SOUVENIR

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C. G. Bhakta Institute of Biotechnology

A journey through a decade...

2005 - 2015
A Decade Celebration
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Shri Bhagubhai Patel

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Prof. Dr. R Krishnamurthy

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Pritesh Patel  Rushabh Shah  Jemisha Mistry
Shreya Desai  Anoop Markande

Cheers!
here’s to the
last ten years!

The Event
Conceived by
Meonis Pithawala

Programmed by
Dhruti Mistry

Co-ordinated by
Rajashekkhar Ingalhalli

A journey through a decade...
It gives me a sense of gratification that C. G. Bhakta Institute of Biotechnology (CGBIBT), started in 2005, is marching forward creating milestones without looking back. Our dedicated service to the mankind, the love and affection to the cause of education has brought us up to this platform. Our success story has been written by our students, who are now all over the globe and are contributing constructively to the society. I personally feel teachers are the backbones for any academic institution. The growth of any institution will be at stake if due recognitions are not given to them. These capabilities include not only first rate infrastructure and facilities but also a teaching faculty which is as good as, if not better than, any of the best colleges in India. We pride ourselves in our unique teaching methods and learning environment which enhance hands-on knowledge, skills and positive attitudes as well as work ethics of our students which would make them highly sought in the global society. I, on behalf of the management, request the co-operation of public at large and the student community in particular to make this institution more beneficial to the mankind.

We invite those who wish to share our vision.

It gives me great pleasure to welcome the present students, alumni, teaching faculty members of CGBIBT and all the guests on the occasion of celebrating tenth anniversary of the founding of CGBIBT. The CGBIBT which was founded on 1st August 2005 was the fruit of many visionaries among whom special mention must be made of Bhakta family Mahave who have liberally contributed in establishing the Institute. Over the past ten years our student strength has grown steadily and now stands at 900. It was decided at the outset that the main objectives of the Institute were to provide quality education to the rural students in Biotechnology who used to either go to the cities in Gujarat or major cities outside Gujarat and to develop sufficient facilities to maintain and improve the standards set in the past. I am proud to say that over the past ten years the Institute has worked with enthusiasm to realise these twin objectives. I will conclude by taking this opportunity to thank those who have contributed to the success of today’s function and to all our activities during the past ten years.

Shri K. G. Bhakta

Shri Shashikendu Patel
Since its founding in 2005, the C.G. Bhakta Institute of Biotechnology (CGBIBT) has grown to become South Gujarat’s flagship, research-led Institute, known for the excellence of its teaching, its research, and its service to local, national and international communities. We have more than 900 students in the disciplines of Biotechnology and Microbiology of whom 200 are postgraduate. 

As we look to the future one thing is certain – knowledge will be a key resource and will be highly sought-after within India and around the world. Our challenges is to help generate ideas that will benefit society, and educate and train people to work in fields where they will be valued both for their specialised knowledge, and for their ability to research, communicates and solve problems.

To meet these challenges we need to build on the alliances and collaborative partnerships with business, government, and other research institutions. I am happy to acknowledge that CGBIBT has been registering exemplary all-round progress and I congratulate the Director and faculty members of CGBIBT.

When C. G. Bhakta Institute of Biotechnology (CGBIBT) was started 10 years back, everyone was filled with a sufficiently high degree of anxiety and self-doubt to (fortunately) cause all of us to compulsively over achieve. Our motto then was (as it is now): Promise Big and deliver even Bigger. Indeed, in 2005, we were surrounded by larger, better funded, and aggressive competitors who saw us as just being in their way.

But, we had something that they could not easily acquire to overtake us. We had extreme persistence coupled with a brilliant faculty comprising of only outstanding academics and professionals who worked tirelessly and exclusively for us. We also were fortunate to have a set of highly committed and dedicated sponsors who provided not just financial support, but also passionate encouragement and who utilized their own business networks to help us succeed at every turn. They never turned down any of our requests. We deeply appreciate their contribution to our institute.

I take this opportunity to extend a special thank you to all of our graduates and current delegates who continue to be vocal advocates for CGBIBT and its various programs. Your continuous support and choice of CGBIBT for your future career and certification is the real reason why we are so successful. But I also know why you chose us and why you are so generous in the accolades you extend to us … it’s because “CGBIBT is South Gujarat’s one of the Best in applied science education”.

I feel extremely privileged to be the Director of this esteemed institute. I have been blessed by phenomenal colleagues, associates, and delegates who have given me incredible support in shaping this institute as an Advanced Research Center. I envision that CGBIBT will become a landmark globally in the coming years.

I just humbly pray to the Almighty to shower His Grace on all those who are directly or indirectly associated with this institute.

With Best Wishes

Dr. D. R. Shah

Prof. Dr. R. Krishnamurthy
Key-note Speakers
Dr. JITENDRA PRAKASH
M.Sc., Ph.D., D.Sc., M.I. Biol. (Dublin)

1. President, invitro International Pvt. Ltd., Bangalore, India
2. Director, Bengal invitro venture Pvt. Ltd. (a joint venture company with the govt. of West Bengal)
3. President, consortium of commercial plant tissue culture laboratories
4. Former deputy director, Twyford Centre for advanced technologies, Cambridge, U.K.
5. Former research director, Biotechnology, AV Thomas & Co., Cochin
6. Former director, Biotechnology, Indo-American hybrid seeds, Bangalore
7. Former member, executive council, Visveswaraya Technological University (2004 – 2007)

AUTHOR/EDITOR:
  (Jointly with K.L. Giles).
- *Low cost options for Tissue Culture Technology in Developing Countries*. Published by IAEA, Vienna, February 2004.

After his doctoral research which was rated as a work of international standards, by Prof. A.I. Gibbons of Australia, Dr. Jitendra Prakash obtained specialized work experience as Virologist and Biotechnologist at University College, Dublin, where he developed a tissue culture seed potato production system for a number of Irish varieties on a commercial scale. He worked out the regeneration systems from protoplasts of potatoes and Freesian and for the first time demonstrated expression of single gene at cellular level.

Dr. Prakash worked for several years as a Cell Biologist, Group leader - Germplasm Development Group - Head - Plant Health Services Division, and Deputy Director of Research, Twyford Plant Laboratories, Somerset, and Programme Coordinator – Twyford Centre for Advanced Technologies, Cambridge, England. As Group Leader - Germplasm Development, Dr. Prakash also headed the Research and Development wing of Asmeer seeds (a subsidiary of Twyford Plant Laboratories), and evolved several new strains of tomatoes, cauliflower and cabbage.

On 15th November 1994, Dr. Prakash invited His Royal Highness The Duke of Kent to try his hand at tissue culture. This event was covered by BBC radio, television and British Press. At Twyford’s Dr. Prakash was not only guiding and coordinating a team of research scientists involved in basic and applied aspects of cell biology, virology and molecular genetics but was also successfully managing a profit centre with a turnover of over 2 million U.S. dollars.

Dr. Prakash returned to India in 1987 as Vice President of A.V. Thomas Group of Companies and was responsible for R & D programmes on tea, coffee, rubber and cardamom. He was instrumental in setting up India's first commercial tissue culture laboratory at Cochin Export Processing Zone. From February 1989 to January 1995, Dr. Prakash worked as Director, Indo-American Hybrid Seeds, a pioneer and innovative seed company engaged in research and development, production and marketing of vegetable seeds, horticulture and greenhouse technology. Dr. Prakash was responsible for its 10 million production capacity tissue culture laboratory, a Genetic Engineering Laboratory and programmes on breeding for vegetable crops like tomato, Morojob, capsicum, okra, and brinjal, flower and oilseed crops in conjunction with cell and molecular biology. During his tenure as Director of the company, Indo-American Hybrid Seeds had made remarkable progress in many spheres of commercial biotechnology and horticulture.

Since February 1995, Dr. Prakash is heading the invitro Group of organizations, comprising of world class horticulture biotechnology enterprises in India. The research and innovations of invitro Group, under the leadership of Dr. Prakash has attracted exclusive contracts with several global leaders in the horticultural sciences. The group is fully owned, promoted and professionally managed by scientists and technocrats. It is responsible for nearly 50% of the country’s tissue culture export market and year after year, its export turnover has been greater than the collective export turnover of 76 operational tissue culture units in India. The company’s efforts and achievements have been duly recognized by the central and state governments, with it being selected for the “Excellence in Export” award, time and again.

Dr. Prakash has more than seventy original research papers to his credit and has presented papers, keynote addresses and chaired sessions at over 50 International Seminars and Conferences all over the world. Dr. Prakash has contributed a number of chapters in reference books and has edited a number of books published by Academic Press (U.S.A.), Kluwer Academic Publishers (The Netherlands), and Oxford & IBH Publishing Co. Ltd., (New Delhi). Dr. Prakash is the founder editor of *Vitamaria*, Editor - Biotechnology Research and Industry Survey, Chief Editor of *International Journal of Biotechnology*, and research papers for Biotechnology, London (U.K.) and other prestigious journals and publishing houses. He is also a member of the panel of the Department of Biotechnology, Department of Science and Technology and Biotech Consortium India Limited, and a number of National/International Organizations / Activities as an expert in Agriculture and Food Biotechnology. Dr. Prakash’s research activities and achievements have been covered worldwide by both electronic and print media innumerable times and he has been rated by leading business magazines as one of the ten foremost commercial biotechnologists in the world (Business India, September 2-15, 1991).

