THE GOLDEN CASKET OF BIOTECH LECTURES

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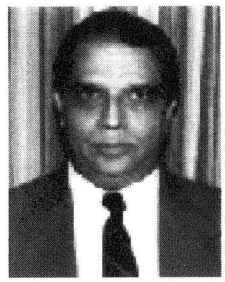

Note from the Chief Editor

Dear Reader

The Institution of Engineers (India), Karnataka State Center (IEI, KSC), Bangalore has over the last two years initiated several activities in the area of Biotechnology after the release of the State's Biotechnology Policy under the able leadership of the Chief Minister Shri. S.M. Krishna. In the policy Biotechnology education is an area where lot of emphasis has been laid upon and hence IEI KSC has taken up this area as its main focus for starting Biotechnology activity.

Over the last two years IEI, KSC has organized a series of lectures titled the GOLDEN LECTURE SERIES ON Biotechnology, where eminent speakers like Prof. H. Sharat Chandra, Prof. G. Padmanaban, Prof. M. Udaya Kumar, Prof. Kameshwara Rao, Dr. T.M. Manjunath, Dr. Jagdish Mittur and several others totaling to 25 eminent personalities from different areas of biotechnology were invited to speak. This valuable collection of speeches, with a short introduction to the speakers is being brought out as THE GOLDEN CASKET OF BIOTECH LECTURES. This book has been printed based on the generous contribution of BIO INFRAA an upcoming enterprise for creating and providing state-of-the-art infrastructure for biotech education and research.

The role of Engineers in the Biotech Industry has been in creating quality instrumentation and infrastructure as per the requirements of the life science. The understanding of the basic biology which was lacking earlier is becoming more and more essential to make the contribution of engineers more meaningful. In this context Visveswaraiah Technological University has taken up the major task of introducing BE Biotechnology, for which IEI, KSC has been making its contribution in the curriculum development and organizing of workshops. In future IEI, KSC will in addition focus on the creation of a centralized quality infrastructure for the purpose of practical training for the teachers and graduates from the different engineering colleges and also life science colleges. This is the need of the hour, as Biotechnology is a practical skill based branch of science, and without quality infrastructure, educational programs in Biotechnology will not be meaningful.

I sincerely hope that the contribution of IEI, KSC will make a quality impact on the progress of Biotechnology education in the state of Karnataka. This valuable document THE GOLDEN CASKET OF BIOTECH LECTURES will go a long way in introducing and educating the public on biotechnology. This is a document which every college student and teacher pursuing Biotechnology education must posses and we will do our best to see it reaches every part of the state. I thank all the speakers who were kind enough to accept our invitation to make their presentations. I also thank Mr. Vinay Kumar for the timely preparation of text and floral graphics for the excellent design and print.

Er. P.K. Shanbhag FIE
Chairman IEI KSC & Chief Editor

KARNATAKA BIOTECHNOLOGY DEVELOPMENT COUNCIL
(Being promoted by Government of Karnataka)

BANGALORE

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Bangalore-560 052
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MESSAGE

I am delighted that a series of recent lectures in various fields of Biotechnology is being brought out as a compendium through the efforts of Dr.Seetharam Annadana. A great deal of R & D work is done in various aspects of Biotechnology and tremendous amount of information is generated every year. Dr. Annadana has selected and edited 25 of the recent lectures by Who's Who of Biotechnology Research and this should make the golden casket of Biotech lectures very interesting and readable not only to the Biotech fraternity but also to the general public. Dr.Annadana deserves to be congratulated for this pioneering effort and I feel such publications brought out periodically should be invaluable to the Biotech professional.

I wish Dr. Annadana success in this venture and in his professional endeavours.

(Divakar Rao)
CEO-designate

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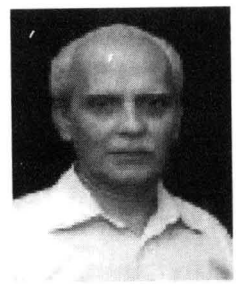
Golden Lecture 1

ETHICAL AND LEGAL ISSUES IN HUMAN GENOME RESEARCH

Profile:



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ETHICAL AND LEGAL ISSUES IN HUMAN GENOME RESEARCH

Write up:

An important goal of current research into human genetics is to identify genetic changes that lead to human disease so that effective interventions can be developed. Towards this goal the molecular biology of human genes is being studied and an ambitious programme - the human genome project - to determine the DNA sequences coding for the approximately 50,000 to 1,00,000 genes estimated to be present in each of our cells has recently been completed. Genes are also being mapped by classical genetic methods, which involve collecting detailed information on families. This approach, called pedigree analysis, permits localization of particular genes on individual chromosomes, estimation of distances between genes on the same chromosome and also the chances that two genes present on a chromosome will be passed on together to a child. Such analyses permit the geneticist to correlate the presence or absence of particular marker genes with the chances of that embryo or child inheriting a disease that is determined by a gene located close to that marker.

The potential benefits of this new genetics are far reaching but there are also risks in terms of unanticipated consequences. The development of routine tests for detecting predispositions to disease and other human characteristics has serious implications for the practice of medicine, for the legal system, for insurance, for employment practices and for numerous other areas of society. This new knowledge combined with rapidly developing technologies in handling human gametes and embryos, will take us into highly complex and unchartered ethical terrains. In this talk I will touch upon a few of these issues as indicators of the type and range of problems that are emerging.

As early as in 1965, long before the advent of the new genetics, there were indications of the type of ethical questions that would emerge as a result of advances in genetic technology. In that year, a paper based on a survey of Scottish prisoners was published in which it was observed that seven out of the 197 prisoners with a history of violent crime that were studied had an extra Y chromosome in their cells. This seemed significant because an XYY child is born, on the average, once in every 1000 male births. Subsequent provocative reports in the news media and scientific journals that men with an extra Y chromosome were destined to lives of criminality created demands for screening of individuals in jails and for prospective studies among newborn and school-going boys with the intention of following their behavioral development. Partly as a result of intervention by the Boston-based Committee on Responsible Genetics, this survey, proposed by a few U.S. scientists, was stopped. The Committee on Responsible Genetics raised the following issues: if a survey of school-going children was conducted as planned, who would be privy to the information that particular boys were XYY? Should their parents be told? Should their teachers and school principal know? Might not such information lead to stigmatization of such boys as 'abnormal'? The parents and the sibs may unconsciously or otherwise begin to treat the XYY child differently from others. Special meaning may be attached by parents and teachers to childhood pranks when they involve XYY children. Who will ensure confidentiality of such information? How might insurance companies and employers react to such information about an applicant? In the face of stigmatization and discrimination, the XYY individual might challenge society's norms resulting in what has been called self-fulfillment of the prophecy.

Since about one in 1000 male newborn children carries an extra Y chromosome, a few prospective studies were eventually done, and the development of such XYY children was followed into adolescence. These findings suggested that XYY children fall within the normal range, but with an array of relatively nonspecific behavioral differences in attention, cognition, motor skills and personality. The only criminal history found was for minor offenses not characterized by violence or aggression. In other words, most male children with an additional Y chromosome grow up reasonably well-adjusted and but for prior knowledge of their chromosome constitution little significance would probably be attached to behavior that may be outside the normal range. Nevertheless, in the U.S., because of the publicity surrounding the XYY chromosome constitution, about 50% of parents have elected to terminate pregnancies for which prenatal diagnostic tests indicated the presence of an extra Y chromosome. Here is a situation in which seemingly normal male foetuses were aborted in spite of lack of a consensus among scientists about genetic predisposition of the XYY male to more violent, antisocial and criminal behavior. These abortions were done on foetuses whose XYY chromosome constitution was unexpectedly diagnosed during prenatal diagnostic studies that were done on account of advanced maternal age or other reasons and not because there was prior interest on the part of the parents or the clinician to avoid the birth of an XYY

child. This case study illustrates how parent's perception (or misconception) of the relationship between genes and behavior can determine decisions about continuation of a pregnancy.

