority of unicellular cyanobacteria contain genomes of 1.6×10^9 daltons, comparable in size of those of other bacteria (1.0 to 3.6×10^9 daltons) (Herdman et al. 1979).

Morphological diversity

There are two major morphological types of cyanobacteria, coccoid and filamentous forms. Coccoid species range from single cells (*Synechocystis*) to colonies (*Chroococcus & Gloeocapsa*) or masses of various shapes. In some, cells are arranged in rows resulting in a flat plate (*Merismopedia*), or they may be radially arranged in spherical colonies or in sarcinoid appearance (*Gomphospharia, Myxosarcina*). Some colonies are loosely attached to the substrata without polarity. Others are firmly attached and have a distinct base and

are established in the skin care market, the main ones being *Arthrospira* (Stolz and Obermayer, 2005). Cyanobacterial extracts can be mainly found in face and skin care products (e.g., anti-aging cream, refreshing or regenerant care products, emollient and as an anti-irritant in peelers). Micro algae are also represented in sun protection and hair care products. One example of commercially available product and their properties claimed by the company; a protein-rich extract from *Arthrospira* repairs the signs of early skin aging, exerts a tightening effect and prevents stria formation. A number of important enzymes are known to be produced in sufficient amounts by cyanobacteria to be exploited as useful commercial ventures. Marine cyanobacteria useful for the large scale production of enzymes such as β- lactamase, protease and lipase have been identified and characterized (Prabaharan et al., 1994).

Analysis of extracellular growth promoting substances liberated by *Nostoc muscorum* and *Hapalosiphon fontinalis* in the external medium was found to be rich in several amino acids like sereine, arginine, glycine, aspartic acid, threonine, glutamic acid, cystine, proline, valine, ornithine, lysine, histidine and iso-lucine (Misra and Kaushik, 1989). In addition, cyanobacteria can be rich sources of several polyols, polysaccharides, lipids, fatty acids, halogenated compounds etc. with varied properties employable as flocculants, surfactants and so on (Becker, 1994). Several marine cyanobacterial strains are known to produce high levels of UVA absorbing compound Biopterin glucoside in response to UV-A irradiation which can be used in skin formulations. The expression of an insecticidal gene from *Bacillus thuringiensis* in a marine cyanobacterium *Agmenellum quadruplicatum* is considered as an attractive candidates for mosquito control.

De (1939) attributed the inherent fertility of tropical rice field soils to the activity of N_2 fixing cyanobacteria. A large variety of cyanobacterial strains colonize the rice field soils and some species are capable to fix atmospheric nitrogen using their specialized cells called heterocysts. Several non-heterocystous cyanobacteria are also capable of fixing atmospheric nitrogen under micro aerophilic conditions. *In-situ* estimations using acetylene reduction technique have shown an addition of 18-15 kg N ha^{-1} yr^{-1} due to the activity of diazotrophic cyanobacteria (Watanabe and Cholitkul, 1979). The role of N_2 fixing cyanobacteria in the maintenance of the fertility of rice fields has been well substantiated and documented all over the world. In India alone, the beneficial effects of cyanobacteria on yield of many rice varieties have been demonstrated in a number of localities (Venkataraman, 1981). Beneficial effects of cyanobacterial inoculation have also been reported on a number of other crops such as barley, oats, tomato, radish, cotton, sugarcane, maize, chilli and lettuce (Dadhich et al. 1969;

phycobiliproteins, characteristic of cyanobacteria have high commercial value. They are used as natural food colourants, as food additives to enhance the colour of the flesh of Salmonid fish and to improve the health and fertility of cattle and in the cosmetic industries. Some of the marine cyanobacteria appear to be potential sources for large scale production of vitamins of commercial interest such as vitamin B complex group and vitamin E. Anti HIV activity has been observed with the compound extracted from *Lyngbya lagerheimii* and *Phormidium tenue*. A compound has been purified from marine *Oscillatoria laete-virians* BDU 20801 has been known for anti-candida activity. Medically important gamma linolenic acid (GLA) is relatively rich in cyanobacteria namely *Spirulina platensis* and *Arthrospira* sp. which is easily converted into arachidionic acid in the human body and arachidionic acid into prostaglandin E2 which has lowering action of blood pressure and the contracting function of smooth muscle and plays a very important role in lipid metabolism.

In studies conducted at National Facility for Marine Cyanobacteria, Bharathidasan University, a marine cyanobacterium *Phormidium valderianum* BDU 30501 was chosen as a complete aquaculture feed source, based on the nutritional qualities and non-toxic nature with animal model experiments (Uma et al., 1998). A technology for mass cultivation of this strain and production of pellet feed was also developed and transferred to industry. Some cyanobacterial species

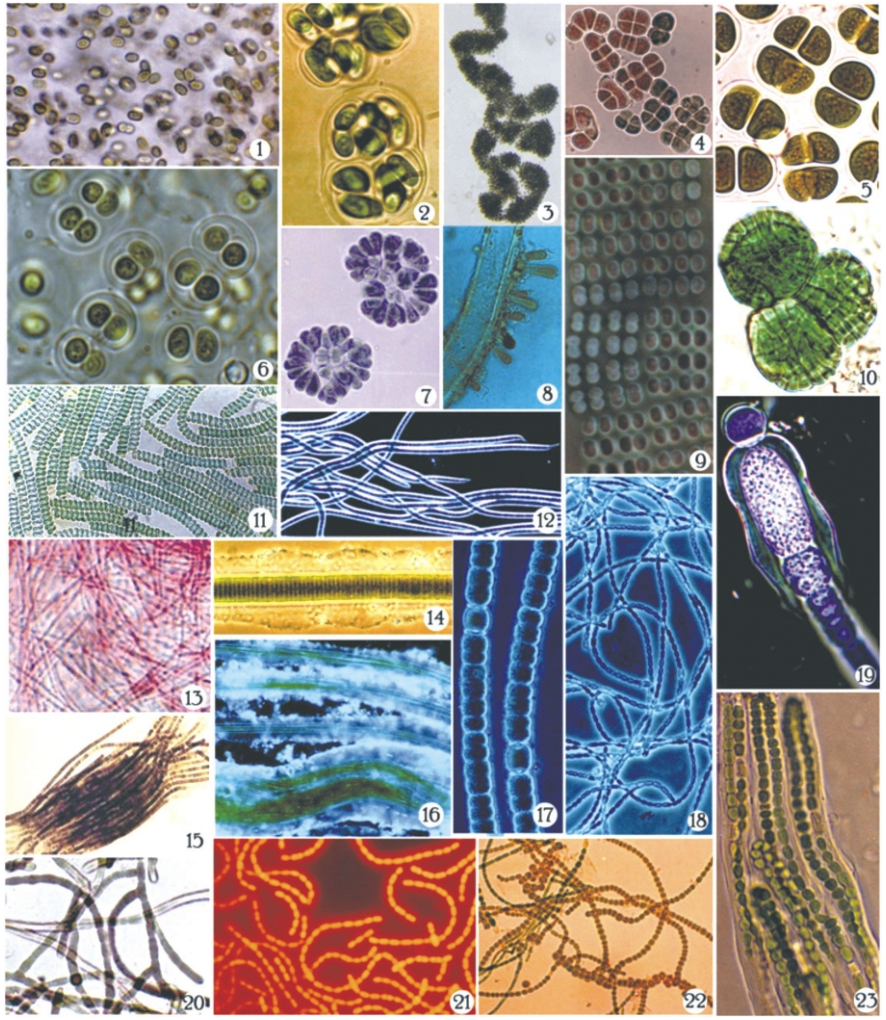
General composition of different human food sources and algae (percentage of dry matter) (Becker, 2004)

Commodity	Protein	Carbohydrate	Lipid
Baker's yeast	39	38	1
Meat	43	1	34
Milk	26	38	28
Rice	8	77	2
Soybean	37	20	20
Anabaena cylindrica	43-56	25-30	20
Chlamydomonas rheinhardii	48	17	21
Chlorella vulgaris	51-58	12-17	14-22
Dunaliella salina	57	32	6
Porphyridium cruentum	28-39	40-57	9-14
Scenedesmus obliquus	50-56	10-17	12-14
Spirulina maxima	60-71	13-16	6-7
Synechococcus sp.	63	15	11

apex (*Chamaesiphon*), often with basal parts that penetrate the substratum. All are enclosed in a gelatinous sheath that varies in consistency and thickness. Filamentous forms produce a row of cells referred to as a trichome, a result of cell division in one plane and failure of the cells to secrete sheath material between the cells or in the plane of division. Trichomes may be simple straight (*Oscillatoria*) or in the form of aggregated bundles (*Trichodesmium*) and or permanently spirally coiled (*Spirulina*). The trichome with the enclosing sheath is referred to as a filament (*Lyngbya, Phormidium*); in some forms, trichomes are enclosed by a common sheath (*Microcoleus*). Some filamentous species are characterized by true cell differentiation and form heterocysts which unlike vegetative cells, lack an oxygenic photosystem, possess extraordinarily thick cell wall, and lack biliprotein pigments and carboxysomes (*Anabaena*). These cells are considered as the sites of nitrogen fixation, provide vegetative cells with combined nitrogen and are not viable when disconnected from the trichome. Many heterocystous cyanobacteria also form a second cell type, an akinetes, which can germinate when conditions are suitable for growth. The filaments are either unbranched (*Nodularia*) or may be branched with uniseirate (*Mastigocoleus*) or multiseriate (*Stigonema*) arrangement of cells. Besides presence of true branching (*Hapalosiphon & Westiellopsis*), false branches may occur in some forms (*Scytonema*).

Marine Cyanobacteria

India has a vast coastline of over 7500 km; in addition it has many lakes, ponds, puddles, backwater areas and a tropical climate that results in abundance of natural populations of varied organisms. Cyanobacteria are widespread and abundant in most marine habitats. Their ability to grow in seawater is presumably related to a preference for alkaline conditions and an ability to tolerate high salt concentrations. The resistance, which many species show towards osmotic shock, extremes of temperature and reducing conditions, suits their existence in a variety of intertidal habitats. Desikachary (1959) suggested that probably 20% of all known cyanobacteria occur in saline conditions and a majority of them are truly marine. However, considerable work has been done to understand the cyanobacterial biodiversity of marine environments of India (Thajuddin, 1991; Thajuddin and Subramanian, 1990, 1991, 1992, 1994, 1995, 2002, 2004; Subramanian and Thajuddin, 1995; Thajuddin et al. 2002, Sudha et al. 2007, Nagasathya and Thajuddin, 2008). A detailed survey was made on the diversity and distribution of marine cyanobacteria of a continuous stretch in south India of over 2660 km of the coast line from Tirakol of Goa state to Bhimunipattanam of Andhra Pradesh encompassing the coastal regions Kerala, Karnataka and Tamilnadu including Andaman, Nicobar and Lakshwadeep group of Islands. This survey included coverage of not only the shore and deeper sea but also stagnant seawater ponds and puddles, backwater and saltpans. A total of 225 species of 58



1- *Aphanocapsa* sp., 2- *Gloeothece* sp., 3- *Microcystis* sp., 4- *Pleurocapsa* sp., 5- *Chroococcus* sp., 6- *Gloeocapsa* sp., 7- *Gomphospharia* sp., 8- Epiphytic *Dermocarpa* sp., on *Lyngbya* sp., 9- *Merismopedia* sp., 10- *Myxosarcina* sp., 11- *Spirulina* sp., 12- *Oscillatoria* sp., 13- *Phormidium* sp., 14- *Lyngbya* sp., 15- *Trichodesmium* sp., 16- *Microcoleus* sp., 17- *Nodularia* sp., 18- *Anabaena* sp., (Phase contrast microscopic view) 19- *Gloeotrichia* sp., 20- *Mastigocoleus* sp., 21- *Anabaena* sp., (fluorescent microscopic view) 22- *Westiellopsis* sp., 23- *Hormothomnium* sp.

fertilizer, medicine, industry, nanotechnology and combating pollution, since the usefulness of cyanobacteria for these purposes has been established (Emodi, 1978; Mitsui et al. 1981; Venkataraman, 1983; Gustafson et al. 1989; Moore et al. 1984; Carmichael, 1992; Bender, et al. 1994; Patterson, 1996; Prabaharan and Subramanian, 1995; Sundararaman et al. 1996; Kulik, 1995; Subramanian and Uma, 1996; Shashirekha et al. 1997; Subramanian, 1998; Jaki et al. 2001, Vijayakumar et al. 2005, 2007b; Nagasathya & Thajuddin, 2008a & 2008b; Mubarak Ali and Thajuddin 2009; Mubarak Ali et al. 2008, 2011a & b, 2012a & b, 2013; Satheesh kumar et al. 2009, 2012, Thajuddin, 2010; Rajeshwari et al. 2012, Reehana et al. 2013; Kumar et al. 2014; Lewis Oscar et al. 2015). Among several cyanobacterial species, *Spirulina* in particular is used as food supplement, due to its excellent nutrient composition and better digestibility due to delicate cell wall. *Spirulina* contain highest protein content (60-71 %) and also rich source of beta carotene and vitamin B_{12}. In addition to their applications as biofertilizer and pollution abatement, several enzymes such as protease, lipase, cellulose, urease, superoxide dismutase etc., amino acids, lipids and fatty acids and an unique sequence specific endonucleases are known from different cyanobacteria namely *Anabaena cylindrica* (*Acy* I), *Anabaena flos-aquae* (*Afl* I & *Afl* III), *Anabaena variabilis* (*Ava* I & *Ava* II), *Anabaena variabilis* UW (*Avr* II), *Nostoc sp*. PCC 7524 (*Nsp* C I), *Microcoleus sp.* UFEX 2220 (*Mst* II), which can be made available in the market at a lesser cost since relative biomass production of cyanobacteria is much less expensive than bacteria or fungi (Elhai and Wolk, 1988).