Besides commercial biotechnology, the use of technologies into sustainable agriculture and rural development is Dr. Prakash’s major interest. He has worked extensively with Prof. M.S. Swaminathan in the initial years of his Bio-village Programme, a concept which was proposed by him in 1991, published by FAO/UNDP, later translated into 6 different languages of Asia and Latin America by the respective National Governments. He has also worked with many programmes of Prof. Vavilov, the Nobel Prize winner for peace, in collaboration with the Free University, Amsterdam.
Dr Prakash has been a Senior Consultant to joint FAO/CARDA Division of Nuclear Techniques in Food and Agriculture, Vienna, Austria, for their research programmes on improvement of banana in Cuba, Ghana, Malaysia and Bangladesh. He was also Scientific Advisor to the World Bank programme on banana research and development. Dr Prakash is also President of Consortium of Commercial Plant Tissue Culture Laboratories (I). He was a member of Executive Council of Vishwakarma Technical University, Belgaum. Dr Prakash is also a Member, Board of Studies in Biotechnology, Karnataka University, Shimoga and Vice Chairman, Board of Studies in Biotechnology, Vishwakarma Technical University, Belgaum, the largest technological university in India. He is also Honorary Advisor to the International Executive Service Corps of USAID (Washington).

Dr Jitendra Prakash has recently received Gold Medal and Certificate of appreciation from the International Society of Horticultural Sciences, for his efforts and achievements in the area of Plant Tissue Culture.

Dr Jitendra Prakash is currently working closely with the Department of Horticulture and Food Processing, Government of West Bengal, in improving fruit crops, vegetable crops, root and tuber crops, flower crops, spice crops, and forestry crops, besides working towards saving the Sunderbans, conservation of bio-diversity of Chhota Nagpur, cleaning of the Ganges and popularizing bio-diesel in the state.

Of the above-mentioned categories, the invitro Group, under the guidance of Dr Jitendra Prakash has already developed low cost tissue culture production protocols for potato, banana, cardamom, tomato, cucumber, orange and the medicinally important Ippeaz to ensure that they are available to the farmers of West Bengal.

In summary, Dr Prakash's work in vitro culture and biotechnology has evolved with the new discoveries in frontier sciences and created innovations leading to most cost-effective and high quality production of billions of in vitro plants of chosen varieties and species, all of which were earlier put to the application of the technology. In vitro plants have triggered accelerated production and distribution (marketing/export) of several crops. Further, his lifetime work on mass production and propagation of genetically transformed elite materials of food and energy crops have great application on sustainability of biofuels, climate changes and food and nutritional security, at the recent time of escalating food prices combined with global financial crisis and emerging climate change issues.

COMMERCIALISATION OF PLANT TISSUE CULTURE TECHNOLOGIES

Jitendra Prakash
In Vitro International Pvt. Ltd., 12/44, Rajiv Gandhi Nagar, Bommanahalli, Bangalore - 560068 Ph: 080 4110 7680 E-mail: invitro@bti.vsnl.net.in

I. Introduction

Biotechnology, as a science and as a commercial activity is very new. In fact it is less than 30 years old. However, in that short period of time, it has dramatically changed the way in which research and development is carried out. It also has changed the way in which commercialisation of products can be achieved. The impact of biotechnology is likely to lead to dramatic improvements in human and animal health, the food supply and the quality of the environment. This expansion of biotechnology has been enthusiastically endorsed by governments and by companies throughout the world, as being essential for economic growth. Biotechnology is being viewed as being essential to competitiveness of countries in both the developed and developing world and is also seen to be essential to the development of the global economy.

Biotechnology in itself is not an industry, but the way in which the technologies associated with biotechnology interact with traditional industries in the three major sectors of pharmaceuticals, chemicals and agriculture, represents the potential for significant growth and expansion. Biotechnology provides a potential to produce new, improved, safer and less expensive products and processes. Pharmaceuticals and diagnostics for human and animals, industrial enzymes, food additives, new crop plants and pathogen degrading microbes are a sample of the new products that can be developed or enhanced through the application of biotechnology.

With the ongoing demand for the economic products of plant origin, relentless efforts are underway to increase the productivity of plants and quality of produce and also to develop plants of agronomic relevance through newer technologies as that of biotechnology. The impact of biotechnology should be felt through solving the basic problems of food, fibre, fuel and medicine. Our main objective should be to improve the plants inherent ability to be more productive, which can be best achieved through a combination of conventional plant breeding technologies and biotechnologies. The tools of biotechnology can help in raising the productivity of crops through increases in total dry matter production, which can then be partitioned in a favourable way to the economic part. The term biotechnology is currently being used to denote a wide variety of biological manipulations. The immediate application would be tissue culture for the fast multiplication of superior clones of various plants. This would open a pathway of continuous improvement in yield, in combination with research, which can further increase the yield ceiling.

II. Use of Tissue Culture Technology by Industries

Tissue culture methods have been successfully used in three main areas:

- Plant Micropropagation
- Production of disease-free propagules
- Novel plant breeding through cellular and molecular techniques including recombinant DNA
- Production of specialty chemicals by cell culture
The most popular application of plant tissue culture is micropropagation, an alternative to vegetative plant propagation. Micropropagation represents the optimum efficiency in terms of vegetative plant propagation and allows a large number of propagules to be produced in a relatively short period of time under controlled conditions, throughout the year in relatively small space (Prakash, 1990a).

The need for healthy stock plants and clean planting materials is of paramount importance for growers, plant propagators, seed producers and exporters. The clean planting material can double the yields of our staple crops which are fortunately clean like: Tobacco, Sweet potato, pea etc. Currently the technology for production of disease free propagule is available on the shelf for more than hundred crops (Prakash, 1990b).

Tissue culture techniques such as protoplast culture, somaclonal variation, endosperm culture, limited gene transfer, embryo rescue techniques, mutation and gene recombination can speed up the process of introduction and induction of variations at the cellular level for most of the economically important agricultural and horticultural crops. Several varieties of rice, maize, tomato, chilies, wheat, oilseed rape,Capsicum and Lilium have been released using technique of anther culture and gynogenesis variation. Similarly, useful mutations have been utilised for the various improvement programs of potato, sugarcane, rice, maize, barley, wheat, tobacco, loblolly, grasspea, alfalfa, rapeseed, oats, tomato, coalers, poplar, grapevine and soybean (Prakash, 1990b).

Similarly, the production of plant species of greater use (such as Rice, Wheat, Maize) can be increased by introducing in their genome, those genes which assume resistance to different stresses such as unfavourable climatic conditions or harmful biological agents like insects, fungi or viruses (Table 1 & 2). One obvious application would be introduction in plants of genes for nitrogen fixation, which would enormously reduce the costs due to the wide usage of chemical fertilizers in agriculture. In plants like cotton, potato, tomato, tobacco, insect resistance can be built using Bacillus thuringiensis (BT) Crystal protein genes. Using recombinant DNA techniques, resistance has been built for a wide spectrum of viruses in crops like Tomato, Alfalfa, Tobacco, Potato, Peatle, Rice etc. Of recent, herbicide tolerant Soybean, Cotton, Corn, Oilseed rape and sugarcane have been developed for herbicides such as bromoxynil, atrazine and glyphosate. The transgenic plants of tobacco, oilseed rape, malt, rice, potato, tomato, sugarbeet, cotton etc. have been under field trial in different parts of the world (Table 3 & 4), and in some countries transgenic products are already available on the supermarket shelves. In future, we may expect plants to tolerate heat, cold, drought, salt and heavy metals.

Furthermore, plants can be engineered to produce molecules of particular value such as drugs of complex protein structure. In this way, expensive drugs could be derived from plants cultured at low price, ensuring continuous and high level production of useful molecules.

Biotechnology industries offer environmental friendly industries to flow. Furthermore, living organisms can be useful to obtain macromolecules to depollute land, for improved utilization of agriculturally valid land in condition of scarcity of water, for the degradation and depletion of organic wastes.
III. Commercial realities: A Global Perspective

During 1990, about 290 million plants were produced worldwide while the figure was only 130 million plants during 1986 (Table 5). Between the years 1986 and 1991 there is an average of about 30% increase in production of tissue culture plants. In 1993, there was an increase in production from 562 million in 1992 to 663 million in 1993, an increase of 17.5%. In 1997, production was 800 million, a mere 2.1% increase. Thereafter, every year the increase has been significant with the exception of the year 2001.

3.1 Commercial micropropagation in the European Continents:

A survey was carried out during 1996-97 of the plant tissue culture laboratories in 23 European countries by COST 822, "Development of In vitro Systems for Large Scale Propagation of Elite Plants using In vitro Techniques". The national representatives on the Management Committee provided the information for their countries. A total of 565 laboratories, 193 commercial and 312 official were located (Table 6). This showed a small recovery in the number of commercial laboratories, but a drop in the official laboratories involved in plant tissue culture for the first time as a slight decrease has taken place (Ribordi, 1997).

In spite of requesting that laboratories avoid listing all cultivars being worked on, as in previous years, the total number of plant names being worked on increased from 1833 in 1993 to 1966 in 1996, an 8.8% increase. Prunus is still the most important plant in both the commercial and official laboratories, having 76 entries for commercial and 112 for official laboratories. The total production was 179 million plants, an increase of nearly 16% since the 1993 survey. This is a mean production per laboratory of 0.54 million. The industry involves 3317 people, which is a small increase in the number over the last three years. But it signifies a small reduction of the mean per laboratory from 7.8 to 7.5. In addition there is a reduction in the number of clean workstations from 7.2 to 5.5. The main interest of 39% of laboratories was rapid multiplication of plants. Basic research was the next most important interest with 21% followed by plant breeding with 15%. Genetic manipulation accounted for only 7% of laboratories (O Ribordi, 1999).