An ethical issue that has been widely discussed in our country is prenatal determination of sex by amniocentesis. This involves removal of a few cells from the fluid that surrounds the growing foetus, usually before the fourth month of pregnancy. From these cells, by appropriate analysis of chromosomes or DNA, the sex of the foetus can be readily told. This technique is useful to prevent the birth of male children carrying sex-linked genetic disorders in families with a history of such familial disease: for instance, Duchenne muscular dystrophy. The procedure would involve not only the verification of the sex of the foetus but also the use of appropriate DNA probes to determine whether the foetus, if male, had inherited from the mother the defective gene that causes Duchenne muscular dystrophy. When amniocentesis was first made available in this country in AIIMS, New Delhi, the first 100 parents who requested this procedure, had no obvious history of familial disease, all had 2 or 3 normal daughters and now wished to have a son. Apparently no couple who had 2 or 3 sons but wanted a daughter asked for such diagnosis. Seeing this distortion, prenatal sexing was discontinued in the AIIMS. As a result of criticism in the press, and demonstrations, mostly by women's groups, the Government of Maharashtra enacted a law banning foetal sexing when no genetic disease is involved. But it is common knowledge that foetal sexing is done widely, especially in the states of Punjab, Maharashtra and Delhi, often using highly unreliable methods. Legislation has been passed by several state governments and the Government of India to prevent the use of amniocentesis solely for the purpose of determining the sex of apparently normal foetuses. But as it often happens in science, this bill, as well as discussions relating to it, have been made irrelevant by technological advances which have nothing to do with genetics. This refers to the noninvasive method of ultrasound imaging which - without amniocentesis, chorionic villi sampling or DNA probes, permits a trained observer to determine whether the growing foetus is male or female during the early stages of pregnancy.

Given the strong emotional, and cultural underpinnings behind parental desire to achieve a balance among the sex of their offspring, we may eventually be required to harmonize the parent's right to have a child of the desired sex and society's attempts to avoid discrimination based on sex. An ethically acceptable approach to this dilemma, and one that avoids termination of pregnancies of the "wrong sex", may become available if current attempts to separate male-determining (Y-bearing) and female-determining (X-bearing) human spermatozoa reach higher levels of reliability.

Kathryn Allen Rabuzzi, writing in the Encyclopedia of Religion, says that "historically and cross-culturally, family in various forms has (until the late twentieth century in post-industrialized cultures) been so basic to human existence as to be a universal symbol of ultimacy". It has also been said that "the significance of the genetic connection between parent and child undoubtedly is part of what makes infertility a painful experience" and why adoption does not appear to satisfy "the yearning to create a version of oneself unfold and develop" (J.L. Hill, 1992). It is therefore understandable that infertile couples often explore all possible avenues - from the religious to the biotechnological - to have a child of their own and raise a family.

Embryo and gamete technology have developed as rapidly as genetic technology, and prenatal genetics is now a very active discipline. Methods of in vitro fertilization and assisted reproduction have permitted many couples to overcome the pain of infertility. This has, however, led to novel family definitions and relationships: children with one biological father and two mothers, one genetic and the other gestational; children with two fathers, one known to the child and the other genetic, often unknown, who donated the sperm. There are also adoptive parents and adoptive children.

A few years ago, a 59-year-old British woman gave birth to so called test-tube twins in an Italian clinic and a 62-year-old woman became pregnant after implantation of a fertilized egg. These cases of reproduction assisted by gamete and embryo biotechnology and the birth of a white baby to a black mother by similar methods have renewed the debate over the ethical and moral dimensions of such reproductive choices. The controversy has become heated lately over the proposal of British scientists to use eggs recovered from aborted foetuses to help infertile couples and overcome the shortage of human eggs. This technique, in effect, would lead to the birth of children whose mothers were never born.

In many Western societies, the concept of the traditional family had come under severe pressure even before embryo technology and genetic testing became available. With the advent of the first, and increasing availability of the second, perplexing ethical and legal questions have arisen. In the U.S., courts in California have distinguished between the genetic parent (i.e., the egg donor) and the gestational parent (i.e., the woman in whose

womb the foetus developed); other courts have made a distinction between the "genetic progenitor" and the "mother". In a landmark custody case in which a woman who had no uterus had her eggs fertilized by her husband's sperm, and hired another woman to carry the pregnancy to term, the justices have ruled in favour of the genetic linkage and said that the couple whose gametes were used for in vitro fertilization were the "genetic, biological and natural" father and mother and therefore entitled to retain custody of the child. In the U.S., the term "natural" or "biological" mother is now widely understood to mean the "genetic" mother. However, it appears, this view, that the gestational mother is no more than a foster parent, is prevalent only in the U.S. and Israel, whereas in several other countries including the United Kingdom, Germany, Switzerland and South Africa the courts have held the view that the woman who gives birth is the child's mother.

In the past, in many societies, the poor, the unpopular and those perceived as disabled (whether or not the perceived disability was genetic in origin) have been targets of discriminatory policies and eugenic measures such as sterilization aimed at "purifying the race". Lancelot Hogben, a geneticist active in the 1930's and 40's angrily reacted to such policies and, referring to the prevalence of haemophilia in the royal houses of Europe, reminded that no one has "publicly proposed sterilization as a remedy for defective Kingship".

The targeting of gypsies and Jews for elimination in Nazi Germany is well known. Less well known, perhaps, is the similar targeting of individuals and their families carrying a specific genetic defect, Huntington Disease. The symptoms of Huntington Disease are rapid and progressive neurological and mental deterioration in adult life leading to death within a few years of onset of the disease. It is an autosomal dominant gene defect, meaning that a child has a 50% chance of inheriting the disease from an affected parent. Since the first symptoms appear when, an individual is in his or her 40's or 50's, the patient would have had children by then who, in turn, would not know whether they are carriers of the defective gene until they themselves reach middle age. The disease poses severe burdens on the individual and the family and presymptomatic detection of the defective gene has therefore been much sought after. This gene has now been mapped and cloned and DNA probes are available for prenatal diagnosis as well as for presymptomatic diagnosis of children and adults. Huntington Disease is one of several psychiatric disorders that were part of an extermination policy in Europe during the Second World War. In July 1933 an act was passed in Germany to enable compulsory sterilization of anyone suspected to carry Huntington Disease and eight other categories of disorder. Professionals, including scientists, lawyers, doctors and others with specialist training in what was called racial hygiene, were co-opted for this purpose. A system of hereditary courts was established and the state established primacy over reproduction. As a result of the facade of legality and expert opinion, appeals to higher courts were rejected (Weindling, 1989). It is estimated that in this well organized campaign, 350,000 to 400,000 individuals were sterilized, among whom there may have been 3000 to 3500 HD patients and their close relatives, and that over 100 HD patients may have been killed in one year in one psychiatric clinic alone (Harper). I need hardly remind this audience of the recent case, in Poona, where a number of young women in an institution for the mentally handicapped, were involuntarily subjected to hysterectomy.

The type of pedigree analysis and DNA research that led to the identification of the Huntington gene reveals information about the health status of individuals belonging to the family whether or not they had agreed to be investigated. Information about such individuals would become part of the records of the investigator and the institution conducting study. Disclosure of such information, deliberately or inadvertently, can lead to complex legal and ethical problems. Should a member of the family, who was not part of the formal study, be told, for instance, that he is a carrier of the disease? Who owns this information? There have been cases in which particular members of a large family wanted all information about themselves, their spouses, and their children, deleted from records. If at some future date it develops that the only means of linking an individual to a crime is through DNA data collected as part of a scientific study in which the individual had unwittingly participated, is such evidence admissible, and is it ethical to disclose such information?