A large number of antibiotic compounds, many with novel structures, have been isolated and characterized. A wide range of antibacterial, anti-fungal and antiviral properties from the extracts of cyanobacteria have been reported. Some of the marine cyanobacteria are a potential source of long chain polyunsaturated fatty acids, especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), found useful in the prevention of cardiovascular disease. It is expected that transgenic cyanobacteria may be exploited as cell factories for the production of valuable recombinant vaccines. Many marine cyanobacteria, especially the feed grade *Phormidium valderianum* has been found to be an excellent source of phycocyanin, a blue natural colourant useful as a phycofluor in diagnostics, a technology for the inexpensive production has been developed. Cyanobacterial single cell proteins (SCP) have also been considered for use as supplement to live stock feed (Litchfield, 1983). Some strains of *Anabaena* and *Nostoc* are consumed as human food in Chile, Mexico, Peru and Philippines. *Nostoc commune* contains a high amount of fiber and moderate amounts of protein, suggested its potential use as a new dietary fiber source which plays important physiological and nutritional role in human diets (Jeraci and Vansoest, 1986). The carotenoids and

conserved stem sequences are particularly useful), the highly variable internal-transcribed spacer section of the ribosomal RNA cistron (ITS, separated by the 5S ribosomal RNA gene into ITS1 and ITS2 regions), the mitochondrial cytochrome c oxidase 1 (CO1 or COX1) gene and the chloroplast ribulose bisphosphate carboxylase large subunit (rbcL) gene. The relative utility of these can be rated by: (i) their ease of isolation from a sample; (ii) the likelihood of within individual variation; (iii) the ease of alignment and analysis; (iv) the number of sequences already known from identified specimens; and (v) the potential universality of a barcode based thereon. For all these targets, PCR facilitates amplification from even single cells, and their multicopy nature is also advantageous. Sequencing is essentially equally easy for all DNA fragments barring extreme base composition biases, polynucleotide runs and stable secondary structures. It is well accepted fact that only a few number of microorganisms were so far identified and studied when compared to the total biodiversity of microorganisms existing in the environment. Only organisms that are able to grow under the conditions imposed in the microbiology laboratories are often selected and identified, but most of the microbes remain inaccessible for traditional identification and cultivation methods. It is, therefore, widely believed that fewer than 20% of the microorganisms have been discovered. With the development of molecular level barcoding techniques in general and 16S Ribisomal RNA gene (rRNA) sequencing in particular, it has been possible to identify and compare the cultured and uncultivable microbes. The collection and analysis of molecular information have only now begun and conclusions are still limited by the relatively small amount of data available. It is expected that more laboratories will become interested in rRNA sequencing and other molecular techniques and this molecular information should be integrated with other characteristics of the strain. This will form the basis for polyphasic taxonomy that will not only be of practical use but will also reflect, as much as possible, on the evolutionary relationships of the strains.

Applications

Cyanobacteria being a prokaryotic organism having potentials of both bacteria and algae and can be easily manipulated like bacteria which shows promising in the field of cyanobacterial biotechnology. Though they have a long history of existence, and were till recently in the oblivion uncared and unrecognized, have shot into fame and popularity owing to a host of their innate properties that make them ideal organisms for use in a variety of ways to meet our needs and to promise us a bright future. The emerging and often unfulfilled demand for pharmaceutical compounds from natural sources, forces the search for alternative bioresources for these bioactive components. Cyanobacteria constitute a vast potential resource in varied areas such as mariculture, food, feed, fuel,

genera belonging to 14 families of cyanobacteria were recorded from the survey of which 35 species were heterocystous and 190 species were non-heterocystous with the members of the family Oscillatoriaceae being predominant in the marine habitats. As many as 21 species of 10 genera all are non-heterocystous could be considered as most versatile species since they occurred in all the regions of the entire area of survey (Thajuddin and Subramanian 1991 & 1992). This survey also resulted in the establishment and maintenance of marine cyanobacterial germplasm collections. Identification of several species new to India and several suspected new species are under investigation. To exploit marine cyanobacterial potentials for the benefit of mankind, a unique National Facility for Marine Cyanobacteria (NFMC) with 300 strains of marine cyanobacterial germplasm was established at Bharathidasan University by our beloved teacher Professor G. Subramanian, an eminent cyanobacterial biotechnologist with the financial support from the Department of Biotechnology, Govt. of India during the year 1991.

Trends in Taxonomy

The taxonomy of cyanobacteria both bacteriological as well as traditional botanical approach rely primarily on morphological characteristics and a few biochemical traits. The main problem met with in applying morphological criteria in cyanobacterial classification arises from the considerable variability of morphological features with different environmental conditions (Praveen Kumar et al. 2007; Pandiaraj, et al. 2012). The biochemical characters were shown to be variable in changing media and environmental conditions. Several chemotaxonomic methods viz. protein & fatty acid fingerprinting, carotenoids, phycobilisomes, protein-isozyme patterns, etc. were used for species and strain level differentiation with little success. In addition, the traditional taxonomy has in the past met human needs in biodiversity conservation and management, agricultural production and human health. However, as the demand for taxonomic services increases, traditional taxonomy is becoming inadequate in coping with the demand. The issue is aggravated by the fact that the number of taxonomists is significantly getting reduced globally. To evaluate the validity of their classification and to investigate their genetic relationships among cyanobacteria, different PCR based molecular tools may be used. Among the various molecular techniques, the determination of 16S rRNA gene sequences is widely used because these sequences are universally present in living organisms and this molecule is composed the regions of higher and lower evolutionary conservation and more variable regions that have been used in differentiation of genera and species. In addition, other PCR based methods namely Randomly Amplified Polymorphic DNA (RAPD), Short Tandemly Repeated Repetitive Sequences (STRR), REP-PCR fingerprinting group I introns (Muralitharan and Thajuddin, 2008) etc. may be used in all taxonomic levels.

The PCR-based RAPD fingerprinting technique of utilizing arbitrary oligonucleotides to prime DNA synthesis at low annealing temperatures to divulge genomic diversity is a particularly powerful typing method. Unlike the traditional PCR analysis, which requires specific knowledge of sequences, RAPD does not require any specific knowledge of the DNA sequences of the target organism (Thajuddin and Muralitharan, 2008). This makes it a tool of great power and general applicability. It has been shown that as few as three primers used separately provide enough polymorphic information to identify species of the symbiotic genus *Anabaena* and to create a phylogenetic tree with topology similar to that derived with 22 primers.

Repetitive sequences constitute an important part of the prokaryotic genome. The Repetitive Extragenic Palindromic (REP) and Enterobacterial Repetitive Intergenic Consensus (ERIC) sequences were originally described for the family Enterobacteriaceae but later found in several gram-negative bacteria and close relatives in the same phyla. For cyanobacteria, distinct families of repetitive sequences, the short tandemly repeated repetitive (STRR) sequences, have been described. The STRR sequence was demonstrated to be a valuable tool for identification and characterization of cyanobacteria. In addition, a 37-bp Long Tandemly Repeated Repetitive (LTRR) sequence has recently been identified in *Anabaena* strain PCC 7120 and was detected in both heterocystous and non-heterocystous cyanobacteria.

In M13 PCR technique, a single primer (5'- GAGGGTGGCGGTTCT -3') specific to the core sequence of phage M13 minisatellite DNA is employed to amplify hypervariable genomic DNA sequences. Because a part of the sequence of this bacteriophage is known to be present in many organisms, it would not be considered similar to RAPD-PCR. M13 fingerprinting has already been used with success for the identification of other organisms at species and strain level differentiation.

Cyanobacteria possess a ribosomal RNA (rRNA) cistron comprised of three genes; the 16S small subunit (SSU), 23S large subunit (LSU) and the 5S subunit, each separated by an internal transcribed spacer region (ITS). A genetic marker often used in phylogenetic studies is the 16S rRNA gene. Within cyanobacteria, sequence information from this gene is widely regarded as one of the most valid criterion for determining relationships between closely related groups, such as species or genera. It is the basis for systematic assignment in the latest edition of Bergey's Manual of Systematic Bacteriology and has been useful in distinguishing broad taxonomic groups as well as individual species. Direct sequence analysis of this region has been fundamental in phylogenetic studies of cyanobacteria and has resulted in a large expansion of the molecular data that is available in public databases such as GenBank (Thajuddin, et al. 2007).

Following steps can be used to study the 16S rRNA gene sequence based phylogeny of cyanobacteria,

1. Extraction of genomic DNA from cyanobacteria through alkaline lysis method.
2. Amplification of 16S rRNA gene using universal eubacterial 16S rRNA gene coding regions in the genomic DNA.
3. Cloning of amplified 16S rDNA into pGEM-T vector using T4 DNA ligase for getting recombinant plasmid DNA.
4. Transform these recombinant plasmids to high efficiency competent *E. coli* (JM109) cells for the separation and further multiplication of 16S rDNA.
5. Inoculate the transformed *E. coli* cells on the solid medium containing Ampicillin, X-Gal and IPTG. Since the JM109 *E. coli* cells are ampicillin sensitive, the untransformed cells may not grow on the ampicillin amended medium. Due to the presence of AmpR genes in the pGEM -T vectors, only transformed cells can grow on the ampicillin containing medium. The cells containing recircularized plasmids were appeared as blue coloured colonies and those containing recombinant plasmids appeared as white coloured colonies.
6. Isolate the recombinant plasmids from the white colonies using standard plasmid isolation method.
7. Sequence the plasmids containing cyanobacterial 16S rRNA gene by following dideoxy sequencing method using radio-labeled α35S dATP.
8. Compare the 16S rRNA gene sequences with other sequences available in GenBank and understand their phylogenetic relationships using suitable bioinformatics tools.

DNA Barcoding

It is currently clear that DNA barcoding technique is increasingly complimenting the efforts made by taxonomists. It is becoming a tool that could settle conflicts arising among taxonomists on species identities. DNA Barcoding is a new science technique and global standard for the identification of biological species using a short gene sequence from standardized position in the genome. It provides a way to identify the species to which a plant, animal or fungus/bacterium belongs. With a sequence-based molecular taxonomy, a single technique is applicable to all taxa: Dr. Paul Hebert and his colleagues at University of Guelph in Ontario, Canada first proposed and initiated DNA Barcoding of life forms particularly eukaryotes based on mitochondrial cytochrome C oxidase I (CO1) in 2003. The sequences used thus far for molecular barcoding are the nuclear small subunit ribosomal RNA gene (SSU, also known as 16S in prokaryotes, and 18S in most eukaryotes), the nuclear large-subunit ribosomal RNA gene (LSU, also known as 23S and 28S; the highly variable expansion loops that are flanked by

CO_2 Incubator

qRT-PCR

Fermenter

Thermocycler

Gel Documentation Unit

Centre for Plant Tissue Culture

-20°C Deep Freezer

Centre for Plant Tissue Culture

SOUVENIR

POPULAR LECTURE SERIES in BIOTECHNOLOGY

01st October 2015

Sponsored by

सत्यमेव जयते

Department of Biotechnology
Govt. of India
New Delhi

Organized by

UKA TARSADIA university

Imparting Knowledge. Awakening Wisdom. Transforming Lives.

C. G. BHAKTA INSTITUTE OF BIOTECHNOLOGY

Maliba Campus, Bardoli, Dist. Surat, Gujarat, Pin : 394350

POPULAR LECTURE SERIES in BIOTECHNOLOGY
Programme Schedule

Lecture 1: Biodiversity and biotechnological applications of cyanobacteria
Date:19/09/2015

Dr. N. Thajuddin
Professor & Head
Department of Microbiology
Bharathidasan University
Tiruchirappalli-23, Tamil Nadu

Lecture 2: Arbuscular Mycorrhizal fungi: Application in plant production system & monitoring its quality parameters
Date: 01/10/2015

Dr. Mahaveer P. Sharma
Principal Scientist
Directorate of Soybean Research (ICAR)
Indore, Madhya Pradesh

Lecture 3: Plant Tissue Culture methods with special reference to cultivation of medicinal plants
Date: 12/10/2015

Dr. R. Krishnamurthy
Director
C.G. Bhakta Institute of Biotechnology
Uka Tarsadia University
Bardoli, Surat, Gujarat

Research Facilities at CGBIBT


Green House Facility


Electrophoresis Unit


Fluorescence Microscope


Inverted Microscope


Net House Facility

Fortin JA, Becard G, Declerck S, Dalpe Y, St Arnaud M, Coughlan AP and Piche Y (2002). Arbuscular mycorrhiza on root-organ cultures. Can. J .Bot. 80: 1-20.

Nichols, KA and Wright SF (2005). Comparison of glomalin and humic acid in eight native United State soils. Soil Sci. 170:985-997.

Rillig, MC, Wright SF, Allen MF and Field CB (1999). Rise in carbon dioxide changes soil structure. Nature. 400:628-628.

Sharma MP and Sharma SK (2008). On-farm production of arbuscular mycorrhizal fungi. Biofertilizer Newsletter. 16 (1) 3-7.

Sharma MP and Buyer JS (2015) Comparison of biochemical and microscopic methods for quantification of mycorrhizal fungi in soil and roots. Applied Soil Ecology 95: 86-89.

Sharma MP, Gupta S, Sharma SK and Vyas AK (2012) Effect of tillage and crop sequences on arbuscular mycorrhizal symbiosis and soil enzyme activities in soybean (*Glycine max* L. Merril) rhizosphere. Indian J. Agricultural Sciences 82: 25-30.

Sharma MP and Adholeya A (2011) Developing prediction equations and optimizing production of three AM fungal inocula under on-farm conditions. Exp. Agriculture. 47 (3): 529-537.

Sharma MP and Adholeya A (2004) Influence of arbuscular mycorrhizal fungi and phosphorus fertilization on the *post-vitro* growth and yield of micropropagated strawberry in an alfisol. Can J. Botany. 82 (3): 322-328.

Sharma MP, Gaur A, Bhatia NP and Adholeya A (1996) Growth responses and dependence of *Acacia nilotica* var. cupriciformis on the indigenous arbuscular mycorrhizal consortium of a marginal wasteland soil. Mycorrhiza. 6: 441-446.

Wright SF and Upadhyay A (1996) Extraction of an abundant and unusual glycoprotein from soil and comparison with hyphal protein from arbuscular mycorrhizal fungi. Soil Sci.161: 575-586.

POPULAR LECTURE SERIES in BIOTECHNOLOGY

Title

Arbuscular Mycorrhizal Fungi: Application in Plant Production Systems and Monitoring its Quality Parameters

Speaker

Dr. Mahaveer P Sharma

Principal Scientist (Agri.Microbiology)
Agricultural Res. Service
ICAR-Directorate of Soybean Research
(Erstwhile; National Research Centre for Soybean)
Department of Agricultural Research & Education, Min. of Agriculture
Khandwa Road, Indore (M.P)

Sponsored by

सत्यमेव जयते

Department of Biotechnology
Govt. of India
New Delhi

Organized by

UKA TARSADIA university
Imparting Knowledge. Awakening Wisdom. Transforming Lives.

C. G. BHAKTA INSTITUTE OF BIOTECHNOLOGY
Maliba Campus, Bardoli, Dist. Surat, Gujarat, Pin : 394350

01st October 2015

Future direction of research in AMF

It has also been established that combined uses of both AMF and plant growth promoting microbes (PGPMs) including biocontrol agents increases the efficacy towards higher growth, mineral nutrition and cope with abiotic and biotic stresses. But the magnitude of response varies with the rhizosphere-to-rhizosphere, which need to be deciphered and customized. There is need to modulate the mycorrhizosphere to maintain higher hyphosphere activity to manage resident AMF and PGPMs for improving the plant and soil health and should be the key aim of the applied area of microbial uses in the future. The combined application of AMF and PGPMs and their inoculum load required to enhance the growth and nutrient and the underlying mechanisms are yet to be streamlined and need rigorous evaluation. The use of modern biotechnological tools should be employed to study the shift in the microbial population and identify the microbes playing negative and positive role in maintaining healthy mycorrhizosphere engineered by the inoculation of AMF and other microbes. The large-scale production of resident AMF is still yet to go a long way. The root organ culture (ROC) technology can only be viable and fast technology option available before the microbiologists but there is need to use location specific strains under *in vitro* production which is yet to be optimized. Besides production of AMF, their mode of application with increased efficacy under field conditions is questionable which needs rigorous evaluation. The compatibility studies of AMF with other PGPMs and biocontrol agents such as entomopathogenic nematodes should be assessed before they release as commercial formulations. Finally, the potential candidates (commercial formulation) would then be subjected to regulatory requirements, quality checks eventually to register as commercial biofertilizer/bioinoculants products.