Since 2003, there has been significant increase in the number of commercial tissue culture laboratories in Western Europe and the production of tissue culture plants has been shifted to China. Eastern European countries and to a limited extent to India. The Commission of the European Communities has not published any recent data on commercial micropropagation in Europe.

3.2 Commercial micropropagation in North America:

Orchids were the first crops produced commercially by micropropagation in North America, starting about 1965. There are about 100 labs, 9.5 percent of these labs in the US are small, producing fewer than 1,000,000 plants per year. Between 1984 and 1986 five large labs were constructed, each with a capacity of 15-20 million plants per year. Another 6-10 have a production capacity of 2-10 million plants per year, accounting for 25-35 per cent of the US production. The total production capacity of North American labs probably exceeds 150 million plants per year, with less than half of this capacity currently utilized.

3.3 Commercial Production in Asia:

Asia is principally covered by agricultural and forestry land. The annual demand for plant material of agricultural crops and forestry is, therefore, enormous. About 162 commercial labs are now in operation throughout Asia (Table 7). The total tissue culture production in selected Asian countries in 1987-88 was estimated at 74 million units. Orchids were by far the largest item, with an estimated production of 44 million plants per year accounting for 60 per cent of the total production.

Following orchids are temperate flowering crops (10 per cent), ornamental and foliage plants (10 per cent), and plantation crops (7.5 per cent). In Korea, bananas and orchids are the main crops. Gerbera is known to be increasing. The remainder (5 per cent) includes tropical flowering crops, fruit crops, spices and forest trees.

Tropical orchards are predominantly produced by tissue culture. The annual production in 1987-88 was estimated at 38 million plantlets, equivalent to 90 per cent of the total orchid tissue culture, whereas temperate orchids accounted for the rest (Prakash, 1993).

3.4 Commercial Production in Australia and New Zealand:

There are about 88 micropropagation companies in Australia and New Zealand producing forestry species (Australia, one; New Zealand, one), orchids (Australia, 2; New Zealand, 10) and other horticultural plants (Australia, 39; New Zealand, 6). Seven companies were producing more than one million plants per annum and four exported more than 50 per cent of their production.

Table 5. Growth trend in micropropagation industry

<table>
<thead>
<tr>
<th>Year</th>
<th>Millions of plants produced</th>
</tr>
</thead>
<tbody>
<tr>
<td>1986</td>
<td>135</td>
</tr>
<tr>
<td>1990</td>
<td>196</td>
</tr>
<tr>
<td>1993</td>
<td>256</td>
</tr>
<tr>
<td>1996</td>
<td>344</td>
</tr>
</tbody>
</table>

Table 6. Devolution of Plant Tissue Culture Laboratories in the 1994 COST 822 Survey

<table>
<thead>
<tr>
<th>Country</th>
<th>Official</th>
<th>Commercial</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>China</td>
<td>112</td>
<td>76</td>
<td>188</td>
</tr>
<tr>
<td>France</td>
<td>27</td>
<td>22</td>
<td>49</td>
</tr>
<tr>
<td>Germany</td>
<td>54</td>
<td>38</td>
<td>92</td>
</tr>
<tr>
<td>Italy</td>
<td>20</td>
<td>14</td>
<td>34</td>
</tr>
<tr>
<td>Japan</td>
<td>16</td>
<td>12</td>
<td>28</td>
</tr>
<tr>
<td>Malaysia</td>
<td>10</td>
<td>9</td>
<td>19</td>
</tr>
<tr>
<td>Poland</td>
<td>8</td>
<td>5</td>
<td>13</td>
</tr>
<tr>
<td>Portugal</td>
<td>2</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Korea</td>
<td>12</td>
<td>8</td>
<td>20</td>
</tr>
<tr>
<td>India</td>
<td>11</td>
<td>5</td>
<td>16</td>
</tr>
<tr>
<td>Indonesia</td>
<td>36</td>
<td>26</td>
<td>62</td>
</tr>
<tr>
<td>Thailand</td>
<td>10</td>
<td>7</td>
<td>17</td>
</tr>
<tr>
<td>Total</td>
<td>632</td>
<td>439</td>
<td>1071</td>
</tr>
</tbody>
</table>

Table 7. Number of commercial labs in Asia

<table>
<thead>
<tr>
<th>Country</th>
<th>Official</th>
<th>Commercial</th>
<th>Total</th>
</tr>
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<td>Korea</td>
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<td>Malaysia</td>
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<td>Philippines</td>
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<td>7</td>
<td>17</td>
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<tr>
<td>Australia</td>
<td>39</td>
<td>5</td>
<td>44</td>
</tr>
<tr>
<td>New Zealand</td>
<td>6</td>
<td>7</td>
<td>13</td>
</tr>
<tr>
<td>Total</td>
<td>632</td>
<td>439</td>
<td>1071</td>
</tr>
</tbody>
</table>

The survey's impact is also evident in other continents, with similar trends and numbers of laboratories conducted.
3.5 Commercial micropropagation in South and Central America

There are about 94 institutes that utilize new biotechnologies. However, the volume of production is not known. The main crops produced are carnation, chrysanthemum, gerbera, heliconia, orchid, banana, anthurium, physostegia, and rose and strawberry. Emphasis in the commercial lab appears to be on virus indexing and micropropagation of indexed stock plants, primarily of ornamental flower crops. More than 100 million plants of banana, sugarcane and pineapple are produced in low cost biofactories of Cuba mainly for domestic use.

3.6 Commercial micropropagation in India

India enjoys a unique position and offers many advantages over other countries in the world for setting up micropropagation industry with its rich land resource spread over diverse agro-climatic zones, profuse solar radiation throughout the year, good quality water, availability of skilled manpower as well as labour at much cheaper rates.

As compared to the West, in India, micropropagation industry has started late by 10 years and has expanded exponentially from 1987 to 1993 (Prakash, 1993 a,b). But the facilities created now are at par with those available in leading countries like Netherlands and the US. The first Indian commercial plant tissue culture unit was set up in 1987-88 by A.V. Thomas and Co., in Cochin Export Processing Zone with a 5 M capacity. Since then, there has been a vertical expansion in the number of units as well as production capacity per annum with about 71 industrial units having been set up or in the pipeline with annual production capacity reaching 500 million plants.

The existing laboratories can be classified by the product type they offer, stage 2 or 3 cultures in vessels, in vitro rooted plantlets, or even hardened plants. Indian industry generally produces hardened plants for domestic use and in vitro rooted plantlets for the export market.

An analysis of the micropropagation in India indicates that the plants belonging to the horticulture segment of the ornamental industry are the major items being produced, which is also the international trend. Besides, Indian industry is working on a few fruit plants such as banana, pineapple, papaya, mango, and vegetables like tomato and potato; spices including cardamom, turmeric, ginger, tamarind; plantation crops of sugarcane, vanilla, tea, coffee, and a few forest species – Eucalyptus, Teak and Paulownia.

IV. Problems associated with Micropropagation Technology

Biotechnology is a highly capital intensive industry and since the delivery of large volumes at targeted time is generally not made possible, in most cases, the per plant cost becomes prohibitive. When the economics of an industry is under construction, it becomes necessary to scrutinize the factors responsible for its performance over the past years, in order to set priorities of its objectives. Though an extensive range of techniques has been developed during the last 20 years, there are still some major difficulties, especially those associated with the optimization of this technology to a variety of crops (Prakash, 1989). Some of these problems can be discussed under the following headings:

4.1 Production Costs

Initially, in the case of selected crops, conventional planting materials were being sold at a much lower price as compared to tissue culture/propagules. However, later when it was proved that through tissue culture technology, uniform and disease-free plants could be produced in large numbers all the year round, there was no problem for the commercial growers in accepting tissue culture plants at a higher price. The present trend in the micropropagation industry leaves us with a picture that more and more companies are producing only a few selected varieties of plants in large numbers, thereby generating stiff competition in the market. For example, Vietnamese farmers are producing tissue cultured potato plants at a total cost of one US$ or less, as compared to 6 US$ per plant in the USA. The reason is that the current procedure of plant tissue culture is labour intensive. Consequently, the labour inputs (in the form of salaries) form a significant proportion of the total production cost. In order to extend the technology to a variety of crops, one must be able to cut down production costs to the minimum by implementing the technical advances that are made in the research and development field. Given technical support and lower transportation costs, future production centres will shift to countries where labour is less expensive. The standard of living in every country is increasing day by day, and the same trend, as can be seen in Vietnam, may not continue in the future. Hence, the future of the micropropagation industry clearly lies in the ability to develop new technologies such as the application of computer controlled handling systems.

Delpeuch et al (1985) presented a robotic vision system, which locates plantlets on their medium, picks them up and transfers them into containers. To reduce labour costs, automated plant culture systems are being developed where they duplicate the features found in manual plant tissue culture procedures but without the necessity for frequent manual transferring of cultures to a fresh medium. This would occur through computer control, either pre-programmed instructions (Tisserat and Vandercook, 1985). However, the use of computers in this technology is under debate because the whole system cannot be automated. Whenever and wherever precise cutting is again required, automation is difficult. In order to automate these features and computerise quality control at each step of the micropropagation, heavy expenditure in research and development would be required, thereby increasing the cost of each unit considerably. Hence, big multinational companies in the USA and Europe are not sure whether to go for automated technology or to shift the production unit to a place where the production costs would be less due to the availability of cheap labour.