A brief reference to gene therapy and patenting of genes and of laboratory animals into which genes of interest have been inserted, may be in order. By mid-1992, there were over 15 diseases for which gene therapy attempts were being made. In one case, a defect in adenosine deaminase deficiency (ADA) its correction through gene therapy seemed to result in dramatic improvement in the children's health. There seems to be support from ethicists for such application of gene therapy to somatic cells. However, controversy surrounds the extension of such methods to the germline which might enable the individual with the gene defect to bear children inheriting the inserted normal copy of the gene in place of the defective copy. Dr. French Anderson who did the first somatic cell gene therapy for ADA, deficiency says: "Besides the medical arguments, there are a number of philosophical, ethical, and theological concerns. For instance, do infants have a right to inherit an unmanipulated genome, does

the concept of informed consent have any validity for patients who do not yet exist, and at what point do we cross the line into "playing God". The feeling of many observers is that germline gene therapy should not be considered until much more is learned from somatic cell gene therapy, until animal studies demonstrate the safety and reliability of any proposed procedure, and until the public has been educated as to the implications of the procedure."

Many, perhaps most, human geneticists believe that there is a significant genetic component to behaviour, and that not all behaviour is equally influenced by the physical, biological and psychological components of the environment. This does not mean that heredity can be equated with inevitability because genes do not determine 'destiny' in a predictable manner. V. Elving Anderson says "Few, if any, behaviours are completely without genetic influence, and few behaviors are completely without environmental influence". The question has been posed whether, if the genes plus environment equation explains all behaviour, is there room left for individual freedom and moral responsibility? This question, if pursued properly, necessarily, takes us into the realms of moral beliefs and religion. Therefore, prudence suggests that I leave this discussion incomplete. Instead, I will end with a hopeful statement by a theologian, Ronald Cole-Porter, who has asked the unusual question whether there is a genetic basis for the moral agency within us. He concludes his enquiry in the following terms: "genes appear to some to lock us into a vast web of biological determinism that deprives us of what distinguishes us from the rest of nature and so removes what we once thought was essential human. By contrast, it has been argued that far from posing an ominous threat to humanity, behaviour genetics and related fields of research offer to illume more precisely the moral nature of the human situation."

I may be permitted to end this talk by quoting Roger Shinn, Reinhold Neibhur Professor Emeritus of Social Ethics at the Union Theological Seminary in New York: "To the perilous leaps in power associated with war and ecology, we must now add genetic knowledge. Past genetic theories, usually infected with prejudice have brought the world much sorrow. An ethical imagination, this time around, might do better. The historical record gives us no assurance of that heightened insight, but it allows us to hope."

Acknowledgements

This lecture is a slightly modified and abbreviated version of the B.V. Narayana Reddy Memorial Lecture delivered by the author. In preparing this text, I have made extensive use of 'The Genetic Frontier : Ethics, Law and Policy' and 'Ethical and Legal Issues in pedigree Research' (both edited by M.S. Frankel and A.H. Teich and published by AAAS, Washington, D.C.) and the papers by Peter J. Harper on Huntington disease. I thank Sreeganga S. Chandra for making these and other documents available to me at short notice and Mustafa Saifi for assistance in other ways.

Golden Lecture 2

BIOINFORMATICS- AN EMERGING FIELD IN BIOTECHNOLOGY

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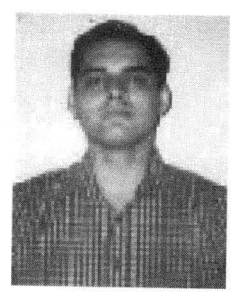

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BIOINFORMATICS - AN EMERGING FIELD IN BIOTECHNOLOGY

Write up :

Role of Bio and Chemo informatics in Rational Drugs design.

The rapidly emerging field of Bioinformatics is new. But there is a general recognition of its great importance and exciting potential for accelerating drug discovery. Bioinformatics is playing an increasingly important and central role in biology research, As such , Bioinformatics has been absolutely vital for every success in genome sequencing, disease gene discovery, functional genomics and proteomics, among the most exciting and fast-moving areas in biology.

Bioinformatics is coming to occupy the central role of the "glue" that integrates together the many disparate bodies of data from fields as diverse as enzymology, genetics, structural biology, medical and animal models of disease, etc. The critical question of how to find the important relationships between these data to solve complex biological problems is being solved via Bioinformatics, by linking all data through their gene associations. As a practical example of this, NCBI, NIH's **Bioinformatics center, receives and processes on their Bioinformatics** website nearly _3 million requests per day from scientists worldwide.

'This revolution could result in a decreased risk and time to market for many new products that are based on genes, proteins, antibodies, small molecules, vaccines, RNA, and antisense therapy. Identifying human gene sequences, however, is only the first phase in developing useful drugs to treat complex diseases. Genomics will need to be combined synergistically with proteomics, functional genomics, Bioinformatics, chemoinformatics and pharmacogenomics in order to rationally select which of the hundreds of thousands of potential gene- or protein-based candidates to further characterize and develop.

The challenges that pharmaceutical companies face because of a drastically increased volume of scientific data are compounded by the economic pressures to enlarge their product pipelines in the face of increased global competition, a more demanding market place, and more stringent regulation. The ability to make better and faster research decisions is a key goal for all research organizations. Thus, pharmaceutical companies are turning to new technologies such as data mining and data visualization to gain knowledge and insights from research data quickly and efficiently as well as to increase the likelihood of identifying targets or lead compounds. In this environment, cherninformatics has emerged as a crucial discipline that encompasses the design, creation, organization, storage, management, retrieval, analysis, dissemination. visualization. and use of chemical information. Cherninformatics solutions enable multidisciplinary research teams to capture, analyze, and communicate the increasing volumes of biological and chemical data created in the search for new lead compounds and drug candidates.

The Current State of Drug Discovery

The 2000 worldwide pharmaceutical market, according to IMS Health, totaled approximately $317 billion and could reach $3 trillion by 2020. However, the pharmaceutical industry can achieve such growth only if it brings many fundamentally new drugs to market. This challenge requires a remedy for the current pharmaceutical productivity crisis that is greatly slowing the process of identifying novel drug targets and propelling the related therapeutics to market. Now, Bioinformatics offers pharmaceutical companies the chance to participate in the front end of drug discovery. Experts estimate that genomics-based research could identify 3,000- 10,000 new targets for small-molecule drugs-a huge increase over the approximately 500 proteins targeted by current drugs. In addition, many disease related genes and proteins are expected to have significant diagnostic, pharmacogenomic, and agricultural applications. To take advantage of this opportunity, pharmaceutical companies are developing a variety of strategies to access and exploit the research

A drug company's objective is to : identify genetic disease targets Manage the rapidly expanding information available in genomic and other databases Apply the breakthrough TOOLS developed within disciplines such as Bioinformatics and Chemoinformatics. Screen immense libraries of natural and chemical compounds for desirable biological and therapeutic activity.

The other objectives of pharma Industry today is to increase the probability of success in a drug discovery program, to reduce the time and expense required to identify and optimize lead compounds, and to eliminate

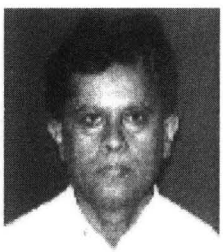
wasteful and costly development efforts on poor drug candidates. Bio and chemoinformatics tools, which can fulfill this need, would be of great benefit for drug discovery. Due to this computational tools have become increasingly important in the drug discovery and design processes. Tools such as Molecular Operating Environment (MOE) which can identify and fetch the relevant target information and which can help to design efficient drugs for those targets are the need of the hour.