Selected references

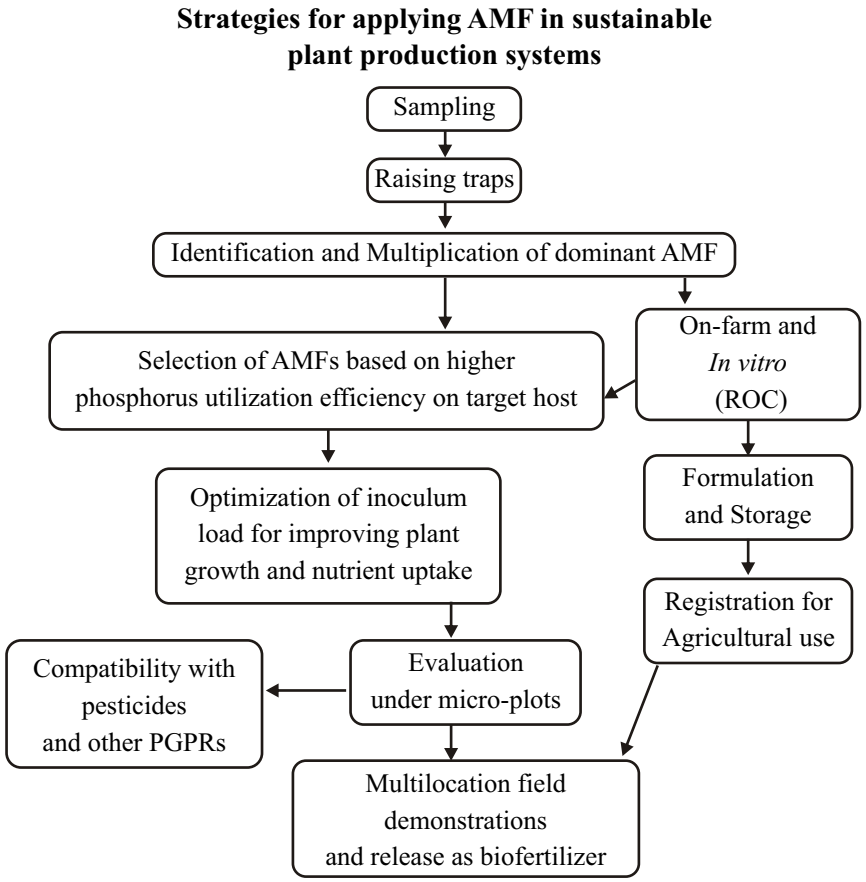
Agnihotri R, Pandey A, Ramesh A, Patra AK, Hazela K and Sharma MP (2015) Soil Carbon sequestration through glomalin production by arbuscular mycorrhizal fungi in soybean-maize intercropping system under organic and inorganic farming practices. In : Proceedings of National workshop on carbon sequestration in forest and non forest ecosystems, held in Jabalpur, India, Feb 16-17, 2015 pp 38.

DJ Bagyaraj, Sharma MP and Maiti D (2015) Phosphorus nutrition of crops through arbuscular mycorrhizal fungi Current Sci. 108 (7): 1288-1293

Douds DD Jr, Nagahashi G, Pfeffer PE, Reider C, Kayser WM (2006) On-farm production of AM fungus inoculum in mixtures of compost and vermiculite. Bioresour Technol. 97:809-818

and biochemical methods seems to be more promising than microscopic methods as unlike microscopic methods the reproducibility of such method is not much affected by person to person skill variation.

Figure 1: Scheme of mycorrhiza management for sustainable production of crops



Organizing Committee

Patrons

Dr. D. R. Shah Shri. Kiritbhai Patel Shri. Bhagubhai Patel

Chairman

Dr. R. Krishnamurthy

Co-ordinator

Dr. N. Amaresan

Discipline Committee

Dr. Meonis Pithawala Prof. Rajashekar I
Dr. Sankar Narayanan

Registration Committee

Dr. Parul S
Ms. Urjita Sheth
Dr. Vijayalakshmi G
Ms. Dhruti H. Mistry
Ms. Nilofer Sheikh
Ms. Amita Rathod

Stage Committee

Dr. Prasant Kumar
Dr. Anoop Markande
Dr. Mitesh K Dwivedi
Dr. Gopal Jee Gopal
Dr. Srivathsa N
Mr. Bhrugesh Joshi

Advertisement Committee

Ms. Nimisha Patel
Ms. Avani Kasture
Ms. Sonal Vashi
Ms. Vrutika Lad
Ms. Divya Patel
Ms. Shraddha Saha
Ms. Swetal Patel
Ms. Trusha Mistry
Ms. Avani Pandya
Mr. Harsh Desai
Ms. Shreya Desai
Ms. Jemisha Mistry
Ms. Sandipa Thesiya

Food Committee

Mr. Prittesh Patel Mr. Rushabh Shah

About the Institutes

Uka Tarsadia University

The establishment of Uka Tarsadia University (UTU) in 2011 by the Bardoli Pradesh Kelavani Mandal (BPKM) is an effort towards meeting the growing demand for quality education. The University offers admission in a comprehensive array of academic programs across the disciplines of Pharmacy, Management, Architecture, Nursing, Physiotherapy, Computer Science, Engineering & Technology and Applied Science. The university provides educational opportunities to over 7500 students. More than 350 faculty members of the university represent an eclectic mix of professional and academic, national and international experiences. UTU has established MoUs with several Foreign Universities, like Cape Breton University (Canada), University of Ilorin (Nigeria) and University of Prince Edward Island (Canada).

C. G. Bhakta Institute of Biotechnology

CGBIBT of Uka Tarsadia University (UTU) is ably managed by Bardoli Pradesh Kelavni Mandal (BPKM) which needs no introduction about its significant contribution in providing collegiate education over the last 60 years. CGBIBT established in 2005 for initiating exclusive education programs in Biotechnology and Basic Science. Since its establishment, CGBIBT has been striving to emerge as a center of excellence in imparting career based knowledge to students to realize their professional ambition. In addition, CGBIBT has also taken up a mission to support the local farming community by supplying quality planting materials. For this purpose, a sophisticated plant tissue culture laboratory and climate controlled Green House have been established. The institute is offering five years Integrated M.Sc Biotechnology / Microbiology; two years M.Sc Biotechnology / M.Sc Microbiology as well as Ph.D program in Biotechnology / Microbiology. The institute is equipped with basic as well as advanced instrumentation facilities. As a part of academic exchange programme and research collaborations, CGBIBT, UTU has signed an MoU with University of Ilorin (Unilorin) Nigeria & IITA, Nigeria and many private industrial organizations.

mycelium/hyphae. The active form of inoculum i.e., the one having on-going symbiosis has to be applied with caution. The ease in application of AMF inoculum is one of the most crucial points to get its maximum benefit. Another point to remember is the presence of a live root so as to initiate and continue symbiotic relationship. The absence of such a condition will lead to the death of AM propagules. Material like sand, farmyard manure is some of the carriers which aid in lab scale production of AM inoculum whereas industrial production uses material like calcined clay etc. A single propagule is considered to be the one which has a granule of carrier material attached to AM propagules. Now a day's many commercial companies are selling AMF products which are being applied as seed treatment and as well as soil application.

Testing the quality of AM inocula

The quality of AM inocula determines its efficiency to serve various symbiotic functions as well as its production in bulk quantity. A particular mycorrhizal species could be dominant in a particular region like *Glomus* dominates in agro-ecosystems. However in some cases *Funneliformis mosseae* has been found to dominate in tilled soils and *Glomus* dominated in undisturbed ones (Avio et al, 2013). This generates the need to study the indigenous/native AM population of a particular soil so as to get maximum positive results of inoculation as well as mass production of that particular strain. Recently in Gazette 2010 by Ministry of Agriculture, Govt. of India, AM fungi has been notified as AM fungal Biofertilizers. The quantification of AM population is the prerequisite for maintaining its quality and protection from spurious AMF products (Figure 1).

The AM propagules are routinely examined by quantification of spore density and root colonization. In the commercial inocula the most probable number (MPN) method (Porter 1979) and infection unit method (Liu and Luo. 1994, Sharma et al. 1996) are mainly used. But these methods rely on utilization of specific stains to visualize the AM-roots and then quantification based on person's skills in microscopic observations and often lacks reproducibility of quantified data by different observers. More recently molecular techniques have also been developed to determine AM fungi based on gene copy number using taxa specific primers and probes (Thoner et al., 2012). Other biochemical techniques involving analysis of signature lipid biomarkers mainly NLFA and PLFA 16:15 in roots and soil (Olsson, 1999; Sharma and Buyer 2015) and sterols (Fontaine et al., 2004) in roots are becoming reliable and useful tools to quantify the live biomass AM fungi in inoculum and infected roots/soil rhizosphere samples. However, these methods need to be re-visited with a known AMF species with a known count so as to identify the most suitable analytical method and highly reproducible. Molecular

propagation of some AMF strains on ROC allowed the cultivation of monoxenic strains that can be used either directly as inoculum or as starting inoculum for large-scale production. *In vitro* propagation on ROC consists of excised roots that proliferate under axenic conditions on a synthetic nutrient medium supplemented with vitamins, minerals, and carbohydrates. The excised roots are the Ri T-DNA-transformed, which have been used to obtain colonized root cultures. For the first time the use of Ri T-DNA transformed roots of *Daucus carota* as host by *Agrobacterium rhizogenes* has permitted to sporulate *G.mosseae*. Transformation is done by inserting *A. rhizogenes* in transformed root copies of T-DNA (transfer DNA), which are found in a large plasmid of *A. rhizogenes*. The transformed roots have an ability to grow profusely and vigorously (negative geotropism) and to provide opportunity for AMF hyphae to make contact (Becard and Fortin 1988). Several sub cultures (3-4) of these roots are necessary in this medium enriched with antibiotics such as carbenicillin or ampicillin to obtain free living roots without bacteria and for establishing dual culture. Monoxenic cultures are developed through extraradical spores and infected root fragments. In most cases surface sterilized spores (Becard and Piche, 1992) isolated from the field or from traps have been successful for establishing dual cultures under *in vitro* conditions. Spore sterilization can also be achieved with a solution containing chloramine T (oxidizing agent) and Tween 20 (surfactant) (Fortin et al. 2002) and rinsed in a streptomycin-gentamycin antibiotic solution (Becard and Piche 1992). While the success of spore germination is solely dependent on sterilization, the presence of root exudates and 2% CO_2 can stimulate germination and post germination hyphal growth. The infected roots, which come from trap culture roots, can be used as a section of vesicles for *in vitro* establishment. The root sections can be disinfected in an ultrasonic processor under aseptic conditions and incubated in MSR (Modified Strullu- Romand) medium (Declerck et al. 1998). The most widely used media for establishing *in vitro* AM root cultures are M (Minimal) (Becard and Fortin 1988; Fortin et al. 2002) and MSR (Declerck et al. 1998) media. Regular subculturing is required to maintain higher infectivity. Continuous cultures can be obtained by transferring mycorrhizal roots to fresh medium either with or without (Declerck et al. 1996) spores. It is always preferable to use actively growing roots.

Inoculation method

Mycorrhizal inoculum is applied to soil in such a way that it resides in the rhizosphere to get contact with the active root and should begin to colonize. It is mainly applied by layering method just below the seed and prior to sowing. Inoculum is processed in many forms where it contains infected root bits, spores,

Biography

Dr. Mahaveer P Sharma
Principal Scientist (Agri.Microbiology)
Agricultural Res. Service
ICAR-Directorate of Soybean Research
(Erstwhile; National Research Centre for Soybean)
Khandwa Road, Indore (M.P)
E mail: mahaveer620@gmail.com
mahaveer620@yahoo.com

Dr. Mahaveer P Sharma has born on 5th September, 1966 at Ajabpura, Alwar, Rajasthan. Currently, he is working in Agricultural Research Service as Principal Scientist (Agricultural Microbiology) at Directorate of Soybean Research, Indore (Indian Council of Agricultural Research-DARE, Ministry of Agriculture, Govt. of India). Dr. Sharma started his research career in mycorrhizal fungi research while working at University of Delhi and then he moved to TERI, New Delhi where he further gained expertise in arbuscular mycorrhizal fungi (AMF) research and completed his doctorate. Dr. Sharma is graduated in Agriculture from Rajasthan Agricultural University during 1991 and is recipient of gold medal award during his Master's programme. He has obtained post graduate diploma in Intellectual property rights in 2009 from Indra Gandhi National Open University, New Delhi.

Dr. Sharma has several awards to his credit like best paper presentation, travel grant awards for visiting abroad (USA, Australia, Portugal, Germany, Switzerland etc.,) in various scientific meetings/conferences. Recently Department of Biotechnology (Govt. of India) has awarded him CREST Award-2013 under that he deputed to USA for 6 months and worked in USDA-ARS at BARC, Beltsville (Washington DC) on "signature fatty acid biomarkers to assess live biomass of AMF and soil microbial community changes in long-term farming system trials" managed at USDA-ARS, Beltsville. Recently he was deputed to Portugal for participating in International symbiosis congress and to have discussions at FiBL and ETH institutions in Switzerland to develop possible linkages in the area of mycorrhizal quality and soil health aspects.

Dr. Sharma has published 67 national and international research articles in refereed journals, magazines and reviews articles in books of international repute. He has also written one book and technical bulletins as well. He has published several abstracts in national and international conferences. In addition, he has many microbial accessions with NCBI database and cultures deposited in International

Microbial Repository Authorities. He is also serving as an editorial board member in ARPN Journal of Agricultural and Biological Sciences, Journal of Agriculture and Crop Sciences and Krishi Sampada (Global Agriculture Magazine). He is reviewer in several national and international journals such as Indian J. Microbiology, European J. Soil Biology, International J. of Phytoremediation, Archives of Agronomy and Soil Science, Applied Soil Ecology etc. Dr. Sharma is contributing as a member to several expert panel/advisory committees and Professional societies such as PAC, MP Biotechnology Council, Govt. of MP, Madhya Pradesh, Indian Society of Mycology and Plant Pathology, MPUAT, Udaipur, Member Mycorrhiza Net work Asia, TERI, New Delhi, Member Soybean society for research and development, Member International Association of Mycorrhizologists and Association of Microbiologists of India.