4.2 Seasonal Nature and Heavy Peak Demands:

Most of the crops are seasonal and are in heavy demand only at certain times of the year. To overcome this problem and to meet the requirements of extremely a large number of plants, efficient methods must be developed. Also, most commercial laboratories produce a mixture of plants required at different times in order to maximise the use of the facilities throughout the year. These definitely cut down the production cost.

4.3 Personnel/Organisational Problems:

Since large-scale micropropagation is a labour intensive process, one must correspondingly increase the number of employees to
increase the number of plants produced. As the activities in any commercial laboratory expand, organisational problems will arise and at times, become critical. To reduce these problems, the management structure must be well planned. Supporting information systems such as inventory control, production scheduling, space utilisation, daily targets and actuals should be well defined for better management. For the successful large-scale micropropagation of quality products, there must be perfect co-ordination among the technicians, supporting staff, supervisors and the researchers. The job description and reporting system should be very clear. In addition to these, personnel training is a critical component for successful large-scale production, which need to be periodically reviewed on the basis of the customers needs. Inventory control must be computerised. All these features will add considerably to the management responsibilities.

4.4 Contamination Control

Close attention has to be given to this aspect, to avoid much of the industrial effort going waste. For example, if in an Indian commercial laboratory, 10 percent of the one million plants produced through micropropagation methods perish due to contamination, then the loss in direct income would amount to approximately Rs. 6,00,000. This loss of cultures during the culture period would increase the production cost of the remaining plants to a large extent.

Contamination in cultures could be caused mainly due to the following:

a) Natural contamination occurs from any of the following agents: dust, air-borne salts, metal filings, chippings, spares, seeds, vegetable matter, fibres, animal hair etc.

b) Man-made contamination occurs mainly from body and clothing. It was calculated that each operator will generate a minimum of 5,000,000 to 2,000,000 particles (greater than 0.5 mm diameter) per minute.

For reducing contamination rates and increasing productivity, a clean indoor room environment for culture work is essential. In the work area (where aseptic manipulations are carried out), three sources contribute to the area particle contamination of the room air: They are the outside air, the liberation of particles by machinery and process and the liberation of particles by an operator. The objective of clean room is to establish and maintain an internal atmosphere devoid of particulate contamination in the working environment. Air cleanliness is measured as particle counts, such as Class 1, Class 10, Class 100, Class 1,000, Class 10,000 etc., that is, on a particle concentration (number of particles per volume).

For the high cleanliness area of Class 100 or better, low turbulence displacement flow, also called laminar flow boxes to be employed where the air moves along parallel flow paths and permits the particles liberated in the room to be carried quickly and as directly as possible. This sort of facility may be established by introducing the supply air into the room through the walls or ceiling built up from HEPA or ULPA filters or through special clean air distribution elements. Since the installation and running cost of laminar flow cabinets is high, it is advisable to design it according to the plant species to be worked on, so that the working space is utilised effectively. To achieve this, a perfect understanding is needed between researchers, engineers and architects. Open minded collaboration between the supplier of the clean system and the end user is absolutely essential, for optimum balance between quality and flexibility on the one hand and investment and running cost on the other, to be achieved (Brussard et al., 1983; Schlicht, 1985).

Modern high quality clean rooms have environmental control systems in the widest sense. Besides cleanliness, temperature and humidity control, they encompass advanced air handling technology, vibration protection and many other aspects. In addition to this, correct and well-defined working procedures are necessary. All this should be backed by performance monitoring of the operation as a whole. Wearing gowns, which is mandatory in Class 100 clean rooms, reduced the contamination from clothing, skin and hair.

Maintaining cleanliness in the working area is equally important. Apart from contaminants appearing in the cultures and those carried along with the plants, there is a continuous entry of contaminants along with the worker and through air entering the room. A definite protocol should be developed in order to reduce contaminations in the laboratory and this varies with the location of the laboratory.

V. Conclusion

Biotechnology could help solve some of the most pressing problems of the developing countries like: health, nutrition, industrial development, environment protection and energy production. Several plant species are poor in protein, quantitatively and qualitatively. Introduction of specific genes into those plants can help production of great quantities of proteins, which are as nutritional as human milk.

The non-seasonality of biotech production could help ease out violent price fluctuations. This can help the farmer to plan his agriculture accordingly and get out of speculative trading. This also has its advantages in microlevel agricultural planning in altering the crop balance (Prabhakar 1992c).

Further larger volumes of plant delivery in time, at affordable prices could be made possible by this technology. The commercial scale-up, however, has to come from the entrepreneurial R & D. Simultaneously, rapid advancements in the newer and advanced frontiers of plant biotechnology by the academic institutions and their commercial upgradation by the entrepreneurial research and development could revolutionize Third World agriculture subsequent to that of the Green Revolution.

At present, demand for most of tissue culture plants exceeds production by commercial laboratories, which is presently approximately 1600 million plants, resulting in a turnover of approximately US $ 460 million, as against an estimated potential global market of $ 15 billion annually.

This clearly indicates that there is enough opportunity for young graduate biotechnologists to enter into business of plant tissue culture at the following level:

i) Micropropagation

ii) Primary hardening

iii) Secondary nursery

iv) Input supplier

v) Marketing and extension
Besides micropropagation, the areas which offer opportunities for young entrepreneurs are as follows:

i) Disease indexing, maintenance and supply of clean plants
ii) Production of double-haploid plants
iii) Creation of novel varieties using in vitro mutagenesis
iv) In vitro pollination and embryo rescue for long distance process
v) Maintenance of mother plants for tissue culture and hybrid seed production
vi) Seedling production and supply
vii) Production of medicinal plants
viii) Hybrid seed production
ix) Selling and control crosses
x) Simple fermentation
xi) Extraction of fruit and vegetable juices
xii) Fresh vegetable packing for super market
xiii) Simple processing of fruits and vegetables
xiv) Encapsulation of essential components of plant origin
xv) Biofuel and Bioenergy
xvi) Mushroom cultivation
xvii) Vermiculture
xviii) Biopesticides
xix) Aquaculture

The above list of technologies are just to name a few. The business of biotechnology offers endless opportunities and much greater than discussed in this presentation.

References


Recent Advances in Molecular diagnosis of plant Pathogens

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Disease diagnosis and pathogen detection are central to the ability to protect crops and natural plant communities from invasive biotic agents. The early and accurate diagnosis of plant disease is a crucial component of any crop management system. Plant diseases can be managed most effectively if control measures are introduced at an early stage of disease development. Increasing globalization, travel and the international trade in plants and plant products will continue to pose a threat to plant health through inadvertent introduction of exotic pests and pathogens. Seeds and planting material provide an efficient means in disseminating plant viruses and virus like diseases. Reliable identification is a prerequisite to the study and control of plant viruses and virus like diseases. Plant health is of strategic importance to government and industry by protecting vegetable crop industries from the increased risk of emergency plant pathogens that could have adverse effects on production, trade and market access. Detection of viruses, viroids and phytoplasmas in plant material, vectors or natural reservoirs is essential to ensure safe and sustainable agriculture. Reliance on symptoms is often not adequate, as symptom expression can be highly variable. Biological techniques for disease diagnosis are usually highly accurate but too slow and not amenable to large-scale application. During the last two decades in addition to biological indexing, immunoassays particularly ELISA using polyclonal and monoclonal antibodies became widely accepted as valid tools in the diagnosis and detection of plant viruses. Immunoassays have now been developed for nearly all groups of plant viruses. New molecular detection techniques such as nucleic acid hybridization, blotting techniques using radio and novel non-radioactive labeled probes, variants of PCR, and real-time monitoring of amplicons or quantitative PCR have been used for diagnostics of viruses. Considerable emphasis is now being placed on generic platforms such as DNA microarrays and sequencing (Metagenomics), which will facilitate diagnosis of unknowns. Electronic technology is also finding its way into disease diagnostic and pathogen surveillance systems. In addition to digital systems widely available for disease diagnosis, several free forms of software can be used to map and share the presence of diseases. In addition to technology development, efforts are to be made to transfer technology, products, and skills to stakeholders in national research and extension services. Availability of these diagnostic methods provides greater flexibility, increased sensitivity, and specificity for rapid diagnosis of virus diseases in disease surveillance, epidemiological studies, plant quarantine, seed certification, germplasm indexing, phenotypic evaluation of germplasm and breeding for resistance programs. They are also used in collecting baseline information and monitoring shifts in pathogen and pest dynamics due to changes in agriculture systems and climate change effect. Many diverse approaches have been tried to minimize the losses caused by these diseases.
Introduction:
Plant diseases continue to impact on the productivity of crops and quality of crop products worldwide despite many years of research and development on improved methods for their control. It has been estimated that an average of 0.20–0.30 of crop yield is lost annually from the field (Enke, 2006), even in crops where pesticides and cultivars with improved genetic resistance to diseases are used. The losses may be substantially greater in subsistence agriculture, where crop protection measures are often not applied.