Pharmaceutical companies have traditionally used computerized information systems to store and manage reams of data related to drug development, such as small molecule screening, pharmacological information, and clinical data. Researchers now need to access, integrate, and exchange data among these systems as well as hundreds of external public and commercial databases. Most companies lack the trained personnel. expertise, and tools needed to fully exploit this growing, wealth of information. Pharma companies having trained people with PhD level qualification can analyse these data and bring efficient drugs faster in a novel way. The burgeoning Bioinformatics and Chemoinformatics fields are promised to provide new ways to bring efficient drugs faster in a rational way to treat many diseases.

Golden Lecture 3

BIOTECHNOLOGY APPROACHES TO CORRECT MINERAL NUTRITION DEFICIENCY

Profile:



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BIOTECHNOLOGY APPROACHES TO CORRECT MINERAL NUTRITION DEFICIENCY

Write up

More than half of the global population is in short supply of two micronutrients namely Fe and Zn. There is growing evidence that a staggering burden of disease is caused by chronic micronutrient deficiencies in people. Most vulnerable groups are infants, reproductive age women especially in developing countries.

There is an urgent need to address this problem, since it is causing > 50% child mortality increasing content or bioavailability of micronutrients in the diet rather than short-term aim to increase consumption of animal products and improving food-processing techniques can eliminate this.

Some attempts are made to improve the Iron status of plant and to our knowledge there are no attempts being made with regard to Zinc. Research should focus on food-based interventions to alleviate nutritional deficiency.

Background

Humans depend on plant and plant depends on soil for their nutrition requirement. So, the availability of inorganic nutrients in sufficient quantity is crucial not only for crop growth and productivity but also for well being of humans. Since, plants provide all the nutrients required for human health except Vit B_{12} and D. The demand by ever-growing population resulted in doubling the cereal yields during green revolution and also breeding of high yielding varieties of major staple crops displaced traditional crops, which has led to substantial reduction in crop diversity. This has contributed to micronutrient deficiency although caloric output is increased (Combs, *et al.*, 1996).

In view of this, plant foods do not contain all the essential nutrients nor do they contain in sufficient quantity to meet daily intake. And also many people do not consume diverse diet and their major diet composed of only staple foods, which are poor source of some macro and many micronutrients (Calloway, 1995).

According to an estimate, 250 million children are at risk for Vit A deficiency (of which 250-5,00,000 will suffer from irreversible blindness every year), 2 billion people (33% of the world population) are at risk for Iron deficiency (Infants, children and women of reproductive age) and 1.5 billion people are at risk for Iodine deficiency (FAO, 1997), And a recent survey indicates, Zn deficiency is the most wide spread of all micro nutrients globally (nearly 49% of population do not meet their daily intake).

This information suggests that, nutrition deficiency prevails globally both in developed and developing countries. However, the people in developed world meet 70% their requirement through meat and dairy products. Limited micro nutrient availability in the soil, not only reduces crop productivity but also quality of food grains, especially the content of micronutrients. Now is the time for researches to improve the nutritional quality of plants, with respect to both nutrient composition and concentration of micronutrients (Graham, 1988). Iron and Zinc stand alone as the two important micronutrients, which are in short supply for human nutrition. This has been substantiated by the fact that in US itself 92.3% of the soil samples taken in 50 states showed medium to serious Zn deficiency. Although, some attempts are being made to improve Fe levels, to our knowledge no such attempts are made with regard to Zinc.

Role of Zn in plant and Human Nutrition

Though the requirement of Zn is only 15 mg/day as per RDA and 0.8 mg /kg soil for plants, its deficiency can cause several impacts on human nutrition. Nearly 40 different food grains and 400 germplasm lines of finger millet analyzed for Zn content showed medium to low Zn content, reiterating the fact that the plant foods contain low Zn (Shankar, 2001).

Zinc is a component of more than 300 enzymes in both plants and humans. It is a component of many important enzymes namely Carbonic Anhydrase, hence the Zn deficiency can cause 50% reduction in photosynthesis, decrease protein synthesis, cause membrane leakage, poor seed set. In humans marginal deficiency itself can cause

several conditions namely difficulties at birth, foetal growth failure, depression, anorexia, reduced growth of infants and delayed male sexual development etc.

Interventions to overcome nutritional deficiency

1. Attempts to increase content and bioavailability

2. Nutritional interventions

   -Supplementation
   -Food fortification
   -Dietary diversification

3. Food processing

   -Reduce ANF's (Phytate, Polyphenols, Saponine and Lactine)

4. Agriculture Interventions

Genetic improvements Strategies to increase uptake of micronutrient by plants Increase of micronutrient availability in soil Of the many proposed strategies, increasing consumption of animal food products or by increasing content or bioavailability of micronutrients in plant derived foods seems logical and reasonable. Other strategies namely dietary improvement, supplementation and fortification do not seem to be practical and cost effective. But look attractive on targeted group in small scale.

Agricultural intervention to improve higher accumulation of micronutrients in edible parts and especially in staple food crops seems to be a workable proposition.

With the advent of new techniques in molecular biology and the available literature suggests that it is possible to improve human nutrition through agricultural interventions.

Molecular Approaches

Advances in plant biotechnology offer great opportunities to increase content and bioavailability of micronutrients (Thoenissen, 2001). Iron and Zn contents are known to vary between varieties / genotypes and among species and are genetically controlled (Graham and Welch, 1996). Using molecular markers it is possible to 'tag' the genes associated with the high Fe and Zn content, and then use these markers to facilitate crossing these genes into new breeding lines.

In recent years, greater emphasis has been given to understand the physiological and molecular aspects of Zn transport in hyper accumulating plant species *Thlaspi caerulescens*, which accumulates to an extent of 3% Zn on dry weight basis in shoots without any toxicity symptoms. A Zn transporter *ZNT 1* gene is sequence analyzed and is known to be similar to Fe transporter *IRT 1* in *Arabidopsis thaliana* and *ZIP Zn* transporter. It has been reported that constitutively high expression of the *ZNT 1* gene in roots and shoots enabled enhanced Zn transport in *Thlaspi caerulescens*. Similarly ZAT protein (Zn transporter of *Arabidopsis thaliana*) has been isolated in Arabidopsis. These studies opens up a new possibility to incorporate these genes into targeted crop species and lead to a better understanding of molecular regulation, control of micronutrient levels and the mechanism of Zn homeostatis in plants (Pence, *et al.*, 2000, Kupper, *et al.*, 2000, Lasat *et al.*, 2000, Shen *et al.*, 2000).

Prospects

The development of nutritionally targeted improved crops has immense significance to mankind. Plant based food with enough concentration of nutrients especially the micronutrients which are in short supply is the best option rather than the other remedial approaches namely supplementation, fortification etc. As Thomas Jeffason wrote " **The greatest service which can be rendered by any country is to add an useful plant to its culture**". There is a need for multidiscipline approach involving human nutritionists and plant scientists to achieve this goal.

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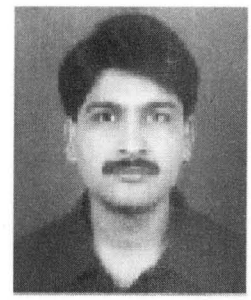
Golden Lecture 4

GREATER SCIENCE, FASTER



Profile:

Name : K K Bhagchandani

Address :Sales Manager - India
Accelrys
916, 3rd Cross, 13th'H" Main
Doopanhalli, HAL 2nd Stage,
Banalore-560008,1NDIA.

Academic Qualification : MSc (Physics)
MBA (Marketing)

Work Experience : Worked for 9 years in Pharma industry at various level of management in Sales & Marketing, out of which 7 years were with Ranbaxy & 2 years with Pfizer.

Present Posion :Working for last 2 years with Accelrys (earlier known as MSI), a subsidiary of Pharmacopeia as Sales Manager looking after the operations of SAARC region.