Dr. Sharma has organized a short-term training on basic techniques of AM fungi for biofertilizer industry production staff from June 14-17, 2011 at DSR, Indore. He has also been deputed by TERI under a MoU agreement to organize a training on basic aspects of mycorrhizal research for Agricultural scientists at Soil and Water Research Institute, Ministry of Agriculture, Republic of Iran, Tehran (Feb 21st-9th March 2001). He has also acted resource person in the area of mycorrhizal research during ICAR and UGC refresher courses/winter/summer schools on various occasions.

Dr. Sharma has supervised 12 Master's students and co-supervised one PhD student for their theses. He has successfully executed 11 research projects as PI and Co-PI, funded by TIFAC, Department of Science and Technology, New Delhi; DBT, Ministry of Science and Technology; ISCB, Indo-Swiss govt.; DSR (ICAR) Indore. Currently, he is executing three research projects funded by DST, New Delhi; DSR-ICAR; ICAR-NBAIM.

Significant achievements:

- Development of a gene pool of soybean rhizobia from Malwa Region of Central India out of which identified two potential root nodulating soybean rhizobia capable of enhancing nodulation and soybean growth which are under commercialization for field utilization in soybean.
- Standardization of PLFA profiling for studying microbial community in soil and Optimized FAME-MIDI system for identifying culturable microbes.
- Standardization of low cost "On-farm production system for mass production of native Arbuscular mycorrhizal fungi biofertilizer" at DSR Indore (National Centre of Organic Farming (earlier national Biofertilizer centre), Ghaziabad published special issue of "Biofertilizer News Letter" on this technology.
- Optimization of single spore culture technique of mycorrhizal fungi suitable for conventional multiplication of mycorrhizal fungi.
- Optimization of protocol of glomalin extraction in soil to study soil C sequestration by AM fungi.

Current research interests:

Dr. Sharma is currently engaged in soil microbiological research involving the

Pre-colonized plants on sterile substrate are needed for these systems. However, plants can be infected directly in the aeroponic system. In hydroponics inoculum can be propagated using the nutrient film technique (NFT) by growing pre-colonized plants in a defined nutrient solution, which flows over the host roots. In an aeroponic system, (a fine mist of defined nutrient solutions) pre-colonized roots are suspended in air and are bathed in (Hung and Sylvia, 1998). This method of AM multiplication is free from any substrate and can be sheared which resulted in to very high propagules number.

Production in Aeroponics

AMF production is also can be produced aeroponically (Sylvia and Hubbell 1986). The aeroponic system was adopted for mycorrhiza production by the utilization of seedlings with roots pre-colonized by an AM fungus and the use of modified Hoagland's nutrition with a very low P level. In the aeroponic system, colonization and sporulation was superior to that reported in soil-based pot culture. Jarstfer and Sylvia (1995) further determined the viability and density of aeroponically produced inocula after shearing. *Entrophospora kentinensis* was successfully propagated with bahia grass and sweet potato in an aeroponic system by Wu et al. (1995). Mohammad et al. (2000) reported the production of *Glomus intraradices* in an aeroponic system where they compared the conventional atomizing disc with the ultrasonic nebulizer technology as misting sources.

Root-organ culture or axenic culture of AM fungi

The root organ culture is dual culture (plant roots and fungus) in which root inducing transfer DNA -transformed roots of a host plant is used to develop the symbiosis under *in vitro* on a specific medium (Fortin et al., 2002). The hairy roots formation occurs due to transfer of root inducing Ri-plasmid from the bacterium, *Agrobacterium rhizogenes*. This system offers a rapid, clean and more efficient method and opens up the avenues for AM fungi commercialization and field application.

The root organ culture (ROC) is a most attractive mass multiplication method for providing a pure, viable, rapid and contamination free inoculum using less space and has an advantage over the pot culture multiplication/conventional system (Fortin et al. 2002; Cranenbrouck et al. 2005; Tiwari et al. 2003). The mono-specific strains available can be used directly as starting material for large-scale inoculum production, a sole Petri dish culture being enough to generate several thousand spores and meters of hyphae within four months. Douds (2002) reported monoxenic culture of *G. intraradices* with Ri T-DNA transformed roots in two-compartment Petri dishes as a very useful technique for physiological studies and the production of clean fungal tissues. Fortin et al. (2002) developed successful

A greater share of glomalin to soil organic carbon has been found when compared to the share of microbial biomass carbon (Rillig, 2004). The dry weight of AMF (0.03 to 0.35 mg/g) constituted major part of microbial biomass carbon as estimated through Phospholipid fatty acid analysis (Olsson, 1999). Treseder and Turner (2007) also found that glomalin and carbon dynamics were linked. A more promising approach to study the contribution of glomalin to SOC is Total glomalin stocks to SOC stocks ratio. Bai et al (2013) found this ratio in the range of 6.91 to 23.97%. A significant positive correlation between glomalin and carbon stocks was found to be present in AM inoculated soybean- maize intercropping system managed with organic farming, suggesting the role of glomalin in maintain C-stocks (Agnihotri et al, 2015).

Methods of producing AM inocula

One of the major bottlenecks for large-scale application of mycorrhiza is the unavailability of adequate inoculum, since AM fungus is an obligate symbiont and it requires a host to complete its life cycle. There are various methods currently being used to mass-produce these fungi.

Conventional method or soil-based inoculum production

Culturing AM fungi on living plants growing in disinfected soil has been the most frequently used technique for increasing the number of propagules. The conventional methods involve the use of starter inoculum/material and grow on soil in pots and beds using suitable trap plants (Douds et al. 2006; Sharma and Sharma 2008; Sharma and Adholeya, 2011). Further to bulk up on mass scale at DSR, Indore the potential AMF was propagated in raised beds at on-farm under field conditions. The use of on-farm system for the production of AM fungi can reduce the cost of AM fungi production, enhances its utilization for producing quality seedlings and wider acceptability to the farmers/growers. The trap plants namely, maize, marigold, sorghum, fenugreek and barseem grown in succession on raised beds amended with vermi compost enhanced the AM fungi infectious propagules from 1.02 to 16.83 and 12.10 IP/g soil in pots and raised beds, respectively (Sharma and Sharma , 2008). The on-farm production method found to be more economical and can also be used for production of quality seedlings of horticultural plants.

Soilless substrate based system

AMF can be cultured on plants in suitable soilless media with appropriate nutrients. Hoagland nutrient solution is currently being used routine to grow AMF with inert materials in pots. Bark, calcined clay, expanded clay, pertile, soilrite and vermiculite have been used inert substrates. Pre-colonized roots, spores and hyphae of AMF could be produced in hydroponics or aeroponics systems.

uses of plant growth promoting microbes particularly AMF role in C-sequestration, drought tolerance and improving the productivity of soybean. In particular, his research activities include:

- Fatty acid methyl esters (FAME)/PLFA profiling of microbes in soil, identify signature fatty acids for active AMF symbiosis, and characterize microbes and communities using MIDI system.
- Development of gene pool of plant growth promoting microbes particularly soybean rhizobia and mycorrhizal fungi capable of functioning at high temperature yet enhancing soybean growth.
- Understanding the microorganisms for their potential uses towards mitigating drought and minimising global warming by sequestering carbon in soil.
- Development of ROC/invitro mass production technology of AM fungi.

Arbuscular Mycorrhizal Fungi: Application in Plant Production Systems and Monitoring its Quality Parameters

Dr. Mahaveer P. Sharma

Introduction

Taking the current food grain production scenario into consideration, India has a compulsive need to raise food production by 5 million tonnes per year as against 3.1 million tonnes per year achieved over the past 40 years. This is a great challenge before the Indian agriculture. Since the land to man ratio is narrowing rapidly, there is almost no scope for horizontal expansion to meet the future demand of production. The unilateral approaches viz., intensive use of agro-chemicals, high yielding varieties, resources, monoculture etc., adopted for increasing the production have generated environmental and biological imbalance which are the major challenges to be met for a sustainable agricultural system. The declining trend in carrying capacity of land and other natural resources have drawn the attention of environmental, agricultural and social scientists in present time to look for an alternative approach for sustaining the resources supporting the ecosystem and human life as a whole. Also, the ever-growing population of India triggers the transition from traditional practices to modern biotechnological-based agriculture. In the doorsteps of 21st century, Indian agriculture is facing challenging task to provide food security as well as nutritional security for all. Agricultural practices have begun to integrate emerging technologies like biotechnology and biofertilizers with traditional practices like organic farming. Biofertilizers are very useful when used in combination with organic manure and inorganic fertilizers in a balanced proportion. Among biofertilizers, the mycorrhizal fungi particularly arbuscular mycorrhizae (AM), the most common fungal association formed nearly in all cultivated plants whether they are agricultural, horticultural or forestry plant species is gaining importance. It is one of the fungal biofertilizer has proven potential in plant production sustaining the low input and organic agriculture. Mycorrhizal biofertilizer has a broad spectrum, benefiting majority of the crops and plants. It benefits plants through a multiple action via absorption of nutrients, particularly P, water absorption, disease resistance, heavy metal toxicity, resistance to salt stress etc. Mycorrhizal biofertilizer can also be applied in stress environments like eroded soils, salt stress or in soils with heavy metal toxicity. Significant yield enhancement through field application of AM fungal inoculum has been recorded in cereals, fruits, ornamentals and timber plants. Augmentation of the mycorrhizal fungi along with traditional practices and integration with inorganic fertilizers is one of the important approaches in sustaining plant productivity.

selected for *in situ* multiplication of native AMF to harness the maximum utilization for large scale reclamation activities. A variety of hyper accumulator-AM mediated herbaceous plants like vetiver grass, bahia grass, *Imperata cylindrica*, maize, *Dianthus maryizii*, Sorghum, *Festuca brigantina*, sunflower, castor and forestry species like Jatropha, Gamhar, Poplar etc., was screened as potential phyto-bioremediator plants using mine-spoil/fly ash substrates. The pilot study was consisted of a fly ash site where plants were established through AMF inoculation and developed a thick green belt yet reclaiming the ash by arresting heavy metals (Sharma and Adholeya, 2005). The technology involved application of mycorrhizal fungi along with organic manures with customized tree-herbaceous species combination which helped in developing greenery at a faster rate at the same time reduced the heavy metal contamination in fly ash and adjoining areas. The demonstration consisted of four tree species viz., *Gmelina arborea, Casuariana equisetifolia, Melia Azadirch,* and *Albizzia procera*, planted in combination with seasonal herbaceous species viz., *Vetiver, Mentha, Tagetus, Polianthes, Helianthus* etc. and heavy metal and nutritional properties was tremendously improved after 24 months of inoculation and establishment of plants.

Role of AMF in soil carbon sequestration through production of glomalin

Nearly 80% carbon is found in soil and is about 2500 GT which has 1550 GT organic fraction and 950 GT inorganic carbon (Lal 2004, 2008). AM fungi via their extra radical hyphae help in soil aggregation where carbon gets securely stored inside soil aggregates. Plant roots associate with ERH of AMF thereby forming stable soil aggregates which in turn gets supported by glomalin (Wright and Upadhyay, 1996) which is a glycoprotein produced abundantly on AM hyphae about 80% of which is present on spores and hyphae and 20% is released into the soil (Driver et al. 2003). Various factors such as land use management practices, nutrient management, climate, nature of crop (mycorrhizal or non mycorrhizal), microbial inputs decide the extent of C-sequestration inside soil. AMF has been found to make significant contribution to soil organic carbon (Rillig, 2004). Earlier it was considered that AMF contributes to soil aggregate stability and carbon sequestration, through hyphal mediated entrapment of soil aggregates. But AMF produces an extremely thermostable glycoprotein called glomalin which maintains better soil quality and aids in soil carbon sequestration by making stable soil aggregates within which soil carbon gets sequestered (Wright and Upadhyay, 1996; Wright and Upadhyay, 1998). Except for the genera *Sclerocystis*, all AMF species have been found to produce glomalin (Wright and Upadhyay, 1998). The residence time of glomalin in soil is found to be 6-42 years (Rillig et al, 2001).

Management of mycorrhiza in the stressed ecosystems

AMF are being used in the remediation of environmentally vulnerable ecosystems and carbon mitigation strategies through sequestering soil carbon in the rhizosphere of plants.

Role of AMF in the remediation of fly ash overburden sites

In India, of the total, about 21% area is under wastelands. Wastelands generated through industrial activities such as mining coal, copper, iron and zinc has created a real challenge to reclaim and rejuvenate such environmentally vulnerable sites. Coal-mining results in a huge dumps of overburden material known as coal-mine spoil, which is a physically, nutritionally and microbiologically impoverished habitat. The fly ash (burned coal) is also a major chuck continuously being generated through thermal power plants of India. Given the growth plans of power sector, the fly ash production is going to be about 170 million tonnes per annum by 2012. Already about 1500 million tonnes of ash accumulated and has occupied around 25,000 hectares of productive land. Further more productive lands will be needed to accommodate the ever burgeoning fly ash.

Not only this, the polluting industries which are involved in creating such environments have a threat of global warming. Once the parties to the Kyoto Protocol to the United Nations Framework Convention on Climate Change decide to approve it, each industry will require exploring the mitigation strategies to gain carbon credits to reduce the global warming. Hence, therefore is need of an hour to look a biological solution to rejuvenate such denuded ecosystems yet enhancing carbon sinks for addressing the issue of climate change.

There is great potential to utilize AM fungi for stress alleviation, to bioremediation in sites polluted with heavy metals. These fungi play a vital role in metal tolerance in AM-mediated plants and have ability to survive plants growing on heavy metal contaminated soils. External mycelium of AM fungi provides a wider exploration of habitat by spreading several meters beyond the root exploration zone, thus providing access to greater volume of heavy metals present in the rhizosphere. The extrametrical hyphae provide a route for movement of metals from the habitat to the plant. Another important feature of this symbiosis is that AM fungi can increase plant establishment and growth despite high levels of soil heavy metals due to better nutrition, water availability and soil aggregation properties associated with this symbiosis. The AM-inoculated plants established at such sites sequester more carbon through the secretion of carbohydrate-glue like substance called 'glomalin' which act as a carbon sink. Besides rapid growth of due to AM-plants, the glomalin also helps in aggregation of fly ash and other eroded / denuded mine-spoil particles. Under a pilot study, niche-based native AMF was

Major benefits

- Enhanced root absorption capacity due to increased absorption area through mycorrhizal hyphae, which increases the mobilization and transfer of nutrients (P, N, S, Cu, Zn) from soil to plant
- Promotion of P-solubilizing bacteria and thereby enhancing rhizobial-legume symbiosis in myco-rhizosphere
- Decreased plant susceptibility to soil-borne pathogens due to antibiotics secreted by mycorrhizae
- Increased synthesis of phytohormones, and
- Altered soil/plant water relations, which could enhance plant adaptation to extreme situations, such as drought and heavy metal contamination.