The accurate and rapid identification of the organism(s) that cause plant disease is essential for effective disease control. It enables more informed decisions to be made about cultivation choice and how and when chemicals can be used most effectively to control disease epidemics. Pathogen detection and identification is important for preventing the spread of disease by screening seeds and other plant material and is needed to implement quarantine regulations. It is also important more generally in many areas of plant pathology research, for example in: studies of disease epidemiology; studies of yield loss disease relationships; and in designing new strategies for disease control. Pathogen or disease diagnosis is therefore, fundamental to virtually all aspects of plant pathology. Traditional approaches to disease diagnosis generally first involve the interpretation of visual symptoms. This may be followed by laboratory identification, using for example selective media and microscopy, to confirm the diagnosis. In some cases these methods are still the cheapest, simplest and most appropriate.

1.0 Importance of Diagnosis: Disease diagnosis and pathogen detection are central to the ability to protect crops and natural plant communities from invasive biotic agents (Miller et al. 2009). Increasing globalization, travel and the international trade in plants and plant products will continue to pose a threat to plant health through inadvertent introduction of exotic pests and pathogens (Branham 2004). In addition to detecting new invasive species, rapid and accurate diagnostic tests are required to monitor the emergence of new variants of well-known pathogens, such as yellow rust (Milne et al. 2009), the Ug99 race of black stem rust (Singh et al. 2008) that is now threatening Africa, the Middle East and South West Asia and more aggressive pathotypes of potato blight in the USA and Europe. Improved surveillance methods will be vital to safeguard food security in the face of such well-known threats, as well as previously minor; or unknown diseases emerging as a consequence of climate change or other environmental shifts, or due to new agricultural practices.

Numerous detection methodologies are now available, but regardless of the approach, important questions need to be answered prior to their inclusion into experiments. These include sensitivity, accuracy, robustness, frequency of testing, and cost. Despite many novel technologies being available, challenges remain to identify as yet uncharacterizable fungi, to detect cryptic pathogens, and to characterize the assemblage and diversity of fungal communities in different environments without bias. There is always a need to characterize fungi quickly and accurately. No one knows how many fungal species exist, but sequencing of environmental DNA may improve the accuracy of current estimates (Hanselworth et al. 2001). Next-generation sequencing and pyrosequencing approaches will also provide promising ways of enlarging the scope of molecular detection studies.

2.0 Traditional Diagnosis: Conventional methods do, however, have a number of drawbacks, which has prompted the search for alternative diagnostic techniques. Traditional methods generally require skilled and specialized microbiological expertise, which often takes many years to acquire. There is a need to use more generic techniques that can be taught quickly and easily to relatively unskilled staff. Methods that involve culturing can often take days or weeks to complete and this is not acceptable when rapid, high throughput diagnosis is required. The results are not always conclusive, e.g. where similar symptoms can be caused by different pathogens or physiological conditions. Closely related organisms may be difficult to discriminate on the basis of morphological characters alone. It may also be necessary to discriminate between populations of the same pathogen that have specific properties, e.g. fungicide resistance, toxin production or differences in virulence. Traditional methods may not be sensitive enough (e.g. where the detection of pre-symptomatic infection is needed) and as such much effort has been devoted to the development of novel methods for detecting and identifying plant pathogens over the last decade. The greatest efforts have been on the development of diagnostics based on antibodies and nucleic acid technologies (Duncan & Terrance, 1992; Schutz et al., 1994; Ward, 1994; Martin et al., 2000; McCartney et al., 2003).

3.0 Antibody based Diagnostic Techniques: Antibodies are molecules, produced by mammalian immune systems, that are used to help identify invading organisms or substances. If antibodies can be generated that recognize specific antigens associated with a given plant pathogen they can be used as the basis of a diagnostic tool. The use of antibody technology is well established for detection of phytopathogenic bacteria, fungi, Phytobacteria, and Viruses. There are three routes for the production of antibodies for use in diagnostic assays. Polyclonal antibodies (PAbos) are made by injecting extracts from the pathogen into an animal, usually a rabbit, and then collecting blood from it, sometimes later. Monoclonal antibodies are made by fusing antibody-producing cells (lymphocytes) from the spleens of an inoculated animal (usually mice or rats) with cultured myeloma cells. This generates many hybrid cell lines (hybridomas) which each produce a different single (monoclonal) antibody. Recently, a new method of producing antibodies has been developed, using a phage display (McCarthy et al., 1990). This technique involves the use of functional fragments of antibody molecules which have been amplified by polymerase chain reaction (PCR) from a number of different animal species Immunodiagnostic assays have been developed using this technique for the bacterial pathogen Raoultella solanacearum Race 5 which causes brown rot of potatoes (Griep et al. 1998) and for a number of plant viruses.

3.1. Assay formats: The principal aim of an immunodiagnostic assay is to detect or quantify the binding of the diagnostic antibody with the target antigen. There are a number of different ways of detecting antibody/antigen binding, but often these involve coupling the antibody to an enzyme that can be used to generate a colour change when a substrate is added. Enzyme linked immunosorbent assay (ELISA), a technology developed in the 1970s, is the most commonly used diagnostic technique that uses antibodies (Clarke & Adams, 1977; Voller & Bidwell, 1985). It involves an enzyme-mediated colour change reaction to detect antibody binding.

This is usually done in a microtitre plate where the degree of colour change, usually measured in a computer controlled plate reader, can be used to determine the amount of pathogen present. These assays have the advantages of being simple, cheap and suitable for processing many samples.
The simplest format is the plate-trapped antigen ELISA (PTA-ELISA). In this assay, the microtite plate wells are directly coated with the test sample. This is followed by incubation with a specific antibody which binds to the target antigen. A more commonly used format is the double antibody sandwich (DAS-ELISA). Here, specific antibodies are used to coat the microtite plate, which then trap antigen from the test sample. An enzyme-labelled specific antibody conjugate is then used for the detection. There are other less commonly used variations of ELISA such as competition assays. Many antibody-based assays have been developed for detection of plant viruses (Sward & Englund, 1995; Tomar, 1995) and to a lesser extent fungi and bacteria (Dewey & Thornton, 1995; Spire, 1995; Dewey et al., 1997). Several companies specialize in the supply of antibodies and kits for detection of plant pathogens, e.g. Agdia, Agdia, Bioreva, Leewa and Sigma. There are some good, simple assay formats that are available for antibody-based detection, and quantification of the target pathogen is relatively easy. The tests work well with viruses, but unfortunately it is often difficult to achieve the required specificity for more complex organisms such as bacteria and fungi. The methods may also be specific for a particular morphological form or stage of the life cycle of the pathogen. This may be an advantage or disadvantage, depending on the application.

Other formats are available which employ a similar detection mechanism to that used in ELISA. One such test is the dot immunoassay (binding assay) FIA (IBA, Los Angeles, 1990) used for detecting pathogens in plant tissue. Plant extracts are spotted on to a membrane (usually nylon or polyvinylidene difluoride (PVDF)) which is then followed by colour-based immunological detection.

3.2. Dipstick assay: In this method, the detection is performed, using a simple protocol, on a membrane on the end of a dipstick. In one format, the dipstick is first incubated with the plant soil extract and this is then followed by colour-based immunological detection. Alternatively, a capture antibody, absorbed to the dipstick, is incubated with the plant soil extract and detected using a second antibody. Cahill & Harter (1994b) developed a new method for the detection of Phytophthora cinnamomi zoospores in soil. The dipsticks incorporated antibodies to the zoospores and were incubated in soil. Using this method, it was possible to detect 40 zoospores ml-1 in less than 45 min.

Plant pathogens have been detected in situ by squashing plant tissues directly on to a membrane followed by immunodetection, that is, tissue printing or squash blot systems (Gwin et al., 1991; Shine & Gernstech, 1993). Immunofluorescence (IF) microscopy is another method that allows in situ localization of the pathogen. Plant samples are applied to microscope slides in thick tissue sections and fixed. Detection is achieved by conjugating a fluorescent dye to the specific antibody (direct IF) or to a molecule that detects the specific pathogen (indirect IF). This method has been used to identify specific plant pathogen spores on microscope slides (Dewey, 1996; Kennedy et al., 1999). However, it is not suitable for high throughput screening and it requires specialised equipment (an ultraviolet microscope) and relatively skilled personnel to perform it.

Recently, there has been great progress in the development of very rapid diagnostic tests that are also simple, and do not require any special equipment or knowledge. Most of these use the lateral flow assay

3.3. Microsphere Immunoassay Technology: A novel multiplexes detection method was developed based on microsphere immunoassays to simultaneously detect four important plant pathogens: a fruit biech (Fusarium oxysporum f. sp. subsp. citrulli (Asc), chili vein-banding motile virus (CSVMV, potyvirus), watermelon silver mottle virus (WSMoV, tospovirus serogroup IV) and melon yellow spot virus (MYSV, tospovirus). An antibody for each plant pathogen was linked on a fluorescence-coded magnetic microsphere set which was used to capture corresponding pathogens. The presence of pathogens was detected by R-phycocyanbryn (RPE)- labeled antibodies specific to the pathogens. The assay was able to detect all four plant pathogens precisely and accurately with substantially higher sensitivity than enzyme-linked immunosorbent assay (ELISA) when spiked in buffer and in healthy watermelon leaf extract (Charlton et al., 2013).