GREAT SCIENCE, FASTER

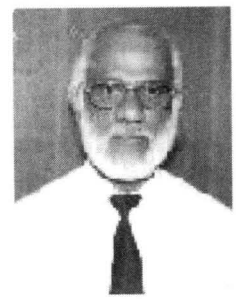
Write up:

Science is always great. However, in the present day's scenario, we sometime need to get things little faster particularly when one is dealing with problems like drug discovery. On one hand, we would always like to get a drug in the market earlier to combat human sufferings. In the other hand, drug discovery is becoming highly expensive due to several reasons. This indicates that emphasis should be given to do such things faster. Scientific software for drug discovery research has emerged as the most useful tool in this regard and Accelrys has the privilege of having a wide range of software products starting from sequence analysis to macromolecular studies to target identification to designing drugs and evaluating their toxicity profiles. Therefore, it is no wonder that most of the leading pharmaceuticals and biotech companies all over the world are using and looking for Accelrys' software tools. Two major emphasis that are put in modern drug design/ discovery efforts are to get a drug in the market in as short a period as possible and to ensure that the drug has least untoward effects. The first problem is related to minimizing the trial and error time involved in experimental works. This not only reduces the time but also the money required to develop a marketable drug. At the same time, evaluation of side/ toxic effects of a drug, preferably at an earlier stage of the discovery process, is extremely important not only for getting a safer drug but also to avoid the risk of withdrawing the drug from the market due to the manifestation of its adverse effects, eventually conceiving a huge loss of time, money and efforts given to develop the drug. All these demand rigorous rationalization of the drug development process. Several mathematical models that have been developed in the last few decades towards this have been found to be quite effective in this regard and success stories are coming out in the literature. Along with this, the development of highly capable computational tools has made the process smoother and faster. In going for faster drug discovery, one of the key factors would be to be able to handle/ manage the huge data/ information, coming out of chemical and biological research, as efficiently and effectively as possible. With a view to address these important aspects, we had delivered, in this presentation, how the state-of-the-art tools onBioinformatics, Cheminformatics, Protein Modeling, Rational Drug Design, Structure Based Drug Design, Pharmacophore based Drug Design and Enterprise-wide Data Management from Accelrys can help accelerate drug discovery process. The presentation was focused on familarising the audience with the Drug-Discovery process step-by-step and emphasizing on how the use of software from Accelrys can reduce the time, effort & money invested in the process. The presentation was focused on ***Insilico Drug Design***, which is the new paradigm of Drug development these days. The presentation was also about what are the techniques used behind the software to ensure error free results and what is the basis of the computat on behind the software. The lecture was an interactive session wherein the participants had asked very interesting and genuine queries pertaining to the way Drug-Development is conducted and what are the application areas of the software. The lecture was also for highlighting how Accelrys (the leading scientific-software company) has integrated the sciences together to provide a comprehensive decision-support system to the research fraternity involved in Pharma, Materials, Biotech, Petroleum, Health-care, Personal-care Research & Development across the globe.

Golden Lecture 5

EVOLUTION OF BIOTECH INDUSTRY IN HEALTH SECTOR

Profile:



Name : Prof. (Dr.) G. Padmanabhan,

Address : Professor Emeritus,
Dept. of Biochemistry,
Indian Institute of Science,
Bangalore.

Academic Qualification : B.Sc., Presidency College, Madras; 1958;
M.Sc., IARI, New Delhi, 1960;
Ph.D., IISc, Bangalore, 1996

Work Experience : Asst.Professor, IISc (1969 – 75),
Assoc.Professor (1975 – 80)
Professor (1980 onwards);
Director (1994 – 98);
Hon. Professor(IISc)/ Emeritus Scientist (CSIR)

Awards : Sarma Memorial Award(SBC, India),
SS Bhatnagar Award(CSIR),
Bhasin Award(Biotechnology),.
Ranbaxy Award,
BR AmbedkarAward (ICMR),
D.Sc(h.c., BHU);
Fellow of all Science Academics in India;
UNESCO Chair in Biotechnology(2000).
CV Raman Professor INSA,

Current Position : Emeritus Professor, Department of Biochemistry.

Publications : Published over 110 papers

EVOLUTION OF BIOTECH INDUSTRY IN HEALTH SECTOR

Write up:

Health care biotech industry started in the late 1970s with a handful of companies in North America. The statistics in 1998 was as follows, There were1280 companies with a market capitalization exceeding $200bn. The sales amounted to $13.4bn and the total revenues reached $18.6bn- The R & D expenditure was $9.9bn and the industry employed 153,000 people. The United States of America 9000 patents and FDA has approved 90 drug products and vaccines, besides scores of raw medical diagnostic kits and use of DNA finger printing in forensic science. About 350 products are in clinical trials and it is projected that the best years are yet to come, particularly in the pharmaceutical trade (Statistics from: Biotechnology - North America: Science dated March 2, 2000).

Changing facet of Biotechnology:

It is obvious that BT in the health sector is a serious industry and not just a hype. It is,a knowledge-based industry and the complexion keeps changing with hewer scientific breakthroughs in the field, Which are not infrequent.

Protein Pharmaceuticals:

Modem biotechnology started with the slogan 'clone a gene and make a million'. The aim was essentially to produce human protein pharmaceuticals in bacteria/yeast through recombinant DNA techniques. Companies such as Genetech, Chiron, Amgen etc. made big news and the products were insulin, growth hormone interferons, blood proteins, streptokinase (to dissolve blood clot) etc. But this effort was not really adequate to sustain the companies. It turned out that while laboratory demonstrations of the production of these proteins was fairly easy, the downstream in processing under GNP (Good Manufacturing Practice) conditions proved to be difficult and expensive. Besides, litigation between the companies for IPR delayed progress.

Molecular Diagnostics:

In the meanwhile, small companies had a mushroom growth in the field of diagnostics. With just a 2-room factory (one room for the laboratory and one for the office) scores of diagnostic kits based on the use of monoclonal/polyclonal antibodies to detect a variety of infectious diseases and cancers were generated- Diagnostics based on nucleic acid probes needed additional infrastructure, but nevertheless have become part of the product portfolio. With the availability of the human genome sequence, use of SNPs (Single Nucleotide Polymorphism) for elucidating disease susceptibility has great scope. Even today the diagnostic kits remain the most successful outcome of the knowledge based growth of biotech industry.

Drug Targets:

Major pharma companies need a constant source of lead molecules. The conventional structure-modification and hit & miss approaches are giving way to high throughput screening involving thousands of molecules generated by combinatorial chemistry approaches against a battery of drug targets. The sequencing of the genome of a variety of pathogens in addition to the human genome is leading to the identification of several new genes that could become potential drug targets. Thus, research based biotech companies would become the knowledge base for the development of new drug targets for. a variety of infectious' diseases and systemic disorders. In addition, biotech companies can also specialize in developing appropriate drug delivery systems for tissue specific localization.

Genomics & Molecular Medicine:

The era of genomics, has come into existence with the announcement of the human genome sequence. With the use of DNA microarrays to quantify gene expression and 2D analysis of proteins to quantify the end products, there is explosive activity to identify candidate genes and their protein products as potential diagnostic and vaccine candidates as well as drug targets. Gene therapy would now have a basket of genes that can be tried against specific genetic disorders and cancers. Knowledge from the functional genomics of pathogens is used to develop DNA vaccines, heralded as the third vaccine revolution. At least two dozen biotech companies are devoted exclusively to developing the field of gene therapy and DNA vaccines. Cancers and cardiovascular disorders have

become the major targets for gene therapy not only in terms of providing useful genes, such as those for cytokines to bolster the immune system to fight, but also to prevent the over expression of unwanted genes. **Interestingly, these researches are** not confined to academic institutions, but vigorously pursued in knowledge-based industries.