Types of mycorrhizal association

The term mycorrhiza literally means “fungus root” which was coined about a century ago to describe the association of plant roots and certain fungi. This association is referred to as symbiotic, which means both the plant and the fungus benefit from the relationship. The fungi grow within the cortex of the roots and send thread-like hyphae out into the soil. These greatly aid the plant roots in taking up the mineral nutrients. Thus, fungi extract nutrients from the soil, which they provide to the plant. The plant, in return, supplies the fungus carbon compounds/carbohydrates. Mycorrhizal colonization of the plant roots is quite common in nature; in fact their occurrence is more of a rule than an exception. There are a number of different types of fungi that form mycorrhizal associations, but in agriculture, it is the arbuscular mycorrhizal fungi (AMF) of the Phylum Glomeromycota that are most important. Approximately 160 fungal taxa of the order Glomales (Glomeromycota) have been described on the basis of their spore morphology (Schussler et al., 2001), although recent molecular analyses indicate that the actual number of AM taxa may be much higher. AMF form a symbiotic association with more than 80% of land plant families. Included in these are many crop species, though crops in the Brassicaceae and the Chenopodiaceae generally do not form mycorrhizal associations (Harley and Smith, 1983). The AMF consists of an internal phase inside the root and an external phase, or extraradical mycelium (ERM) phase, which can form an extensive network within the soil. Adequately defining the arbuscular mycorrhizal (AM) association has proved problematic. These fungi thoroughly invade the living cells of roots and form specialized structures called arbuscules and vesicles. They are invisible without the aid of a microscope. Researchers are able to observe arbuscular mycorrhiza by staining plant roots with a simple biological dye called acid fuschin or trypan blue (Phillips

and Hayman, 1970). The fungal filaments, arbuscules and spores become colored and they are thus visible under the microscope. The spores can be extracted from soil using simple protocol of wet sieving decanting method (Gerdemann and Nicolson, 1963).

The other type of association is visible to the naked eye, as they cover the root surface with a thick mantle of hyphae. These are known as ectomycorrhizal fungi. They associate predominantly with woody plants such as oaks, birches, spruces and pines. Many form fruiting bodies that we recognize as mushrooms. One of the major constraints in realizing the yield potential of crops or high yields is the supply of sub-optimal nutrients. The high cost of fertilizers, the low purchasing power of small and marginal farmers and their adverse effect on the environment has led the agricultural scientists to search and develop alternate strategies. One such approach is the use of beneficial rhizosphere microorganisms called as biofertilizers, which can fix atmospheric nitrogen and solubilize or mobilize phosphorus, zinc and other soil nutrients to stimulate plant growth and improve soil health.

Arbuscular mycorrhiza: a beneficial symbiosis gives up secrets

The symbiotic relationship between AM fungi and plant in ancient and quite common in nature; in fact their occurrence is more of a rule than an exception. There are a number of different types of fungi that form mycorrhizal associations, but in agriculture, it is the arbuscular mycorrhizal fungi (AMF) of the Phylum Glomeromycota that are most important and form symbiotic association with more than 90% of terrestrial plant species (Gadkar et al, 2001). Included in these are many crop species, though crops in the Brasicaceae and the Chenopodiaceae generally do not form mycorrhizal associations. The presence of a mycorrhizal crop is the most important factor that drives AM growth, development and subsequent benefits to crop. Crops like mustard which are of non mycorrhizal origin do not support mycorrhizal growth. In all types of mycorrhiza, fungal hyphae permeate soil and reach beyond the depletion zones developed around the non-mycorrhizal roots. Thus, mycorrhizal roots explore a larger soil volume and have greater absorptive area than non-mycorrhizal roots. Indian soils are usually deficient in phosphorus, an element that is required more as compared to other major nutrients. Moreover, when applied to soils, phosphorus quickly gets fixed and immobilized. Hence plants are unable to utilize it. Mycorrhizae help in mobilization/solubilization and increase the uptake of phosphorus in plants (Bagyaraj et al. 2015). Inoculation of plants with mycorrhizal fungi during seedling stage and then transplantation in well-manured fields can certainly substitute the chemical fertilizer inputs, particularly P, to a large extent. This way

species are often extremely dependent on mycorrhizae for growth. Although finer rooted crops may not be as dependent on mycorrhizae for growth, they are also mycorrhizal and can function to build and propagate mycorrhizal populations to benefit coarser rooted crops subsequently grown in rotation. In agriculture, mycorrhizae form a natural part of cropping systems and of value in phosphorus and zinc nutrition. Knowledge of these factors will enable a farmer to get the best value from fertilizer investments. In a phosphorus deficient soil, poor mycorrhizal colonization will produce a crop with nutritional problems, whereas a crop with good mycorrhizae in the same soil will ensure better growth. Mycorrhizae have a key role in determining the effectiveness of applied fertilizer. Mycorrhiza favourable agricultural practices (such as in zero-till, elevated beds, crop rotation and intercropping system etc.) may result in greater hyphal biomass, improved soil texture and health, increased glomalin with improved crop yields in the soil (Nichols and Wright, 2005). Therefore, whole system involving AM fungi with varied cultivation systems is likely to be important in terms of better functioning of AM fungi to produce more AMF biomass and enhanced aggregate stability and C-sequestration in the soil. Similarly in our research farm, one of the long term trials having mycorrhizal crops and organic as well as integrated nutrient management also showed higher AMF biomass despite of having no AM inoculation as long term cropping involving organic practices favours indigenous AM population.

In a recent study conducted at DSR (Sharma et al. 2012) involving different soybean-based crop rotations and tillage systems influenced the mycorrhizal symbiosis. After completing six cropping seasons, significantly higher mycorrhizal spore count (17.0/g soil) was observed in the soybean grown under soybean-wheat-maize-wheat rotation under conventional-reduced tillage systems as compared to other combinations. On the other hand, in wheat, when compared to crop rotation and tillage combinations, significantly higher AMF population (14.2/g soil and 12.86 g/soil) was observed in soybean+maize-wheat rotation taken under conventional-reduced and conventional-conventional tillage practices respectively. It is interesting to note that in both the crops, the higher AMF population was observed in the plots wherever maize was included in the rotation. The rhizosphere of both soybean and wheat under conventional-reduced tillage systems in soybean based rotations involving maize showed higher AMF spore density and colonization. The infectivity potential (IP) caused due to resident AM fungi was observed higher in the plots of soybean-wheat-maize-wheat rotation under conventional-reduced tillage systems. Overall, higher IP was assessed in the plots of rotation where maize was included. The lower IP in conventional-conventional tillage could be attributed due to breakup of AMF networks (Sharma et al. 2012).

Table 1: Application of AM fungi in some important agricultural and horticultural crops*

Plant species	AM Fungi	Inference	Reference
Agricultural crops			
Wheat	*Glomus mosseae, G. etunicatum*	Improved growth, yield, drought and P and Fe nutrition	Al-Karaki et al. 2004 Sharma et al. 2011
Rice	AM inoculation	Improved establishment and nutrition	Dhillion and Ampompan, 1992; Secilia & Bagyaraj, 1992
Maize	*G. mosseae*	Improved physiological status, salt tolerance	Sheng et al. 2008; Feng et al. 2002
Bean	AMF+*Rhizobium*	Improved growth nutrition	Aryal et al. 2006
Soybean	*G. mosseae*	Enhanced N-fixation	Ganry et al. 1982
	G. intraradices	Drought tolerance	Porcel et al. 2004
Groundnut, Pigeon pea	AM fungi	Enhanced nutrition	Bell et al. 1989; Shibata and Yano, 2003
Horticultural crops			
Onion	Native mixed AMF	Enhanced growth and P-fertilizer saving	Sharma and Adholeya, 2000
Potato, Capsicum	*G. fasciculatum*	Enhanced P-nutrition	McArthur and Knowles, 1993
	G. etunicatum, G. intraradices	Improved growth and bioprotection	Yao et al., 2002 Sensoy et al 2007
	Native mixed AM fungi	P-saving	Sharma and Adholeya, 2004
Strawberry	*Glomus fasciculatum, G. etunicatum, G. claroideum*	Enhanced growth	Gryndler et al. 2002
	Indigenous AMF	Enhanced growth	Vestberg et al. 2006
Apple, peach, and plum root stocks	*Glomus sp.*	Enhanced growth	Sbrana et al., 1994
Cassava (micropropagated)	*G. deserticola*	Enhanced growth	Azcón-Aguilar et al. 1997
Banana (micropropagated)	*G. intraradices*	Enhanced P-nutrition and growth	Declerck et al. 2002
	G. proliferum, G. versiforme, G. intraradices	Enhanced P-nutrition and biomass	Jaizme-Vega et al., 2003
Lilium (micropropagated)	Mixed native *Glomus* sp., *G. intraradices,*	Improved survival and fertilizer savings	Varshney et al., 2002
Citrus limon (micropropagated)	Native AMF, G. mosseae	Improved survival	Quatrini et al. 2003
Sour orange	*Glomus clarum*	Increased P, Zn and Cu nutrition	Ortas et al. 2002

*Modified from Sharma and Adholeya 2007

the production achieved would be sustained without affecting the soil productivity and fertility. AM can directly take up inorganic nitrogen from the soil and transfer it to the host plant, and the biochemical pathway was recently elucidated by Govindarajulu et al. (2005) using 15N- and 13C-labelled substrates. The role of common mycorrhizal networks in the transfer of nitrogen within and between plants was reviewed by He et al. (2003). While plant AM symbiotic relationships are undoubtedly important in nutrient cycling in natural undisturbed ecosystems, their overall importance in intensive agricultural systems is not as well understood.

The AMF consists of an internal phase inside the root and an external phase, or extraradical mycelium (ERM) phase, which can form an extensive network within the soil. These fungi thoroughly invade the living cells of roots and form specialized tree like structures called arbuscules and vesicles which can be observed by staining plant roots with simple biological dyes called acid fuschin or trypan blue. The fungal mycelia/hyphae, arbuscules and spores become colored and they are thus visible under the microscope. The spores can be extracted from soil using simple protocol of wet sieving decanting (Gerdeman and Nicholson, 1963) where spores are visible through their natural colour and the spore wall and hyphal attachment helps in their identification.

Integration of mycorrhizal fungi with other plant growth promoting rhizobacteria: A sustainable approach

Inoculation of legume seeds with Rhizobium spp. is a well-established biotechnology routinely practised by farmers in developed countries, and both national and international efforts continue to promote the technology in developing countries, with outstanding success in some regions (Alves et al., 2003). The need to develop sustainable farming systems with reduced external inputs of pesticides and manufactured fertilizers has encouraged soil microbiologists to seek other microorganisms that can likewise benefit crop production. The roots of many plant species are naturally colonized by arbuscular mycorrhizal (AM) fungi, which are ubiquitous in soil. The most important bio-inoculants for soybean are *Rhizobium* (Brady, Sino, Meso etc.,), phosphate solubilizing bacteria, mycorrhizal fungi etc., and their combined application has been a proven potential (Barea, 1997). Now it has been established that AM fungi can help in taking up of inorganic nitrogen and transfer it to plants it is likely that if AMF join hands with rhizobia, the nitrogen fixation will much higher than using alone. There is need of an hour to have a strong gene pool of microbes from indigenous niche and should be ecologically sound which can be used for further evaluation for their affinity, competitiveness, inoculum load and efficacy for enhancing the growth of soybean yet minimizing chemical inputs.

Application of AMF in the enhanced growth, mineral nutrition, disease protection and conferring drought tolerance to plants.

The symbiotic association with AM fungi allows the plant to access phosphorus beyond the depletion zone through the extraradical fungal hyphae, in addition to the root uptake (Pearson and Jakobsen, 1993). AMF have been shown to improve productivity in soils of low fertility (Jeffries, 1987) and also contribute to the uptake by plant of micronutrients, such as zinc (Thompson, 1990) and the macronutrient nitrogen, both inorganic and possibly also organic (Hawkins et al. 2000; Hodge et al., 2001). Improved P nutrition has been shown to increase in infertile and P-fixing soils of the tropics (Dodd, 2000). Under drought conditions the uptake of highly mobile nutrients such as NO_3^- can also be enhanced by mycorrhizal associations (Ázcón et al., 1996; Subramanian and Charest, 1999). In legume plants the importance of AMF symbiosis has been attributed to high P requirements on the nodulation and N_2 fixation process which requires enhanced P uptake (Barea and Ázcón-Aguilar, 1983; Manna et al., 2006). Mycorrhizal fungi can also improve absorption of N from NH_4^+ -N mineral fertilizers, transporting it to the host plant (Ames et al., 1983; Johansen et al., 1993). Its transport and absorption can also increase biomass production in soils with low potassium, calcium and magnesium (Liu et al., 2002).

In addition to the nutrient uptake activity, the extraradical mycelium also releases substances that cause the soil and its organic components to aggregate (Tisdall, 1994; Bearden and Petersen, 2000). Another impact of AM fungi on the plants, including agricultural crops is their ability to increase their tolerance to drought (Davies et al. 1993) and reduce damage caused by plant pathogens (Whipps, 2004). Hormonal changes throughout the entire plant under the influence of the symbiosis have also been described (Allen et al. 1982). Under some circumstances AM fungi are able to decrease negative effects by heavy metals in plants (Tonin et al., 2001). It has been reported that stress induces some specific proteins and metabolites that confer tolerance to microbes to adapt and survive until conditions return to normal (Ocon et al., 2007). A common response of organisms to drought stress is the accumulation of sugars and other osmoprotectants that helps in stabilizing biomolecules. One such compound is Trehalose, a non-reducing disaccharide, has been found in a wide variety of organisms including fungi and bacteria. Trehalose found to play an important physiological role as drought stress protectant. Trehalose accumulation (as carbohydrate reserve) in an organism protects the cell by stabilizing cell structures and enables protein to maintain their native conformation under drought stress. It has been reported that trehalose may occur in plants colonized by organisms like

AM roots and nitrogen fixing nodules (Garcia et al., 2005). AMF-mediated plants may help in increased levels of trehalose in the plant which eventually enhance the tolerance to cope up with drought conditions.

Besides enhanced plant growth and nutrition, AM also helps in disease protection. The use of AMF as biological means paves the way to manage the nematodes infestations. Mycorrhizal fungi and plant parasitic nematodes (PPN) particularly, control of soil borne diseases, root-knot nematodes which are commonly found inhabiting the rhizosphere and colonizing the common roots of their host plants. These two groups of microbes exert opposite effects on plant growth. Arbuscular mycorrhizal fungi (AMF) form a symbiosis with 80% of all plants and are able to increase plant nutrition and plant health (Dehne, 1982). Mycorrhiza-mediated plants brought changes in the host physiology and enhanced P-nutrition, which deters pathogens to attack, and built plants more tolerant to pathogen attack and reduced nematode infestation (Sharma and Bhargava et al. 1993). Numerous reports were published on the suppression of nematode penetration and development following AMF inoculation (Talavera et al. 2001; Elsen et al.2001; Diedhiou et al.2003). Harrier and Watson (2004) emphasized the role of AMF in organic and/or sustainable farming systems that rely on biological processes rather than agrochemicals to control plant diseases.