4.0 Nucleic Acid-Based Diagnostics: Nucleic acid-based methods (using probes and/or PCR) have increasingly been used in recent years to develop diagnostic assays for plant pathogens (Schots et al., 1994; Ward, 1994). These methods, particularly those based on PCR, are potentially very sensitive. In addition they also offer the potential to be highly specific. Most assays developed for bacteria and fungi have detected pathogen DNA, which is easier to prepare, and more stable than RNA. Various methods are available for DNA purification from pure bacterial or fungal cultures, infected plants, soil or air-samples (McCourtney et al., 2003). However, since detectable DNA may be obtained from dead cells, some workers have opted to start from RNA, since this may more accurately reflect viable pathogens material. When using a PCR assay starting from RNA, the RNA is generally converted to DNA by the enzyme reverse transcriptase before it is used in the detection assay, hence it is termed a reverse transcriptase PCR (RT-PCR) assay. This type of assay is also commonly used in the detection of plant viruses (Wasserhouse & Chu, 1995), most of which have RNA genomes. An alternative, isochronous amplification technique, Nucleic Acid Sequence Based Amplification (NASBA), has also been used for amplification from RNA (Batziak et al., 2002; Van Beekhoven et al., 2002; Cook, 2003).

4.1. Molecular Hybridization: Molecular hybridisation-based assays were first utilised in plant pathology to detect potato spindle tuber viroid (Owens and Diener, 1981) and adapted to virus detection (Hull, 1993). This development in nucleic acid hybridization technology offers a good potential for virus detection (9). The target viral nucleic acid from a plant sample is spotted onto a solid matrix, commonly nylon or nitrocellulose membranes, and bound by baking. Free binding sites on the membrane are blocked with a nonbiological DNA and a blocking agent. Thereafter, hybridisation with a labelled probe is carried out. The label is then detected by autoradiography (for radioactive probes), or by a colormetric reaction if an enzyme label is used. The sensitivity of dot-blot hybridization is about the same as ELISA

4.2. Polymerase chain reaction (PCR): PCR, a method for rapidly synthesising (amplifying) millions of copies of specific DNA sequences includes using colorimetric assays (Beck et al., 1996; Matsa et al., 1996) or fluorometric assays (Fosuso et al., 1999) in a microtitre plate format. PCR is a very sensitive technique, only small amounts of DNA are needed (e.g. from a single spore (Lee & Taylor, 1990)) and often this can be prepared fairly simply.
4.2. Nested PCR: Nested PCR is used to improve the sensitivity and/or specificity of the assay. This involves two consecutive PCR reactions, the second one using primers that recognize a region within the PCR product amplified by the first set. PCR products can also be detected with a probe (Mutas et al., 1995; Knoll et al., 2002). This can improve the sensitivity and specificity of the assay, particularly when the amplified product may not be sufficiently to be seen on an agarose gel.

4.2.1. Multiplex nested RT-PCR: A multiplex nested RT-PCR in a single closed tube has been developed for simultaneous and sensitive detection of the viruses CMV, CLoV, SLRV, and AYN, and the bacteria Pseudomonas savastanoi pv. savastanoi from olive plants using 20 compatible primers in a compartmentalised tube. This newly developed method combines the advantages of multiplex RT-PCR with the sensitivity and reliability of nested RT-PCR carried out in a single closed tube. It enables the simultaneous detection of several viral RNA and bacterial DNA targets in a single analysis, performed with woody plants. It also saves time and reagent costs because it can be performed in a single reaction, although accurate design of compatible primers is needed.

4.2.2. Immunoassays (IC-PCR): Antibodies immobilised on the surface of a microtitre plate or microtube tube are used to capture the pathogens, which are then detected using PCR. IC-PCR can improve the sensitivity and specificity of the assay and reduce problems with inhibitors in the sample. A few plant pathogens have been detected using this approach (e.g., Jacob et al., 1999; Shamblin & Hebard, 1999; Hartung et al., 1996).

4.2.3. Multiplex PCR: Multiplex PCR allows the simultaneous and sensitive detection of different DNA or RNA targets in a single reaction. On the other hand, PCR detection protocols can be designed to verify the presence of more than one pathogen in plant material by looking for common specific sequences in two or more of them, or to detect related viruses or bacteria on multiple hosts (Weir). Multiplex PCR is useful in plant pathology because different bacteria and/or RNA viruses frequently infect a single host and consequently sensitive detection is needed for the propagation of pathogen-free plant material. There are several examples in plant pathology of simultaneous detection of several targets and the amplification by multiplex PCR of two or three plant viruses has been reported. Multiplex PCR has been used to develop an assay that can detect four fowl wheat pathogens (Septoria tritici, Stagonospora nodorum, Puccinia striiformis and Puccinia recondita) [Fraschol et al., 2001].

5.0. Real-time PCR: The process of quantifying target DNA has recently been simplified considerably with the advent of real-time PCR. This method avoids the usual need for post-reaction processing, as the amplified products are detected by a built-in fluorimeter as they accumulate. This is done by using non-specific DNA binding dyes (e.g., Syber Green) or fluorescent probes that are specific to the target DNA [Wittwer et al., 1997]. The principle underlying real-time PCR is that the larger the amount of target DNA present in the sample to be tested, the quicker the reaction progresses and enters the exponential phase of amplification. The amount of PCR amplicons produced at each cycle is measured, using the fluorescent dyes or probes, and for each sample tested the cycle threshold (Ct) is calculated. This is the cycle number at which a statistically significant increase in fluorescence is detected. The Ct increases with decreasing amounts of target DNA. Using specific probes, such as TaqMan oligonucleotide probes (Livak et al., 1995), has the advantage of reducing signals due to mispriming or primer dimer formation. Other types of fluorescent probes and primers, that utilise the advantages of reporter and quencher dyes, have also been used in real-time PCR assays (Madsen et al., 2002; Bates & Taylor, 2001). These include Molecular Beacons (Tyagi & Kramer, 1996) and Scorpions (Thelwell et al., 2000).

It is possible to detect several targets simultaneously by using probes with different fluorescent reporter dyes (Weller et al., 2000). Real-time PCR methods are not yet widely used for plant pathogen diagnostics but many assays have already been developed for detection of fungi, bacteria, viruses and viroids (see Böhm et al., 1999; Schaad et al., 1999; Beesens et al., 2000; Mumford et al., 2000; Beijby et al., 2001, 2002; Schaad & Frederick, 2002). In addition to simplifying quantification, real-time PCR has a number of other advantages over conventional PCR. It is faster and a higher throughput is possible. Post reaction processing is unnecessary, eliminating the risk of cross contamination. It can be more specific than conventional PCR, if a specific probe is used in addition to the two specific primers. Real-time, fluorogenic PCR assays have recently shown great promise in the diagnosis of many plant pathogenic bacteria.

6.0. Loop-mediated isothermal amplification (LAMP): Loop-mediated isothermal amplification (LAMP) is another type of isothermal amplification that is being increasingly used in the diagnostic field offering sensitivity and economic costs (Nuttom et al., 2009). The method requires a set of four specifically designed primers that recognize six distinct sequences of the target and a DNA polymerase with strand displacement activity. The amplification products are stem-loop DNA structures with several inverted repeats of the target and cauliflower-like structures with multiple loops, yielding >500 g/ml. The LAMP reaction was enhanced by the addition of loop primers (Nagamine et al., 2002), reducing the time and increasing sensitivity.

7.0. Microarrays: Microarrays consist of multiple probes (nucleic acids, proteins, carbohydrates, antibodies) deposited or directly synthesised on a surface in an ordered fashion. This approach has several advantages over classical probe based methods: high-throughput, parallelism, miniaturisation and automation. Conventional DNA microarrays consist of nucleic acid probes deposited on a planar glass surface. The surface is usually coated with chemically reactive groups (epoxy, poly-L-lysine or aldehyde) to ensure efficient binding of nucleotidic probes on the surface. To assess the presence of target genes, nucleic acid samples are labelled, either chemically or by an enzymatic reaction. Labelled samples are then hybridized onto the array, and washed using different stringency buffers. The resulting signal resulting from specific interactions between probes and target nucleic acids is measured using a confocal microarray scanner. Only probes hybridized to a labelled target will yield signal, thus retaining the presence of the cognate nucleic acid motif in the sample. High-density DNA microarrays have been used in a broad variety of applications such as transcriptomics, comparative genome hybridization (CGH), resequencing, drug discovery, microbial community characterization or single nucleotide polymorphism (SNP) analysis. The field of application depends primarily on the strategy and the marker genes used to design the probes. A variety of genes (virulence factors, phylogenetic markers, antibiotic resistance genes, etc.) have been employed on microbial diagnostic microarrays. Microarray-based technology has been used for plant virus detection since 2002 (Eitenm et al., 2010; Abdullah et al., 2009; Wei et al., 2009; Pasquin et al., 2008; Lee et al., 2008; Abdullah et al., 2005; Dongen et al., 2005; Bystracka et al., 2005; Beesens et al., 2005; Lee et al., 2003). DNA microarray provides the highest capability for parallel specific testing which can be used to detect individual plant virus or combinations of many plant viruses and/or virus-like pathogens (Haddad et al., 2004; Haddad and Barua, 2006; Barua and Haddad, 2007).
8.0. Protein Microarrays: The increasing use of protein microarrays for proteomic studies has spurred the advancement of various detection technologies. This gel-free approach has found an increasing number of applications due to its ability to rapidly and efficiently study thousands of proteins simultaneously. Different protein microarrays, including capture arrays, reverse-phase arrays, tissue microarrays, lectin microarrays and cell-free expression microarrays, have emerged, which have demonstrated numerous applications for proteomic studies including biomarker discovery, protein interaction studies, enzyme-substrate profiling, immunological profiling and vaccine development, among many others. Conventional label-based approaches like fluorescence, chemiluminescence and use of radioactive isotopes have witnessed substantial advancements, with techniques like quantum dots, gold nanoparticles, dye-doped nanoparticles and several bead-based methods now being employed for protein microarray studies. In order to overcome the limitations posed by label-based technologies, several label-free approaches like surface plasmon resonance, carbon nanotubes and nanowires, and micro cantilevers, among others, have also advanced in recent years, and these methods detect the query molecule itself.