Stem Cell Research :

Stem cells are pluripotent cells meaning that they are the precursors for different cell types in the body. If these pluripotent cells can be guided towards specific lineages such as liver cells, neuronal cells, pancreatic cells, intestinal cells etc, then they would be of immense use to treat a variety of specific tissue disorders through transplantation techniques. While, stem cells of the bone marrow and peripheral circulation have limited fates, embryonic stem cells are the ideal precursors to generate a variety of cell types. The human embryonic stem cells are a hot commodity, but difficult to manipulate. Due to ethical considerations, public funding for work with human embryonic stem cells is under serious debate; but substantial research is being carried out with mouse embryonic stem cells

Another contentious area has been 'human cloning" arising out of the success in animal cloning, using somatic cell (eg. udder cell) nucleus and egg cell cytoplasm. On the one hand, private corporations are interested in animal cloning with a useful gene to produce a human protein pharmaceutical in milk- but production of human embryos by this technique to obtain stem cells or through regular *in Vitro* fertilization technique has become a serious ethical issue.

Stability of BT-Companies :

It is obvious that knowledge-based biotech companies are always in a flux and need to be constantly updated based on new knowledge. The initial slogan 'clone a gene and make a million' did attract investment in the early 1990s. But, when it was realized that the returns would be slow to come, investors drifted to dot. coms and the latter has also burst in recent times. However, the biotech companies were saved by the stepping in of Pharma companies. Only few of the original biotech companies are stand-alone companies today. Thus, biotech companies can concentrate on innovative research and generation of lead molecules, while the big pharma companies would take care of clinical trials, financing and marketing. Biotech companies or divisions concentrate on basic research, licensing promising leads to the major partner. Many others are toolbox companies that undertake service or contract research for major pharma or seed companies or public funded institutions. A large number of biotech companies in the western world provide a back up for research in terms of sophisticated reagents as well as services such as protein & nucleic acid sequencing, cloning and expression of genes, oligonucleotide and peptide synthesis, DNA microarray analysis etc. With the availability of human and other genome sequences, Bioinformatics has become a hot subject. This data mining exercise from the mind boggling DNA and the derived protein sequence available will be profitable, only if it is undertaken as a contract project or is linked to a down stream 'wet lab', where the theoretical predictions are converted into actual lead molecules or processes. Companies can establish a niche in specific areas, but keep updating the deliverables.

Public perception is an important element in the eventual success of biotech companies. Ethical debates on gene therapy, human embryo research and genetically modified foods do slow down investments, although areas such as diagnostics, rational drug design, Bioinformatics, transgenic crops to improve nutrition etc. should pose very few ethical questions. Even in contentious areas, there is public pressure for new therapeutic options and societal demands and therefore, overnments come around to grant approvals after evolving suitable safeguards on the basis of risk-benefit analysis.

The Indian Scenario :

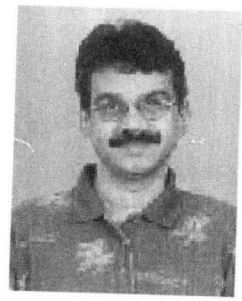
Modern biotechnology has received support during the last 15 years.. Thanks to major support from the Department of Biotechnology and other agencies such as CSK DST, ICMR & ICAR, a reasonable level of infrastructure and competence in the area has been built. The response of the industry was lukewarm but it is now picking up consequent on the initiatives of central and state governments and the slow down in the IT sector. The 1990s have been a period of learning exercise for academia-industry collaboration. Presently, indigenous technologies are used to make recombinant hepatitis B vaccine and diagnostic kits for HIV, hepatitis C & others of this group, cysticercosis etc. A live vaccine against leprosy has become a commercial proposition. There is some commercial initiative in the indigenous production of recombinant insulin, interferons, streptokinase, EGF etc. A live vaccine for cholera and a DNA vaccine for rabies are on the anvil. These are modest gains, but aggressive

progress can only be achieved with the participation and encouragement of the health ministry. Right now, the policies are not very conducive for encouraging indigenous technologies in the health sector.

With the multinational companies having very little interest in vaccine production due to poor economic viability, India has a great chance to move in and work hard to assume global leadership for which it has to compete vigorously with countries such as china, cuba etc. A proper strategy in this area would put India as a major player in the global trade, besides addressing a highly relevant social concern. After A vaccination is the cheapest mode of protecting one's health, especially the poor and modem biotechnology affords a great opportunity to develop and manufacture modem vaccines against a wide variety of diseases.

Golden Lecture 6

BIOINFORMATICS AND ACCESS TO GENOMICS



Profile:

Name : Mittuur N Jagadish

Address : Monsanto Research Centre
Malleswaram , Bangalore

Academic Qualification : Post Doctoral work at Rutgers University and Cornell University, USA and University of Bielefeld, Germany
Ph.D. (Yeast Genetics - 1979), Dublin, Ireland
M.Sc. (Botany) Bangalore, India, 1974
B.Sc. (Hons.) (Botany) Bangalore, India, 1972

Work Experience :

Programme Lead - Genome Knowledge Enhancement Program - Monsanto Research Center, Bangalore - Current from January 1999
Proect Lead - Astra Biochemicals, Bangalore - 1998
Visiting Scientist - NCBS (TIFR), Bangalore - 1997
Senior Scientist - Vittal Mallya Scientific Research Foundation, Blre -1996- 97
Principal Research Scientist - CSIRO, Division of Biomolecular Engineering, Melbourne, Australia - 1985- 1996
Research Associate - University of Bielefeld, Germany- 1984-85
Post Doctoral Research Associate - Boyce Thomson Institute, Cornell University, Ithaca, NY, USA - 1982-84
Post Doctoral Research Associate - Waksman Institute, Rutgers University, Piscataway, NJ, USA - 1980-82

Awards : 1970-71 R.P.D.B.P. Mahadevaiah prize, 1975-76 Junior Research Fellowship, UGC, 1977-79 Overseas Scholarship, The Royal Commission for the Exhibition of 1851, London, UK., for Ph.D., 1979-82 Johanna-Busch Postdoctoral fellowship, 1982-84 Postdoctoral fellowship at the Boyce Thompson Institute, 1995 / 97 Member of teams that won CSIRO, Australia, Chairman's gold medals.

Current Position : Associate Director and Program Lead

Publications :35 research publications in peer reviewed international journals and 6 in Proceedings and books.

BIOINFORMATICS AND ACCESS TO GENOMICS

Write up:

A vast amount of biological data has come into existence since the elucidation of principles of genetics by Mendel in the late 19th century and the discovery of DNA as the molecule of inheritance in mid 20th century. The pace of deployment of gene-, protein-, mutant- related data in the literature has rapidly increased since the advent of molecular biology, protein chemistry and protein engineering.

The number of genomes that have already been sequenced and being sequenced is growing at a rapid speed. As of now, the complete genome sequence of more than 30 highly diverse biological systems (prokaryotes, lower and higher eukaryotes) have been published and information on many genomes is pending for publication. *Mycoplasma genitalium*, one of the simplest pathogens, has less than 500 genes to exist as a self-replicating organism. The sequence details of yeast (*Saccharomyces cerevisiae*) a nematode worm (*Caenorhabditis elegans*), a fly (*Drosophila melanogaster),* plants (Arabidopsis thaliana, *Oryza saliva* [rice]), and man (*Homo sapiens*) are available.. The approximate sizes of the genomes stretch from 4.2 mega bases (each letter in GATC being a base) in bacteria *Bacillus subtilis*, 13. 5 Mb in yeast *S. cerevisiae*, around 120 Mb in *C. elegans, D. melanogaster* and *A. thaliana*, 400 Mb in rice *O. sativa* and 3000 Mb in Humans.