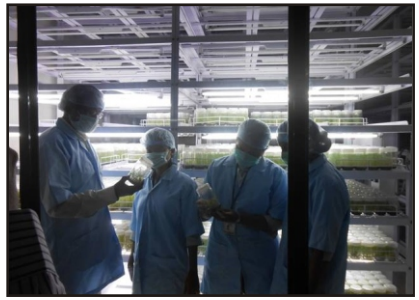
Since, AMF response to marginal soils and environment, legume crops are generally grown in poor environments, it is likely that AMF will respond favourably when compared to cereal crops. Nair et al. (1990) reported that higher level of AM infection was beneficial for plant growth of cowpea (*Vigna unguiculata* L.) under field condition. Hamel and Smith (1991) found that mixture growth of both corn (*Zea mays* L.) and soybean plants was greatly enhanced when inoculated with mycorrhizal fungi. Alloush et al. (2000) found that chickpea plants inoculated with mycorrhizal fungus *Glomus versiforme* had higher number of nodules; shoot phosphorus content, shoot dry weight and grain yield than uninoculated chickpea plants. A detailed list of references on AM response on the growth, nutrition and tolerance to drought has been given in the table-1.

Management of mycorrhizae in cropping systems

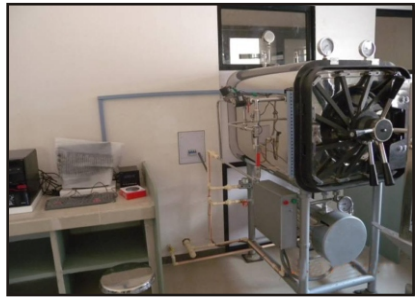
Indian soils are usually deficient in phosphorus, an element that is required more as compared to other major nutrients. Inoculation of plants with mycorrhizal fungi during seedling stage and then transplantation in well-manured fields can certainly substitute the chemical fertilizer inputs, particularly P, to a large extent. Some crop species do not become mycorrhizal, and so do not benefit from mycorrhizae. Among these are many species within the cruciferae-the cabbage family. However, most other crop species are mycorrhizal. Thicker rooted crop

Maliba Research Centre for Excellence in Tissue Culture


Inoculation & growth room




Autoclave & media room




Green House


SOUVENIR

POPULAR LECTURE SERIES in BIOTECHNOLOGY

12th October 2015

Sponsored by

Department of Biotechnology
Govt. of India
New Delhi

Organized by

UKA TARSADIA university
Imparting Knowledge. Awakening Wisdom. Transforming Lives.

C. G. BHAKTA INSTITUTE OF BIOTECHNOLOGY
Maliba Campus, Bardoli, Dist. Surat, Gujarat, Pin : 394350

POPULAR LECTURE SERIES in BIOTECHNOLOGY
Programme Schedule

Lecture 1: Biodiversity and biotechnological applications of cyanobacteria
Date:19/09/2015

Dr. N. Thajuddin
Professor & Head
Department of Microbiology
Bharathidasan University
Tiruchirappalli-23, Tamil Nadu

Lecture 2: Arbuscular Mycorrhizal fungi: Application in plant production system & monitoring its quality parameters
Date: 01/10/2015

Dr. Mahaveer P. Sharma
Principal Scientist
Directorate of Soybean Research (ICAR)
Indore, Madhya Pradesh

Lecture 3: Plant Tissue Culture methods with special reference to cultivation of medicinal plants
Date: 12/10/2015

Dr. R. Krishnamurthy
Director
C.G. Bhakta Institute of Biotechnology
Uka Tarsadia University
Bardoli, Surat, Gujarat

Research Facilities at CGBIBT


CO_2 Incubator


qRT-PCR


Fermenter


Thermocycler


Gel Documentation Unit


Fluorescence Microscope


-20°C Deep Freezer


Inverted Microscope

POPULAR LECTURE SERIES in BIOTECHNOLOGY

Title

Plant Tissue Culture Methods with Special Reference to Cultivation of Medicinal Plants

Speaker

Dr. R. Krishnamurthy
(Former Director of Zandu Group)
Professor & Director
C. G. Bhakta Institute of Biotechnology
Uka Tarsadia University
Bardoli-394 350, Dist.-Surat, Gujarat, India

Sponsored by

सत्यमेव जयते

Department of Biotechnology
Govt. of India
New Delhi

Organized by

UKA TARSADIA university
Imparting Knowledge. Awakening Wisdom. Transforming Lives.

C. G. BHAKTA INSTITUTE OF BIOTECHNOLOGY
Maliba Campus, Bardoli, Dist. Surat, Gujarat, Pin : 394350

12th October 2015

Organizing Committee

Patrons

Dr. D. R. Shah Shri. Kiritbhai Patel Shri. Bhagubhai Patel

Chairman

Dr. R. Krishnamurthy

Co-ordinator

Dr. N. Amaresan

Discipline Committee

Dr. Meonis Pithawala Prof. Rajashekar I
Dr. Sankar Narayanan

Registration Committee

Dr. Parul S
Ms. Urjita Sheth
Dr. Vijayalakshmi G
Ms. Dhruti H. Mistry
Ms. Nilofer Sheikh
Ms. Amita Rathod

Stage Committee

Dr. Prasant Kumar
Dr. Anoop Markande
Dr. Mitesh K Dwivedi
Dr. Gopal Jee Gopal
Dr. Srivathsa N
Mr. Bhrugesh Joshi

Advertisement Committee

Ms. Nimisha Patel
Ms. Avani Kasture
Ms. Sonal Vashi
Ms. Vrutika Lad
Ms. Divya Patel
Ms. Shraddha Saha
Ms. Swetal Patel
Ms. Trusha Mistry
Ms. Avani Pandya
Mr. Harsh Desai
Ms. Shreya Desai
Ms. Jemisha Mistry
Ms. Sandipa Thesiya

Food Committee

Mr. Prittesh Patel Mr. Rushabh Shah

About the Institutes

Uka Tarsadia University

The establishment of Uka Tarsadia University (UTU) in 2011 by the Bardoli Pradesh Kelavani Mandal (BPKM) is an effort towards meeting the growing demand for quality education. The University offers admission in a comprehensive array of academic programs across the disciplines of Pharmacy, Management, Architecture, Nursing, Physiotherapy, Computer Science, Engineering & Technology and Applied Science. The university provides educational opportunities to over 7500 students. More than 350 faculty members of the university represent an eclectic mix of professional and academic, national and international experiences. UTU has established MoUs with several Foreign Universities, like Cape Breton University (Canada), University of Ilorin (Nigeria) and University of Prince Edward Island (Canada).

C. G. Bhakta Institute of Biotechnology

CGBIBT of Uka Tarsadia University (UTU) is ably managed by Bardoli Pradesh Kelavni Mandal (BPKM) which needs no introduction about its significant contribution in providing collegiate education over the last 60 years. CGBIBT established in 2005 for initiating exclusive education programs in Biotechnology and Basic Science. Since its establishment, CGBIBT has been striving to emerge as a center of excellence in imparting career based knowledge to students to realize their professional ambition. In addition, CGBIBT has also taken up a mission to support the local farming community by supplying quality planting materials. For this purpose, a sophisticated plant tissue culture laboratory and climate controlled Green House have been established. The institute is offering five years Integrated M.Sc Biotechnology / Microbiology; two years M.Sc Biotechnology / M.Sc Microbiology as well as Ph.D program in Biotechnology / Microbiology. The institute is equipped with basic as well as advanced instrumentation facilities. As a part of academic exchange programme and research collaborations, CGBIBT, UTU has signed an MoU with University of Ilorin (Unilorin) Nigeria & IITA, Nigeria and many private industrial organizations.

Biography

Dr. R. Krishnamurthy
Professor & Director
C. G. Bhakta Institute of Biotechnology
Uka Tarsadia University
Bardoli-394 350, Dist.-Surat, Gujarat, India
E.Mail: Krishnamurthy@utu.ac.in;
krishnashanti@gmail.com

Dr. R. Krishnamurthy, is a native of Tamil Nadu, India. Currently, he is serving as Director of C. G. Bhakta Institute of Biotechnology at Uka Tarsadia University, Bardoli, Gujarat, India. Dr. Krishnamurthy has done graduation (1981) and post graduation (1983) in Botany from University of Madras, Tamil Nadu. He was awarded Ph.D., degree in 1988 from Department of Botany, M. S. University of Baroda, Vadodara, Gujarat for his research work in the area of "Plant and Cell Physiology of Rice".

Dr . Krishnamurthy started his research and academic career as UGC-JRF, CSIR-SRF, CSIR-RA, CSIR-Pool Officer, Lecturer in M. S. University of Baroda and Poona University affiliated colleges during April, 1983 to February, 1995. Dr. Krishnamurthy has 13.5 years of industrial R & D Experience on Herbal and Medicinal plants with ZANDU group (Vapi/Mumbai) laboratory (approved by DSIR-SIRO, DST). He served with ZANDU group as Director during March1995 to September 2007. Then, he executed his duties as Professor and Director at Bhagwan Mahavir College of Biotechnology (affiliated to Veer Narmad South Gujarat University), Bharthana, Surat, Gujarat, from July 2007 to August 2011.

Dr. Krishnamurthy has successfully handeled six research projects as PI and Co-PI funded by DBT, DST and National Medicinal Plants Board, Govt.of India, New Delhi (Total Project Cost: Rs. 151 Lakhs). In addition, he also successfully executed 3 Joint Venture Commercial Projects funded by Foreign Companies from Japan, Germany and Singapore (Total Project Cost INR 162 Lakhs).

Dr. Krishnamurthy has also been active in International Research Collaboration as Project Advisor & Project Incharge of 'Domestication, field performance and yield potential of tissue culture plants of five Indian popular Banana (*Musa paradisiaca* L.) varieties in Ilorin, Kwara State, Nigeria' in joint collaboration with C. G. Bhakta Institute of Biotechnology, Uka Tarsadia University, India, University of Ilorin, Nigeria. He has been associated as Technical Advisor to the many Foreign Company Projects including ZAHAB General Trading & Contracting Corporation, Safat, Kuwait and M/S Hamdani Farm Limited, Ilorin, Kwara State, Ilorin, Nigeria.

He has supervised several master students for their theses and currently, 6 students have been registered and conducting research for their Ph.D. Dr. Krishnamurthy has published 96 research articles in peer reviewed national and international journals. He has also been serving as reviewer in many reputed journals and as an editorial board member. He has participated in several national and international conferences. In addition, while attending several national and international conferences/symposia, Dr. Krishnamurthy chaired several technical sessions and received guest of honour. Nonetheless, he has been a resource person in delivering expert talks/plenary lectures during national and international conferences/symposia. He visited UK, Germany, Kuwait and Nigeria for Research Purpose.

Dr. Krishnamurthy has conducted international conference, national level seminars, Farmers' Meets and Overseas Inter-Faculty Lectures. Besides these, Dr. Krishnamurthy has also conducted research training programmes on Tissue Culture training for teachers and students of other Institutes.

He was a member of Academic Council and Senate (2007-2011) bodies to Veer Narmad South Gujarat University, Surat. He was also a Member of Board of Management (2011-2014), Uka Tarsadia University. He is currently a member of Academic Council and Board of Studies (2011- till date), Uka Tarsadia University, Bardoli and also a committee member of Gujarat State Medicinal Plants Board, Govt of Gujarat, India. He is Involved with Farmers of Gujarat, Maharashtra, Karnataka and Tamil Nadu States, India for commissioning Agro-Technology of Medicinal Plants Cultivation and also supporting Farmers by providing Tissue Culture Medicinal Plants, Banana, Sugarcane and some fruit crops since 1995 till date.

Significant Achievements/Awards:

- Awarded Best Project for Transfer of Technology to Industry on Medicinal Plant Kaucha (*Mucuna pruriens*) Breeding by DBT, Department of Science & Technology, New Delhi, on 11, May 2002.
- Developed 69 breeding lines of Mucuna pruriens in the DBT sponsored project and short-listed five deserving lines for improved quality (yield and L-Dopa, a molecule used for Parkinson disease) and higher nutritional composition and standardized for field operations.
- Identified three high yielding lines (viz. code: ZFMP 1305, ZFMP 1604 and ZFMP 0603) with improved and other agronomical traits and promoted for large scale commercial cultivation and post-harvest operations.
- Filed Three patents jointly with RRL scientists, Jammu (CSIR Laboratory Govt. of India), out of which one patent 027086164 -2123-IN0200057 has been taken at European patent office. Other patents filed jointly: (i) Process for the isolation of immunoside and immunomodulatory agent from *Asparagus racemosus*. (ii) A process for the isolation of an antioxidant and free radical scavenging fraction from *Terminalia chebula*.

Current research interests:

- Conducting field and laboratory R & D Programme on various medicinal plants with reference to domestication, cultivation, improvement of yield & quality, and laboratory studies of plant based products.
- PCR based molecular studies of medicinal plants.
- Technological support to farmers/growers for cultivation of medicinal plants.
- R & D and commercial projects using Tissue Culture and Micropopagation Technology.
- Application of micro-organisms as bio-fertilizers related to nitrogen fixing (*Rhizobium*, *Azospirillum* and *Azotobactor*), phosphate (*Aspergillus, Bacillus* & VAM Fungi) and potash-nutrients uptake and improve nutrients availability to plants, and improvement of yield.
- Plant based products for Iron deficiency and Nutraceutical medicinal plants in collaboration with University of Ilorin, Nigeria.

Plant Tissue Culture Methods with Special Reference to Cultivation of Medicinal Plants

Dr. R. Krishnamurthy

Medicinal Plants in India and Global Scenario

Every nation has its unique traditional medicinal wisdom. However, due to overriding current technological advances, many nations across the globe have lost their heritage. But the Indian Traditional System of Medicine - Ayurveda literally meaning the science of life, has not only retained its traditional-know how but also popularized the practice across the world. Due to health consciousness and its awareness, people are adopting Indian System of Medicines (ISM) like Ayurveda, Siddha, Unani, Homeopathy and other traditional medicinal systems worldwide. The demand for medicinal plants and herbs has been rapidly increasing since the last decade and it is still growing at a faster pace. It is estimated that over 80% of the world population currently relies on traditional plant based medicines for their basic health care needs (WHO report). The global trade demand for plant based products is estimated to be at 62-120 billion US dollars and it is expected to go up to above 7 trillion or 7000 billion US dollars by the year 2050 (NMPB, 2015).