9.0. Metagenomics: At the present time there are some plant diseases where the causal agent in unknown or the disease symptoms are associated to different pathogens or abiotic agents. In this sense metagenomics provide a new rationale and effective methodology to identifying the primary causative agents. The study of untargeted microbes may carry out by metagenomics, which gives a broad sight for the investigation of microorganisms origins and function in the environment. Metagenomics is based on studies of ecological diversity of uncultured and molecular biological data. For the metagenome analysis of microbial populations in phytopathology, the total content of nucleic acids involved with the disease is used.

10.0. Next Generation Sequencing & Genomics: One of the major uses of next generation sequencing technology is non-targeted analysis of biological components in a sample for diagnostic and detection purposes from a range of sample material of plant, bacteria, fungi, virus, virloid and phytoplasma. This has successfully used these novel technologies for detection of previously unknown pathogens and is using it for microbial community profiling in a multitude of research projects. Novel developments in DNA sequencing technology have delivered an increase in achievable read length of DNA sequences which is a prerequisite for reliable species identification. Furthermore, bacterial genomics with focus on plant and foodborne pathogens forms an integral part of our sequencing portfolio.

11.0. Recent Developments and Future Directions: Over the past decade there have been significant advances in plant pathogen diagnostics. Antibody-based and/or nucleic acid-based diagnostic methods are now available for most of the important crop pathogens found in developed countries. The approaches for developing new diagnostics are well established, and extensive databases of DNA sequences exist that can be used in designing and checking the specificity of diagnostic methods. There have also been recent advances in the technology available for detection; for example, the lateral flow assays available for antibody-based detection allow rapid, on-site detection by untrained personnel.

12.0. Concluding Remarks: The new molecular-based diagnostic techniques that have been developed can be more rapid, sensitive and accurate than traditional methods. Immunological and DNA-based diagnostic tools are playing an increasingly important role in plant pathological research and will continue to do so. The specificity and sensitivity of these methods will open new opportunities for research on the interactions between pathogens and their hosts. For example, the ability to target specific pathogen genes could lead to better understanding of host pathogen interaction or the dynamics of fungicide resistance development within fungal populations. Diagnostics that can discriminate pathogens in disease complexes will improve our understanding of these complexes, which may lead to more efficient control strategies.

The application of novel diagnostic methods to inoculum detection will allow progress to be made in understanding the temporal and spatial dynamics of epidemic development, and open up new opportunities for disease forecasting and management. However, new management tools, such as efficient sampling strategies, will have to be developed to make the best use of these new diagnostic methods. The methods will also play an important role in restricting the spread of disease via infected seeds and other plant material.

The future prospect of developing biosensors able to identify pathogen inoculum, either through specific sequence amplification, or biochemical signatures present on spores or cells, or released during germination of propagules. There are considerable technical challenges in producing a sensor of sufficient specificity and sensitivity that can detect disease agents in real time without the need for downstream sample processing. Signal amplification from very small quantities of biological target material and transduction into an electrical readout that is proportional to the initial chemical concentration are two key issues. Advances in nanotechnology and sensor design suggest that these challenges should be met in the near future. Already, electrochemical devices are available that exploit changes in electromagnetic waves (surface plasmon resonance) when biopolymers such as DNA or proteins adsorb to the sensor chip surface. Such devices can incorporate the specificity of antibody-antigen or nucleic acid molecular interactions. It is anticipated that advances in biosensor technology will increasingly impact on fields as diverse as healthcare, food science, agriculture and bioscience.

Suggested References:


A journey through a decade…
2005 - 2015

Path Traversed
by
CGBIBT…
The idea of founding this Institute was conceived on December 2003 when Bardoli Pradesh Kelavni Mandal formed a committee of enquiry into assessing the demand of having an educational institute in the field of Biotechnology. During this inquiry, the committee put in a submission which highlighted the immediate need to establish such a centre in south Gujarat region. The philanthropist, Shri Narani Bhakta from Mahua came forward to found the centre in the fond memory of his late elder brother Shri Chhaganbhai Bhakta. Thus, the institute came formally into being in the year 2005 in the name of C G Bhakta Institute of Biotechnology (CGBIBT).

The institute first opened to students on 1/8/2005. The first integrated course in Biotechnology was started with affiliation to Veer Narmad South Gujarat University, Surat. CGBIBT grew over the years with rapid expansion in intake of students and the courses offered. In the year 2011, CGBIBT became one of the constituent premier Institutes of Uka Tarsadia University. Today about 800 students are studying in undergraduate, post graduate and doctoral courses in Biotechnology and Microbiology. More than 400 CGBIBT’s alumni have graduated with degrees in Biotechnology and Microbiology. About 10% of CGBIBT’s alumni study, work and live abroad.

**Major Accomplishments:**

During the past 10 years CGBIBT has achieved remarkable success in furthering its mission and core-values.

- **2005:** The course in Biotechnology was initiated on 1st Aug 2005 and was temporarily accommodated in Maliba Pharmacy College. Foundation stone for the separate building exclusively for biotechnology was laid.

- **2007:** The faculty of Biotechnology began functioning in the separate building.

- **2008:** The new program of M.Sc Microbiology was introduced.

- **2009:** State level competition Gillon was hosted under auspices of CGBIBT.

- **2010:** First batch of Integrated M.Sc Biotechnology came out.

- **2011:** CGBIBT became constituent institute of UTU. Prof. Dr. R Krishnamurthy joined as full-fledged Director of CGBIBT. Five year Integrated M.Sc Microbiology program was initiated.

- **2012:** The foundation for Maliba Research Centre for Excellence in Tissue Culture was laid. MOU was signed between UTU and UNILORIN, Nigeria for academic and research exchange program. Mr David Animasaun was the first overseas faculty research scholar from UNILORIN who stayed for one and half years for carrying out part of his doctoral research work.

- **2013:** Maliba Research Centre for Excellence in Tissue Culture started functioning in the service of farmers.

- **2014:** First International Conference on Current Status and Opportunities in Medicinal Plants and Natural Product Research – ICMPNPR, was hosted by CGBIBT. Another faculty research scholar Mrs Khadija Abdulkareem from UNILORIN, Nigeria joined CGBIBT to carry out part of her doctoral research work. First overseas inter faculty lecture series from members of UNILORIN, Nigeria was organized by CGBIBT. Prof. Dr. R. Krishnamurthy was invited as Visiting Professor at UNILORIN, Nigeria
lasting memories to cherish
Co-passengers in the early journey...
Hidden talent unearthed...
Biotechnology: Something old, something new, finding things out of the blue. We think and do, the things which people find weird to do. Be it bacteria or fungi, we are the real guys researching in the lab. We were the lucky few, who got in the CGBIBIT's queue. After the admission procedure as we got through, we were surrounded with instruments such as colorimeter and centrifuge. We were invincible. If we won't have had the support of our faculty, we'd have got the instruments into pieces!

Bhavnik Desai
Integrated M.Sc. Biotechnology Semester V

Reminded him of his milk suits and queen's year earnings. A thin old blanket to share its freezing low temperature. With just a thought alone the mighty king got to shave. The family worked hard so that penny could add. Seeing their efforts to live the king got sad. The children were bare with rifts peeping out. Suddenly his highness the king found himself scrawny. With simmering thoughts of soul hidden, and by learning a lesson the king walked back. Then to the king, she declared: The powerful and wise. Mind got blank with a question arises. If the blood of mine was red there was not blue, then life we lived was it was just and true?

Diwesh Tachhadiya
Integrated M.Sc. Biotechnology Semester V

Hiren Rathod
M.Sc. Biotechnology 2015

Life...

Life is the name of purpose, struggle, love, dedication and a number of feelings and emotions. Life shows its true colors with time. It depends upon you that how you look towards life and what is your strategy of leading an ideal life. Life takes a number of turns during its whole span. Life may not be kind to you all the times. There are tough times which really test your courage and your capabilities of facing unfavorable situations. How well you tackle these adverse conditions prove your credibility. People who can close their eyes to avoid facing the hardships of life are coward. They do not have courage and stamina to stand firm in front of life's demons. Remember what nothing can be done without trying, without striving hard to get yourself out of the trouble, without facing the situations. Such incidents in life improve your approach towards destiny. These incidents may entirely change one's life. Those who make you think a bit more normal, if there were no such things in life, it would have been quite boring and monotonous. These incidents make you strong willed person. In other words, life tests you all the time.

"LIFE IS DIFFERENT FROM A TEACHER, BECAUSE TEACHER TEACHES A LESSON AND TAKES THE EXAM BUT LIFE TAKES EXAM FIRST AND THEN TEACHES A LESSON."

When you are in love, life changes its meanings, priorities and requirements. Life seems to be confined within the kingdom of your loved one. Life is a gift of God and to love and being loved is the best feeling in life which has no parallel. Everyone wishes to live a blissful life with his/her life partner. Life can be blissful only if you know each other, understand well and stand for each other through bad times. Do not waste your life. Life should have some purpose. Identify your aim, head towards your destiny and spend your entire life in achieving your goals. This is what life calls you. It is a wise saying that time waits for no one. Once it is spent, it is gone. We've got to be wise in choosing our priorities so as not to waste any of our precious time. Keep on trying to get your goals and don't lose hope. It is said by Pablo Coelho:

"WHEN YOU WANT SOMETHING, THE ENTIRE UNIVERSE CONSPIRES IN HELPING YOU TO ACHIEVE IT."