The rapid progress that has occurred in the area of Genomics is largely due to the spectacular developments in the areas of DNA sequencing chemistry, DNA sequencing machines and computational tools (Bioinformatics) required for the analysis of the large amount of data generated from the sequencing projects. Bioinformatics provides software tools for construction of complete catalogues of the ORFS, gene products, functional properties, interaction amongst the gene products, elucidation of pathways, comparison of genomes, similarity searches, and insights towards genetic modification of economically important biological systems to result in new products. The following URLs http://gnn.tigr.org/main.shtml and http://www.sanger.ac.uk/ can be used to access genomics relevant information.

The DNA information provided is in long strings of four-lettered sequences organized into distinct open reading frames (ORFs) associated with additional sequences for governing these ORFs. The ORFs representing all the genes constitute the genetic make-up of that organism. The ORFs translates into twenty-lettered amino acid sequences, which forms the basic framework to determine the functional properties. While many of the translated sequences from ORFs have known functions assigned to them, there are many that are unknown awaiting exciting discoveries which in turn may lead to identification of new biological functions of economical significance. A simultaneous genome-wide analysis of gene expression has been possible by the development of 'DNA chip" technology. This technology is a high through put method used for understanding the behavioral pattern of microbes and higher eukaryotes, including plants, under various environmental conditions.

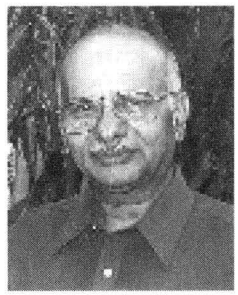
To complement the study of Genomics, there are equivalents such as Proteomics, a study of the complete set of proteins expressed in an organism or under a particular condition, Transcriptomics, a study of messenger RNA expression patterns, Metabolomics, a study of the metabolites at a given time, and Phenomics, a study of phenotypes conferred by protein functions.

Genomics and other related areas described above combined with plant transgenic technology has opened up a plethora of possibilities for making agriculture more productive for everyone. Agriculture technology has a tremendous opportunity to break away from the highly labour intensive and unproductive traditional practices to ensure attractive remuneration for the farmers. At a time when other technologies are making long strides bringing fundamental changes to the way of living, agriculture seems to be at cross roads, as it has been many times before, with farmers forced to stick to old technology or improperly adopted new technology.

Golden Lecture 7

BIOTECHNOLOGY : HOW IT CAN HELP AGRICULTURE

Profile:



Name :Dr. T. M. Manjunath

Address :Monsanto Research Centre (MRC), Bangalore (India)

Academic Qualification :Ph.D. in agricultural entomology from the University of Agricultural Sciences, Bangalore

Work Experience :He worked extensively on a wide variety of insect pests of cotton, corn, rice, sugarcane, pulses, vegetables, coconut and several other crops. He has studied the natural enemy complex of a number of major pests and recorded more than hundred new parasitoids and predators. He studies their bio-ecology and developed innovative mass-production, packing and release techniques for several promising biocontrol agents.
He has been actively involved in the establishment and overall growthof MRC right from its inception in early 1998.

Awards :He is a recipient of two awards at the national level, one from the Plant Protection Association of India in 1994 and another from the Institution of Agricultural Technologists in 1995

Present Position :Director, Monsanto Research Centre

Publications :He has published over 100 research and review papers in national and international journals.

BIOTECHNOLOGY: HOW IT CAN HELP AGRICULTURE

Write up:

The global population, which has crossed 6 billion in 2000, is estimated to be about 9 billion by 2050 of which about 90% will reside in Asia, Africa and Latin America. In India, the population has already exceeded 1 billion by 2001 and our country is projected to be the most populous in the world with 1.5 billion by 2050. We are confronted with several problems: a) 840 million people in the developing countries suffer from malnutrition and 1.3 billion are afflicted by poverty; b) 1.4 billion women (24% of world population of which 60% in the developing countries) suffer from iron deficiency anemia which impairs immunity and causes mental as well as physical weakness; c) 400 million children (7% of world population) suffer from vitamin A deficiency resulting in infant mortality and blindness; d) more than 30% of our crop yields are lost to pests, diseases and weeds despite spending about Rs.30 billion on chemical pesticides annually; e) huge losses of fruits, vegetables and flowers also occur in storage and transport; and f) there is an ever-decreasing resources like land, water, fertilizers, labour etc. These are some of the hard facts for which we have to find solutions. Recent advances made in biotechnology offer tremendous scope in this endeavour.

Agricultural biotechnology can contribute towards increasing crop yields; improving nutritional status; enabling crops to be grown in inhospitable habitats thus adding more land to production base; tolerance to abiotic extremes like drought, heat and cold; designing plants with reduced water requirements; increasing shelf-life of fruits, vegetables and flowers; resistance to pests, diseases and weeds. Transgenic plants bestowed with any of these traits can make a lot of difference to modern agriculture. The recent work on 'Golden Rice' and 'Golden Mustard' has created a lot of interest and hope.

The global adoption rates for transgenic crops by farmers have been unprecedented in the history of agriculture. Between 1996 and 2000, the commercial area planted to transgenic crops increased from 1.7 to 44.2 million hectares. Countries like the USA, Argentina, Canada, China, South Africa, Australia, Romania, Mexico, Bulgaria, Spain, Germany, France, Portugal, Ukraine and Uruguay have adopted this technology. The principal transgenic crops included soybean with herbicide tolerance (25.8 m ha); cotton with insect tolerance (Bt) and also insect tolerance-cum-herbicide tolerance (5.3 m ha); canola with herbicide tolerance (2.8 m ha); and corn with insect tolerance (Bt) as well as insect tolerance-cum-herbicide tolerance (10.3 m ha). These crops have given significant benefits by way of effective pest/weed management, higher yields, greater profits and safer environment through decreased use of conventional chemical pesticides.

In India also, transgenic technology has recently attracted considerable attention with Bt-cotton being in the forefront. Maharashtra Hybrid Seed Company (MAHYCO) has introgressed Monsanto's 'Bollgard' Bt-gene (*Bacillus thuringiensis*) into the Indian cotton hybrids by backcrossing with a transgenic line. These plants, conferring protection against the notorious Indian cotton bollworms (False American Bollworm - *Helicoverpa armigera*; Pink Bollworm - *Pectinophora gossypiella*; Spotted Bollworm - *Earias vittella*; and Spiny Bollworm - *E. insulana*), have undergone multi-location field trials in 1998 and 1999 under the supervision of Dept. of Biotechnology (DBT), Govt. of India. The Genetic Engineering Approval Committee (GEAC) under the Ministry of Environment and Forestry, after scrutinizing the data, has given approval in July 2000 for large scale field trials on 85 ha and for seed production on 150 ha in different geographical locations in the country. Some of these trials are being repeated during the 2001 cotton-growing season also to collect additional data as advised by GEAC. Bt-cotton is the first transgenic crop to reach such advanced stage of regulatory approval in India. Experiments on transgenic plants like brinjal, cabbage, cauliflower, potato, rice, tobacco, tomato and mustard are also being carried out by IARI, various ICAR and other research institutions, universities, private companies etc. However, so far no transgenic crop has been commercialised in India.

The global market for transgenic products has grown tremendously from year to year. The estimated global sale from transgenic crops was US $ 75 million in 1995; it increased to $ 235 m in 1996, to $ 670 m in 1997, to $ 1.6 billion in 1998, to $ 2.3 billion in 1999, and about $ 3 billion in 2000 thus showing about 40-fold increase in a short span of six years. The global market is projected to reach $8 billion in 2005 and $ 25 billion in 2010. The number of countries growing transgenic crops has increased from 6 in 1996 to 9 in 1998, to 12 in 1999 and to 13 in 2000. More countries are showing increasing interest in this technology.