India and China are the two major producers of medicinal plants occupying more than 40% of the global biodiversity. India possesses one of the world's richest medicinal plants heritages with about 8000 plants species being used by rural communities. Medicinal plants of India are utilized by about 60% of the world's population. India ranks 2nd among major exporters : 70% of crude extracts and 30% of finished products. In India, there are 15 agro climatic zones having 17000 -18000 species of flowering pants, out of that 6000-7000 species are having medicinal uses in folk and Indian documented systems of medicines (FRLHT report, 2007; NMPB, 2015). It is estimated that about 960 plant species are in the trade, of which 178 species are utilized and amount to over 100 metric tonnes. A large portion of Indian population not only depends on medicinal plants as a major resource, but also as livelihood and health security. The Indian domestic trade industry does business to the tune of Rs. 80-90 billion and exports their products worth Rs. 10 billion per annum.

Medicinal and Aromatic plants are economically important as they provide basic raw materials for pharmaceutical, perfumery, flavour, soap and cosmetic industries across the world. Due to our living standard and increased population, and several constraint in long term use of modern medicine, there has been continuous demand for traditional medicinal plants and their products in the recent years. Eventhough, India earns considerable foreign exchange from the

medicinal plants, it is still difficult to cope-up with long term supply due to over exploitation from natural resources. India also facing tough competition from other developing countries for the quality of medicinal plants raw materials and their price, and there is an urgent need to encourage the Indian farmers/growers to develop organized cultivation of Indian medicinal plant species. By keeping all these in mind, the foregoing details are made on selected important medicinal plants and sharing our experience with farmers in order to promote and popularize the cultivation of medicinal plants; which in turn supports sustainable availability of quality medicinal plants raw materials and thereby prevent over exploitation from natural resources (Pathak, 2006).

Selected Medicinal Plants and their Cultivation:

Why medicinal plants require cultivation ?

- Ensure quality and purity
- Consistency in active ingredients and chemical constituents
- Better yield and sustainable availability of quality raw materials to user industry
- Systematic cultivation resulted in raising many medicinal crops with maximum content of active constitutes and oils levels, e.g. in Ginger, Turmeric, Liquorice etc.
- Organized and systematic harvesting periods, drying and post-harvesting operations helped to improve quality of crude drugs and value addition, e.g. in Senna, Kalmegh etc.
- Systematic plantation and cultivation of medicinal plants tree species helped to conserve many Dashmool plant species from over exploitation in the wild, e.g. in Arlu, Arni, Gambhiri and Padala

Why medicinal plants need to be produced through tissue culture?

- Tissue Culture Technology (Micropropagation) has been successfully used in raising mass - scale production quality planting materials of many medicinal plants including red listed plants, and other plants witnessing slow propagation rate through conventional methods.
- True to type (Identical to parents)
- Produced from high yielding and superior quality, and disease free mother stock
- Uniformity in growth, maturity and harvesting
- High yield and other agronomical traits like plant duration and other quality parameters
- Large number of plants produced in short span
- Flexibility in accordance with the planting season and market demand

Mandukaparni (*Centella asiatica* L. / *Hydrocotyle asiatica* L.)

It belongs to Apiaceae family. It is a small perennial creeping herb with slender jointed stems and a tropical plant found in damp, moist and marshy areas preferably under partial shade conditions. It is native to India, China, Indonesia, Madagascar, Africa and through tropical Asia, ascending in Abyssinia to elevation of 6000 feet. It also occurs in America from South Carolina to Valdivia, in West Indies, the islands of the Pacific, New Zealand and Australia.

Hydrocotyle is known as Manduka-parni in Sanskrit, Khulakhudi in Hindi. It has long been used in medicine by the natives of Java and Coromandel cost. Boilean, French physician of Mauritius pointed out in 1852 about its virtues in the treatment of leprosy and later on it was tried for leprosy in the hospitals of Madras by Hunter in 1855. Since then the Mandukaparni has been included and placed in the Pharmacopoeia of India.

Mandukaparni (*Centella asiatica*)

Mandukaparni (*Centella asiatica*): Field & Harvest

The plant is propagated through stem cutting comprising a rooted node. In commercial production, it can be cultivated under mango orchards and also under artificial 50% shade-net condition. Dense plantation is maintained at a spacing of 15x15 cm and the same crop is continued for two years. The crop gives about 3 metric tons of dry herb yield/hectare in six harvestings in two years (Krishnamurthy *et al*. 2006). The plant is used for nerve tonic, skin disease, hair tonic, antiulcer, and used in chutneys, leaf vegetable, pickles and refreshing soft drinks in Southern part of India.

The major chemical constituents of the plant is asiaticosides and triterpenoids (1.0%) in leaves and stems. Mandukaparni is vegetatively propagating plant through its rooted nodes and is in commercial cultivation about 1.0 lakh plants are required per hectare for dense plantation. Therefore, producing such a large quantity of planting material under nursery conditions will be difficul it occupies a large nursery area. Micropropagation techniques could be adopted in research and also in multiplying Brahmi genotypes with high triterpenoids content (above 1.0 %) and having characteristics of higher biomass yield.

The National Medicinal Plant Board (NMPB) reported (2015) that the market demand of Mandukaparni is 500-1000 MT per annum and its market price is Rs. 30-35 per Kg. FRLHT, reported that this is one of the 46 wasteland species used in high trading.

Senna (*Cassia angustifolia*- Alexanderian Senna ; *C. actifolia* Thirunelveli Senna)

The two species of Senna popularly known as Alexanderian and Thirunelveli Senna, are used in medicine. They are also known as Sonamukhi among locals. They belong to the family Caesalpiniaceae. The plant grows in Yemen and Hadramaut in Southern Arabia; it is also found on the Somali Cost, in Sind and Punjab, in some parts of India. It is considered as one of the most important source of organic laxatives. The wild plants of Arabia is supplied in the world market.

Senna (*Cassia angustifolia*)

However, the so called Bombay Senna comes from cultivated source and occupies the majority of global market. The crop originally was raised from Arabian seeds and is popularly known as "Thirunelveli Senna" in the drug market.

The elaborate of Carl Martins indicated that the knowledge of Senna cannot be traced back earlier than the time of the Elder Serapion, in the 9-10th Century. Issac Judaeus, who wrote during A.D. 850 - 900 mentioned it as a native of Egypt, the best Senna brought from Mecca. The Senna (known as Ssinen or Ssnen) was enumerated among the commodities liable to duty at Acre in Palestine during the 12th Century. In the year 1542, in France, a pound of Senna was valued at an official tariff at 15 sols as equivalent to the price of pepper or ginger. *Cassia obovata* Coll. was the first species known to Botanists and it was cultivated for medicinal purpose in Italy during the 16th century. Indian companies export Senna leaves and pods of worth over 239 million INR (Ayurbiz, Nov 2006 Jan 2007). The Senna crop is generally cultivated through seeds and therefore, developing planting materials through tissue culture is not required except in multiplication of high Sennosides (above 2.50 % in leaves) yielding accessions.

The crop is sensitive to heavy rainfall and it prefers warm and dry weather during the growing season. It grows well on red loams to course gravelly soils and alluvial soils. The advantage of this crop are that it is best situated for sandy loam, lateritic soils of low fertility with higher pH range of 7.0 - 8.5. The crop is raised through seeds and its first harvesting is done in 90-120 days and the subsequent harvest will be done after 40 days. It is estimated that the crop produces 600-1000 Kg of dry leaf yield and 400 kg of dry pod yield/hectare. The leaves and young pods possess Sennosides (2 - 3%), which are believed to be having laxative principles. It is being recommended as a laxative or purgative since ancient times in Indian System of Medicines and considered as a safe and effective drug for chronic constipation and therefore, available in the modern medical stores. It is estimated that 5000-10000 kg of Senna raw material is used annually in trade at Rs. 33-50 per Kg.

Gudmar/Madhunashini (*Gymnema sylvestre* R.Br.):

Madhunashini is commonly known as Gudmar in Hindi and an important medicinal climbing plant belonging to the family Asclepiadaceae and acclaimed for its anti-diabetic properties. It is found to grow in tropical conditions in Deccan Penninsula, Uttar Pradesh, Madhya Pradesh, Tamil Nadu, Kerala, Punjab and some parts of Asia and Malaysia. This plant is growing abundantly in the forests of Karnataka, Tamil Nadu and Bihar. The National Medicinal Plants Board projected that 500-1000 MT of Gudmar is used annually in trading at the price of Rs. 40-50 per kg. This plant is considered and reported as one of the seventy medicinal plant species in high demand and sourced from tropical forest (FRLHT report, 2007).

Gudmar/Madhunasini (*Gymnema sylvestre*)

Gudmar Leaf Harvesting

1. Small Leaf type - Its leaves are oval measuring 1.0 - 3.5 cm length and 1.5 - 2.5 cm width and very soft, found in dry regions.

2.Broad and Pubescent type - Its leaves are also oval measuring 3 - 6 cm in length and 3.5 - 5.0 cm in width. Leaves are dark green compared to small leaf type and all are pubescent.

The plant is suitable to grow in red sandy loam or medium deep soil and dry soils. It is sensitive to water logging soil condition. Plants can be produced by stem cutting under nursery conditions as its seeds witness poor germination. The vegetative propagation of this plant by stem cutting takes around two months for rooting and therefore, Tissue Culture based Micropropagation method is desirable for raising commercial scale planting materials for larger scale cultivation.

In commercial cultivation, the plantation is done at 2 x 2 M plant spacing and leaf harvesting is done at every six month intervals. The crop yield is about two tones dry leaves per hectares/ year in 3-4 coppicing of leaves. The leaves contain 2.0- 3.5% Gymnemic acid (Krishnamurthy *et al*, 2014). It is believed that the anti-diabetic properties of the plant are attributed to the presence of mixture of triterpines and saponins in the leaves. In addition to this, the plant is extensively used in almost all the Indian systems of medicine as a remedy for rheumatism, cough, ulcers and pain in eyes. In some parts of India, the root of this plant is recommended as a remedy for snake-bite. Indian companies earn revenue in the form of Gymnema Extract to the tune of 25 million INR (Ayurbiz, 2007).

Kalmegh (*Andrographis paniculata*)

Kalmegh (*Andrographis paniculata*):Seed production

Kalmegh (*Andrographis paniculata*) (Burm.f.) Wall.ex Nees :

Kalmegh is an annual tropical herb belonging to Acanthaceae family, 1-2 feet height, common throughout India, growing under the shade of trees. It is found in Ceylon and Java, and it has been introduced in the West Indies. The plant is popularly known as 'King of Bitters'. It is a bitter annual herb and its whole plant is medicinally used. Kalmegh is known in Bengal as Maha-tita literally 'king of bitter', from the Sanskrit 'tikta', and it has been included in the Pharmacopoeia of India.

It grows well in variety of soils i.e. from sandy to loam soils. Kalmegh is propagated either by seeds or stem cuttings. In commercial

cultivation, seeds are used for raising seedlings and planted at a spacing of 30 x 15 cm distance. The whole plant is harvested at 90 days and the second harvesting is done after 60 days after first harvest. The crop gives an average yield of 2.0 - 2.5 ton dry herb yield per hectare. After harvesting, the drying of plants under shade condition is highly recommended (Farooqi *et al*, 2001).

The whole plant is the source of several diterpenoids of which the bitter water soluble lactone Andrographolide is concentrated upto 2.5% in leaves and 0.5-1.0% in the stem. The whole plant drug is used as a liver stimulant (hepto-protective), anti-hepatitis, anti-typhoid. In some parts of India, it is also used as appetizer and in dysentery.

Velvet Bean (*Mucuna pruriens* DC.)

Velvet bean is commonly known as Cowhage and Atmagupta/Kiwach in Hindi. It is an annual and a tropical climber belonging to the family Leguminosae. It is reported to found throughout tropical conditions of Indian regions including Andaman and Nicobar Islands. The pods of this plant bear trichomes/hairs which gives itching sensation upon contact. The seeds of velvet beans collected from wild conditions have shown considerable variation in their morphology and chemical composition including L-Dopa (2-6%), and yield associated characteristics (Krishnamurthy *et al*, 2002 & 2003). It withstands drought and grows well in sandy to clayey-loam soils having adequate drainage. It produces copious foliage and is generally grown as a fodder crop in India (CSIR, New Delhi, 1962). The duration of the crop is 9-10 months and planted in a spacing of 1.0 x 1.0 m. Crop is raised through direct sowing of seeds and the plants require support (stakes) to climb and achieve maximum seed yield (1.0 - 1.5 ton/ha).

Kiwach (*Mucuna pruriens*)

Kiwach (*Mucuna pruriens*) Pods & Seeds

In Ayurveda, the seeds of velvet bean are used as a tonic and for aphrodisiac properties. Seed powder has been tried in Parkinson's disease

(Kampavata) and depression with positive results (Dymock *et al*, 1980 ; Mahajani *et al,* 1996 ; Manyam 1995 ; Manyam and Parikh, 2000). In Indian systems of medicines, the seeds of velvet bean have been recommended as nervine tonic since ancient times. Since the discovery that this plant seeds contain L-Dopa (4-3, 4-dihydroxy phenylalanine) which is an Anti-Parkinson's drug, this crop has drawn great attention of farmers/growers for commercializing this plant.

Shatavari (*Asparagus racemosus* Willd):

Shatavari belongs to the family Liliaceae and its roots (tuber) are used in Ayurveda, Siddha and Homeopathy medicines. It is a tropical and perennial plant found to grow in the forests of Asia, Africa and Australia. The plant prefers to grow well in red loamy soils and sandy loamy soils with adequate drainage. Since it is a shallow rooted (tuberous) crop, it best suited under shallow and rocky soil conditions where available soil depth is upto 30 cm with pH range of 7.0 - 8.0 ; dry soil conditions (50-100 cm annual rainfall) and upto 1400 m altitude. The plant is vegetatively propagated through root suckers with rhizomatous disc for commercial cultivation. The plant produces seeds in the month of Feb - May. In commercial plantation, developing planting materials through root suckers or seeds consumes long periods and therefore, raising plants through Tissue Culture Technology is desirable and could be economical. In dense plantation, the plant spacing is maintained at 1.0 x 0.5 m and the crop is ready to harvest its tubers in 24 months. The maximum tuber yield of 3 ton dry/ha could be achieved with the crop cultivated under systematic cultivation practice, irrigation and manuring in 24 months (Krishnamurthy *et al*, 2005). It is estimated by National Medicinal plants Board that the annual market demand of Shatavari roots in India is ranging 2000 - 5000 MT and with its price Rs. 40-70/- per kg.

Shatavari (*Asparagus racemosus*)

Shatavari (*Asparagus racemosus*):Tuber

Shatavari roots are used mainly as galactogogue, which stimulates the secretion of breast milk. In Indian System of Medicine, it is used as an aphrodisiac, tonic and it supports to maintain the health by balancing immunity. It is considered as a good energy provider to the weak body system during prolonged / chronic illness. The tuber contains main chemical constituents Shatavarin and Saponins.