Life is worthless without hope. Hope is the key element in life which never lets you down and keeps your morals high. Always be optimistic and have faith in God. Everything happens for a reason. Sometimes a slight inconvenience makes us think that we are unfortunate, life is cruel etc. but this is not the reality. Spread optimism instead of pessimism. It will light up your life positively.

Remember that:

"THE DARKEST HOUR OF NIGHT COMES JUST BEFORE THE DAWN."

The inspiration of life is to serve mankind. Spread happiness and you will be blessed!
Woh lamhe jo meri zindagi ke anmol pal ban gaye, woh lamhe jo mere guzre huye kal ban gaye,
Kasah in lamho ko main fir se jee paata, woh lamhe ko meri aankho ke jal ban gaye,
Aankho mein sapne aur dil mein arman liye, ek safar pe chal pade bina kisse ke saath liye,
Raaste mein kuch naye chehra se mulakat ho gayi, Phir to woh aise dost bane jaise ek rishta ho umra bhar ke liye,
Woh lamhe jo mujhe mere chahunme wale dost de gaye, woh lamhe jo na bhula pane wale log de gaye,
Is safar ki shuruvaat hame ne saath ki thi, jaan se pyare yaaron ke sath kitni sari baat ki thi,
Zindagi ke un palon ko bhi hum ne saath jya tha, jin palon ne khushi aur gum dino se mulaqqat ki thi,
Woh lamhe jo ab laut ke nahi aa sakte, woh lamhe jahan hum chah ke bhi nahi ja sakte,
Apne yaaron ke dill ki baat bina kahe jaan lete the,
Kyun pasand hai kisko ye thodhe se jhagdhe ke baad hame maan lete the,
Woh lamhe jo ek dhundhli yaad ban gaye, woh lamhe jo ek yaadgar kitab ban gaye,
College aane ka man nahi phir bhi college aaya karte the, kuch logo gharfi aur baki ko teacher bulaya karte the,
Exam dena ki to jaise aadat si ho gayi thi, phir result dekh ke kuch note to kuch muskuraya karte the,
Ajab the ye lamhe jismein hum harse bhi aur roye bhi, ajeeb the ye lamhe jismein kuch paaye bhi kuch khoye bhi,
In lamho ko ek yaad bana ke apne sath lejaunga, in lamho ki dastaan fir kise sunaunga, kyoon ki........
Kya in lamho ko mein phir jee paunga ? kya aise mahol main me phir se aa paunga ?
Main janta hoen iske jawab to Na hi hoga, kyoon ki likhi hai kitab ko main phir se kora nahi kar paunga
Bus in lamho ko apne dill mein basa loonga aur jahad aayegi in doston ki to naam aankho ke saath

musthara loonga

Rashid hai mera naam aur ye thaa mere dosto ke liye ek chhota sa paigaam
Emotional expressions
Our college is celebrating 10th year of its founding this year. I had the honor and privilege of being a part of CGBIBT for almost 9.5 years, as a student of its 1st batch for 5 years and then as a lecturer for 4 and a half years.

There are countless memories that come back to my mind when I try to recall my days here at CGBIBT and it will be impossible to make justice to all of them through my brief remarks, but the very first one that comes to my mind is, I have had privilege of being one among the 7 students who did “the Bhoomi-pujan” of our college building.

I am one among those who lived the journey of this institute from the beginning with minimal facility in 2005 to the present full-fledged facility what we see today. As a proud and grateful member of CGBIBT family, I convey my best wishes to all of you this wonderful institute on its 10th Anniversary!

As I look ahead and visualize, CGBIBT will strive further in pursuit of higher standards of teaching, research, and shaping the dreams of young talent.

Let us all carry forward the glorious legacy of this institute that continues to inspire us and it also share an unbounded optimism in its continued impact and relevance in shaping and enriching the lives of countless young students making them to think creatively, work collectively, and excel passionately!

I take this opportunity to congratulate our Director, Prof. Dr. R Krishnamurthy and all the teaching and non-teaching staff of CGBIBT on this proud occasion.

Hima Vyas

Being away from home has challenges of its own. It takes some time to get used to living in an unknown place and adjusting to being a student again. The support of my seniors and fellow classmates were crucial to my life at Maliba Campus. Similarly, the faculty and other staff members of CGBIBT are very helpful. Professors are approachable and accommodating; while the office staff is eager to help us even with non-academic matters. Living on campus also has a lot of great benefits. The campus facilities such as WiFi and the library are easily accessible to students and are very useful to my studies.

Prachi Pandya

When I first came to the CGBIBT, Maliba campus, I was amazed at its peaceful beauty. Our campus is situated on the outskirts of Bandoli town in rural atmosphere surrounded by sugarcane fields and mango orchards. This peaceful atmosphere at Maliba campus makes it a wonderful place for studying. Unlike its relaxing surroundings, academic life at CGBIBT is challenging. My first year in the Biotechnology program was flooded with assignments, quizzes, presentations, papers, and exams. However, with great guidance from enthusiastic professors, the tough program made my effort much more rewarding. After one year, I realized that my conceptual understanding had improved greatly in terms of both theory and practice. I strongly believe that the knowledge and skills I have gained from CGBIBT will enable me to advance my career in the field of Biotechnology upon graduation. Maliba campus life is very enjoyable.

The friends I meet and the time we are together in Maliba are invaluable in making my life more meaningful. After one year studying at CGBIBT, Maliba Campus, I knew I made the right decision to come here. If I had to choose again, I would still choose Maliba Campus for its peaceful surrounding, high education quality and diversity of students and faculty members.

Vitika Sharma
Int. MSc Biotechnology Sem III
David Adeyemo Animasaun
Ex-Overseas Research Scholar

It gives me immense joy, great honour and profound pleasure to give my goodwill message and to be identified with you on the occasion of 10th Anniversary of our great institute; G.B. Ishaq Institute of Biotechnology (GBIBIT), Uwa Tarada University (UTU), Baroda, India.

It was indeed a one-man standing ovation when I heard that GBIBIT a Center of Excellence in Biotechnology would turn TEN. Although I could not be physically present due to some commitment here in Nigeria, the joy within me knows no bound and I feel really honoured to send this short message.

I want to sincerely congratulate the entire Management, Staff and Students of GBIBIT on this great occasion of the Tenth Year (Anniversary) of making dreams come true. I recall with great reminiscence my stay at GBIBIT, the special times and beautiful moments we had together, the unspeakable hospitality and the culture of excellence in all endeavours. These are times I treasured most in life and the memories are eternal. It was a great privilege for me to be a part of this great success and still being a part of you till the moment is like a cherished treasure to me. GBIBIT, I was indeed bathed by the shower of your benevolence, discipline, diligence and nobility.

I join my good friends, mentors, colleagues and scholars on this joyful occasion. I believe all our achievements, the laurels we won are just the beginning of our unstoppable international exploits. I see greater future as our dear GBIBIT (Citadel of Excellence) continues to transform lives and play leading role as Global Biotechnology Hub.

Prof. Dr. R Krishnamurthy Sir and your entire team’s hard work and unity of purpose have brought our Institute this far. May each coming year bring even more success to GBIBIT and UTU. My heart goes to my dear friends, colleagues and students who have been keeping the touch on. You all really deserve this magnificent celebration. I am very proud to be a part of you.

Once again, on behalf of my family, the staff and students of the Department of Plant Biology and the TISSUE CULTURE GROUP, University of Ilorin, I heartily congratulate you all. I wish you a fun-filled event.

Thank you.

Khadija
Ex-Overseas Research Scholar

It was a great and memorable experience for me to be in India and at the same time in GBIBIT. I carried out part of my doctoral research work at GBIBIT for six months successfully which is a milestone in my professional career. This would have not been possible without the mentorship of Prof Dr R Krishnamurthy, kind cooperation by the management of UTU and the help rendered by all faculty members of GBIBIT. I am so happy that GBIBIT is celebrating its 10th anniversary and wish all the success for the event and congratulate all faculty members of GBIBIT on this proud occasion.
I have enjoyed the journey of almost a decade in CGBIBT first as a student and then as a teacher. Transforming from carefree into careful was an undertaking and engaging experience. The guidance and blessings of all the teachers have helped me shape my life. The great company of friends while studying from 2006-2014 has emblazoned in me endless memories. I am fortunate to continue to be a member of CGBIBT family.

SOLO RIDE
Something that hits you like tones of water
Gives you tons of pain
And you will have crying face.

Times of missing are working under
Which can make you wonder
Still nothing has changed.

Fix but broken de-faith heart
Never ending nothing apart
Keep on adding
Why do we cry
When we had pour a pure blood
It does mean something to ours
Each and every "try".

New we will prize
And fix our all effort together
A never ending try,
So somewhere we can fly.
Till our soul drown down.

Someday you may need someone to appreciate,
But nobody there to even a praise
That was the moment when you have to taste the glory
And forget all worries
And let the evening fade.

So why do we cry,
When we can have it by alone,
It does mean much more to ours
Each and every smile.

Now we will rise
With what we had shane enough
Never cry for lost behind
And so forward we can lead,
Till our soul drown down.
PROMISES to Keep & MILES to Go...