Vidhari Kand (*Pueraria tuberora* D.C.):

It is commonly known as Indian Kudzu and belongs to family Leguminosae and locally called Vidari (Sanskrit), Sural (Hindi) and Vidharikan. The plant is found in Western Ghats; Dharampur and Saputara of South Gujarat and some parts of Jammu in light to red soils. It is also called Giant Potato and is having woody perennial climbing habit. The plant produces less number of seeds during the month of summer and therefore, our experiments conducted for developing commercial scale planting materials by vegetative propagation through stem cuttings and direct rooting under greenhouse condition was not successful as it took about six months for rooting of stem cuttings. Hence, Tissue Culture Methods of propagation is recommended and for economically viable commercial cultivation. The *P. tuberosa* is short listed as one of the red- data plant and there is an urgent need to produce planting material of this species in bulk through alternative methods like Tissue Culture Micropropagation methods for cultivation.

VidhariKand (*Pueraria tuberora*): Tuber harvest

VidhariKand (*Pueraria tuberora*): Woody (stem) climber with root

In commercial plantation, it is planted at 1 x 1 m spacing (10000 plants/ha) and its tuber is ready to harvest after 3 years. The tuber is rich in carbohydrates and saponins. In many parts of India, the tuber is consumed for tonic, aphrodisiac and weight gaining purposes after prolonged illness and therefore, recommended for general debility.

Chitrak (*Plumbago zeylanica* L.)

In Ayurvedic and other Indian systems of medicine the roots of white flowered lines are used in different formulations. The plant is found in wild in Western Peninsula, Bengal and some regions of Madhya Pradesh, Chhattisgarh forest. It is a tropical plant and performs well under partial shade condition of forest canopy. It is a perennial shrub which grows upto height of 1.5 - 2.0 m. During the rainy season, the plant grows actively and in winter its growth is stopped after reaching flowering and fruiting. However in summer, the plant appears almost leafless. The new shoots develop after drying of old shoots or after cutting the shoot system.

The plant is best suited for well drained, sandy loam, clayey loam soils, having higher organic content. The plants does not withstand water logging or sandy or gravelly soil with lower water holding capacity. The plant is propagated through stem cuttings of 10 - 15 cm long having 2 - 3 nodes. However, this type of vegetative propagation consumes time and requires larger area of nursery set- up for developing commercial scale planting materials. Therefore, it is preferred to utilize tissue culture micropropagation technology for production of economically large number of plants in commercial cultivation. In commercial plantation, the optimum plant spacing of 50 × 25 cm (80,000 plants/ha) is desirable for maximum yield and economics. The crop gives dry root yield of 1700 kg/ha after 12 months. The Indian industry buys Chitrak root at the rate of Rs. 35 to 50/kg. Intercropping of Chitrak with other tree crops is also recommended for commercial cultivation for getting remunerative yield.

Chitrak (*Plumbago zeylanica*): Root harvesting

Chitrak (*Plumbago zeylanica*): Roots

The root of Chitrak contains alkaloid Plumbagin, which is found to stimulate central nervous system, secretion of sweat, urine and bile. The root is believed to be a potent abortifacient when taken orally during the stage of pregnancy. Root is an appetizer and also used in skin diseases. The root oil is effective in rheumatism, joint pain and paralysis. The FRLHT, reported that the Chitrak is one of the high traded commercial source material from wastelands out of 46 medicinal plants listed. The annual demand of Chitrak root is estimated at 2000 to 5000 MT.

Ashwagandha (*Withania somnifera* Dunal):

It belongs to the family Solanaceae. Ashwagandha is an important medicinal plant found in dry region of tropical and subtropical areas of India i.e Rajasthan, MP, UP; South Africa, Egypt, Pakistan and Afghanistan. Its roots are used in Ayurvedic and Unani preparations. Ashwagandha requires relatively dry

season for its good growth and its roots are fully developed in six months and thereby ready for harvesting. The crop grows successfully in sandy loam or light red soils having a pH of 7.5 - 8.0. In commercial cultivation, direct broadcasting of seeds along in monsoon is preferred. Dense population of plants is normally desirable. Plant based industries prefer to buy only cultivated Ashwagandha roots rather than the wild collections due to poor quality root traits. The crop yields 600 Kg of dry root and 50 kg of seed/ha. The major alkaloids in the materials are Withanolides in roots and Somniferin in leaves. In Indian System of Medicines, the Ashwagandha roots are recommended as a tonic and aphrodisiac in human beings. The Indian market demand for Ashwagandha root is estimated at about 5000 MT/annum. Industry buys superior quality root materials at the rate between Rs.70-100/ Kg dry roots. FRLHT (2007) rated Ashwagandha as one the highly commercialy traded source material which comes from cultivation and this is one among 36 medicinal plant species listed in their report. Since Ashwagandha is recommended to cultivate through seeds and there is a less significants for Tissue Culture Technology except in research and plant breeding.

Ashwagandha (*Withania somnifera*): Field and Root harvest

Ashwagandha (*Withania somnifera*): Root & seed harvest training

Sweet Flag (*Acorus calamus* L.)

The *Acorus calamus* commonly known as "Vacha" belongs to the family Araceae and is a perennial plant propagating through rhizome (root). The plant is found in Uttaranchal, HP, Jammu and all over India; China and Arabian countries. The plant is naturally growing along the stream and river boundaries and in low lying areas. The plant is propagated through its rhizomes in nurseries and transplanted in the field at the density of 30 × 30 cm spacing (Kasture Avani and Krishnamurthy, 2015). Since, it is vegetatively propagating plant through rhizome, the Tissue Culture Technology is desirable to apply only in research and large scale propagating elite planting materials and superior accessions. In commercial cultivation, the crop requires frequent irrigations with recommended

dose of fertilizers and other field management practices for maximum yield and quality. Its rhizome gets matured and is ready to be harvested in 12 months under regular cultivation and field management. The Vacha crop yields 1 -1.5 MT dry root yield/ha in 12 months under well planned adoption of cultivation practices. The domestic consumption of vacha root is estimated as high as 500 - 1000 MT annually and it fetches the market price of Rs 35 to 50/kg.

Sweet Flag (*Acorus calamus*): Nursery & Field

Sweet Flag (*Acorus calamus*): Rhizome harvesting and drying

The calamus rhizome is having demand in plant based industries for the purpose of preparation of medicines and for flavoring liquors. The oil extracted from rhizome and leaf also show antimicrobial activity (Kasture Avani *et al*, 2015). It is also used as appetizer and for epilepsy. It is commonly used in agarbattis, insecticides against flies, mosquitoes, bedbugs and for the protection of food grains and also in perfumery and toiletry products.

Shalparni (*Desmodium gangeticum* (L.) DC):

Shalparni is a tall, under shrub bearing broad alternate unifoliate leaves and white-purple coloured flowers and grows 1.0 -1.2 m tall in monsoon rain forests upto 900 m elevation of Indian tropical region. It belongs to the family Leguminosae.

In Ayurveda, roots of ten medicinal plants used together (5 herbs and 5 tree species) as Dashmool are considered high efficacious in management of several common ailments. Amongst the classical formulations, the "Dashmoolarishta" is the most popular and is used as basic ingredient in the manufacture of over 109 drug formulations. It generates a large demand for the raw material in the industry and as a result of this, the roots of some of these species are in short supply. This has led industries to use the roots of allied species and their whole plants (viz. Panchang). The Salparni is one among 10 plants, which grows all over plains in India.

The plant is propagated through seeds and well developed seedlings under the nursery conditions, are planted in the field in a dense population of 49,000 to 55,000 plants per hectare for commercial cultivation. The crop can fetch a profit of Rs. 25000 to 30000 per ha under cultivation over under low fertile marginal lands in

Shalparni (*Desmodium gangeticum*): Field

Shalparni (*Desmodium gangeticum*): Root harvest

high rainfall tract. The shoots can also be sold, adding another Rs.15000 to 20000 to the profit. Therefore, Shalparni cultivation can be introduced in partial shades of Orchards in regions experiencing short and mild winter season (Krishnamurhty *et al,* 2005).

Pristiparni (*Uraria picta* & *Pseudarthria viscida*):

In Dhashmoolarishta**,** both *U. picta* and *P. viscida* are considered as "Pristiparni". In Ayurveda the roots of both species are recommended in Dashmool drug preparation. However, in both Western and Northern parts of India, the industries use *U. picta* or *U. lagopoides* as Pristiparni; whereas in Sourthern parts of India, the *i*ndustry buys raw materials of *P. viscida* as Pritiparni for their drug formulations. *P. viscida* is also having antimicrobial activities (Krunal Naik *et al,* 2014). Since all the Pristiparni species are producing small quantities of roots even after one year under cultivation, and there is an acute shortage of its genuine root raw materials in the market and hence drug manufacturers use 'Panchang' (all plant parts mixed) for their products. Further, *P. viscida* is a Red listed plant species and therefore, there is a necessity for systematic and regular cultivation of this species. The commercial cultivation of *P. viscida* requires around 50000 plants per hectare as planting materials for raising dense plant population and therefore, generating such a bulk, uniform planting material through seeds is relatively time consuming and also economically not viable. Hence, it is advisable to adopt Plant Tissue Culture based Micropropagation methods to produce bulk planting materials to meet the demand of this Red-Listed plant species.

Pristiparni (*Pseudarthria viscida*): Field

Acknowledgements

Dr. R. Krishnamurthy (Former Director of Zandu group) greatly acknowledge his ZFHC's research team members Dr. Rajendra Gupta, Dr. J.M. Pathak, Dr. M.S. Chandorkar, Shri. Maheshbhai Patel and Shri. I. Rajashekar for their Support and contribution in various medicinal plants projects.

SELECTED REFERENCES

1. R. Krishnamurthy, M.S. Chandorkar, M.R. Palsuledesai, J.M. Pathak and Rajendra Gupta (2002). Breeding in velvet been (*Mucuna pruriens*) for improvement in seed yield and quality traits. Indian Journal of Agricultural Sciences, 72, 709-715.
2. R. Krishnamurthy, M.S. Chandorkar, M.R. Palsuledesai, B.G. kalzunkar, J.M. Pathak and Rajendra Gupta (2003). Standardization of cultivation and harvesting stage of velvet been (*Mucuna pruriens var utilis*) for optimum yield and quality. Indian Journal of Agricultural Sciences, 73, 585-589.
3. R. Krishnamurthy, M.S. Chandorkar, B.G. Kalzunkar, M.R. Palsuledesai, J.M. Pathak and Rajendra Gupta (2003). Studies on use of bio-fertilizers on Kewach (*Mucuna pruriens*) and Brahmi (*Centella asiatica*) for yield and content. In "Organic Production of Medicinal Aromatic and Dye Yielding Palnts", Published by FAO pp1-11, one line Journal, http://ecoport.org/ep, Search type = article view & article ID: 145 & page = -2.
4. R. Krishnamurthy, M.S. Chandorkar, B.G. Kalzunkar, M.R. Palsuledesai, J.M. Pathak and Rajendra Gupta (2006). Studies in agronomic practices for growing *Centella asiatica* (L.) Urban in high rainfall localities under open and partial shade of mango orchards in India. Indian Journal of Horticulture, 63, 76-80.
5. R. Krishnamurthy, M.S. Chandorkar, B.G. Kalzunkar, M.R. Palsuledesai, J.M. Pathak and Rajendra Gupta (2005). Studies in for improving tuber yield in Shatavari (*Asparagus racemosus*) as irrigated and rainfed crop. Journal of Medicinal and Aromatic palnts, 26,740-745.
6. R. Krishnamurthy, M.S. Chandorkar, B.G. Kalzunkar, M.R. Palsuledesai, J.M. Pathak and Rajendra Gupta (2005). Diversity evaluation in velvet bean (*Mucuna pruriens*) germplasm for seed yield and associated agronomical traits. Journal of Medicinal and Aromatic Plants, 27,291-296.
7. R. Krishnamurthy, M. S. Chandorkar, J.M. Pathak and Rajendra Gupta (2005). Identification of high yielding germplasm of Dashmool drug plant- Shalparni (*Desmodium gangeticum*). Indian Journal of Horticulture, 62,378-384.
8. Pooja patel and R. Krishnamurthy (2013). Feasibility studies on in vitro mass scale propagation of Indian Aswagandha (*Withania somnifera*) cultivars for commercial. Journal of Pharmacognosy and Phytochemistry, 2, 168 174.

9. Kasture Avani and R. Krishnamurthy (2015). Impact of Planting space, irrigation pattern and different concentration of fertilizers on the yield and yield related parameters of sweet flag (*Acorus calamus* Linn).International Journal of Agriculture, (Photon) 126: (2015) 405-411.
10. Avani Kasture, Shuchi Patel, Jigna Chauhan and R. Krishnamurthy (2015). *In vitro* antimicrobial effect of essential oil from leaf and rhizome of various accessions of *Acorus calamus* Linn. and its phytochemical screening. European Journal of Medicinal Plants, 9 : 1-13.
11. R. Krishnamurthy, M. S. Chandorkar, J. M. Pathak, David A. Animasaun and R. Gupta. (2014). Characterization of foliage and Gymnemic acid yield of *Gymnema sylvestre* accession for selection of elite lines. International Journal of Medicinal Plants, (Photon), In Press.
12. R. Krishnamurthy, David A. Animasaun and Rajshree Patel (2015). Isolation of Gymnemic acid from big and small leaf of *Gymnema sylvestre* R. Br for phytochemical constituents and hypoglycemic effect. International Journal of Biological and Chemical Sciences (Accepted).
13. S.S. Mahajani, Joshi V.J. and Parikh K.M. (1996). Bioavailability of L-Dopa from HP-200, a formulation of seed powder of *Mucuna pruiens*: A pharmacokinetic and pharmacodynamic study. Phytotherapy Research, 10: 245-6.
14. B.V. Manayam (1995). HP-200- A alternative medicine treatment for Parkinson's' disease. Journal of Alternative and Complementary Medicine, 1:249-55.
15. B. V. Manayam and Parikh K . M. (2000). Anti-parkinson activity of *Mucuna pruriens* seeds. International Journal of Neuropsychopharmacology, 3:52.
16. A. A. Farooqi, Khan, M. M. and Vasundhara, M. (2001). Production Technology of Medical and Aromatic Crops. Published by Natural Remedies Pvt Ltd., Bangalore.
17. CSIR, New Delhi, (1962). The Wealth of India : Raw Materilas, Vol 6, pp 639-644, Council of Scientific and Industrial Research, New Delhi.
18. W. Dymock, Warden C.J.H. and Hooper D. (1980). *Mucuna.* Pharmacographia India, 1 : 447-450.
19. J. M. Pathak (2006). *Disha*, The Zandu Group's In-House Bulletin, Vol.7, Issue 2, April June, 1-2.
20. Krunal H. Naik and Krishnamurthy R. (2014).Inhibitory activity of methanolic extract of bark of *Pseudharthria viscida* against microorganisms. International Conference on Current Status,Opportunities and Challenges in Medicinal Plants and Natural Product Research, September 24-26, 2014,Uka Tarsadia University, Bardoli, India, pp.85.