In every country, the prescribed biosafety requirements are to be fulfilled before a transgenic technology is approved for commercialization. All the transgenic crops that have been commercialised so far have gone through and passed extensive safety trials with regard to toxicity, allergenicity, pollen transfer, safety to non-target beneficial organisms, etc. In India, DBT, through its various expert committees, is responsible to ensure compliance of the required biosafety regulations for all genetically modified organisms (GMOs) including transgenic plants. Bt-cotton has undergone/still undergoing such biosafety tests.

Transgenic technology is highly precise and powerful. It is not a panacea, but has the potential to usher in the much-needed second 'green revolution' in the face of burgeoning population. Effective dissemination of correct information and proper guidance are necessary to remove any misconception or apprehension about this remarkable new technology. India, being predominantly an agricultural country with vast land and human resources, has the potential to become a super power in agriculture if modern technologies are suitably adopted.

Bt-Cotton Approved in India

The.Genetic Engineering Approval Committee (GEAC) of the Ministry of Environment & Forests, Govt of India, has approved three Bt-Cotton hybrids of MAHYCO for commercial cultivation in India on March 26, 2002. Thus Bt-Cotton became the first-ever transgenic crop to receive official approval in India. The first commercial Bt-Cotton crops were sown on about 80,000 acres in the second half of 2002.

Golden Lecture 8

GROW MORE SANDAL USING BIOTECHNOLOGY

Profile:

Name : Dr. P.S. Rao

Academic Qualification : B.Sc. (Hons)
M.Sc. Botany
Ph.D. Botany

Work Experience :

1966 Dec	Joined the Bhabha Atomic Research Centre, Govt. of India atBombay as a Scientist
1971-1973	Deputed by Govt. India as a Visiting Scientist to CNRS Lab, France. Worked with Prof. J.P. Nitsch on Plant tissue culture - Chemical control of growth and differentiation
1982-1983	Deputed by Govt. of India as a Visiting Scientist to Max Planck Institute, Koln, Germany. Worked with ProE 0. Schieder on Isolation of plant protoplasts, fusion and hybridization
1999	Superannuated from BARC service. Prior to retirement Dr Rao was the Head of Agriculture and Biotechnology Division at BARC

Present Position : Dr. Rao is working as Vice President (Biotechnology), Indo American Hybrid Seeds Pvt.Ltd. Bangalore.

GROW MORE SANDAL USING BIOTECNOLOGY

Write up:

Micropropogation and Technique Development

MicroPropagation essentially involves removing aseptically a bit of tissue or a few cells from leaf, root, stem, etc. from a healthy donor plant and grow each of them to whole plants on an appropriate nutrient medium in a culture vessel under controlled conditions of light, temperature and humidity. Plants developed under these conditions are transferred to soil in pots, hardened in green house and a shade house and thereafter used as normal plants. Since each cell carries the genetic potential to form a new plant this new technology has generated a lot of interest for increasing productivity. Today, we can actually speed up the multiplication rate of a plant by over ten thousand times and a large number of plants are indeed being routinely multiplied and marketed all over the world by commercial biotech companies.

Technology for high volume production of planting material has been developed for many commercially important plants which is briefly outlined below:

Banana

Banana is an economically profitable fruit crop with large in country consumption and considerable export potential. It is vegetatively propagated through the suckers. Production of suckers is limited and variable. Besides suckers carry a number of pathogens both bacterial and viral. During vegetative propagation the pathogens pass from one generation to other and spread rapidly. Sometimes the entire banana crop is destroyed by these diseases. By adopting shoot tip culture technique, a low cost micropropagation technology for high volume production of disease free banana plants has been developed and standardized for many commercial cultivars. (Trop, Agr. 71, 299, 1994 ; Cuff. Sci. 68, 646, 1995; Ind. Jour. Expd. Biol. 3 5, 96, 1997; Proc. Nad. Acad. Sci., 68, 45, 1998; Curr. Sci. 76, 1228, 1999). Tissue cultured banana plants grown in farmers field have shown vigorous growth, early maturity and increase in bunch weight with better quality of fruits. The technology has been transferred to user agencies.

Grape

Grape (Vitis vinifera) is world's high value added fruit due to its widespread use as fresh fruit, jams, raisins and also in wine industry. Conventionally one vine yields 12 new vines per year, but with tissue culture method, a ten fold increase in production can be achieved. A protocol has been developed for large scale production of grape plants which can be scaled up for commercial exploitation (Plant Cell Rep. J-7, 65, 1997). In a wild species of grape, Vitis lafifolia L.; anther cultures have been established and plant regeneration has been obtained via somatic embryogenesis (Plant Cell Rep. -L8, 617, 1999).

Pineapple

Pineapple (Ananas comosus) is another important fruit which is vegetatively propagated. However, the average production of propagules is two per year. Growing dormant buds of Pineapple on a nutrient medium can yield upto 40-50 shoots each of which can be converted into plants. Several such in vitro obtained plants have been field planted. The process developed can be used for large scale cultivation of elite selections of pineapple to boost the pineapple industry.

Sandalwood

Sandalwood (Santalum *album)* is a commercially valuable forest tree known for its fragrant wood and oil and is a foreign exchange earner. The trees cannot be vegetatively propagated and seed progeny is variable owing to cross-pollination. Therefore, predictions about wood quality and oil content cannot be guaranteed. A tissue culture technique has been developed for mass multiplication of sandalwood plants (Can.J.Bot. *56, 1153, 1978;* Ann.Bot. 44, *629, 1979;* Plant Cell Culture & Crop Improvement, *119, 1983).* Somatic embryos have also been successfully grown in bioreactors (Curr. Sci. *59, 746, 1990).* The technique can be employed for the clonal multiplication of genetically superior trees known for its characteristics of oil and wood and thus has potential applications in afforestation *of* sandalwood plantations.

Mulberry

Mulberry (Morus *alba)* is very important in silk industry and quality mulberry plants are crucial for silkworm feeding. Although vegetatively propagated by cuttings, certain elite mulberry varieties are difficult to root or have low rooting ability. This impedes propagation. Through culture of axillary buds on a defined nutrient medium a protocol has been developed for large scale multiplication of mulberry plants (Z. Pflanzenphysiol. *I I ~ 465 1983,* Plant Cell Rep. *4, 78, 1985).* This would be useful for enhanced biomass production and for multiplication *of* difficult-to-root varieties as well as hybrids. This technology has been transferred to Central Sericulture Research Institute, (Central Silk Board), Mysore.

Technology has also been developed for large scale, selective clonal multiplication of genetically superior varieties of turmeric, ginger, capsicum, (Plant Sci. Lett., 11, 365, 1978), eggplant, (Plant Sci. Lett. 13 ' 57, 1978), tomato (Ann. Bot. 45, 205, 1978), orchids (Plant Sci.Lett. 17, 303, 1980)and mustard (Plant Sci. Lett..6, 111,1982).

Micro and minituber technology in Potato

Potato (Salanum tuberosum) is the fourth important food crop in the World and represents an important part of the diet for more than half billion people. It is vegetatively propagated and is prone to many viral diseases and the viruses permeate perpetually through vegetative propagation. Due to this the seed stocks degenerate rapidly affecting their yielding ability. Production of virus free seed material is essential for getting a healthy crop. Using tissue culture approach (nodal segments, tubers, shoot tips) it was possible to produce potato plants on a large scale. Further, tubers were induced in the tissue cultured potato plants on a large scale. Further, tubers were induced in the tissue cultured potato plants on a specific tuberization medium. Upto 120 microtubers were induced per culture vessel. The microtubers were harvested and grown in the green house and minitubers(5-30g each) were produced which formed the seed material. The advantage is that only So kg of minitubers are required per hectare as against 2000 kg seed tubers. This is a very useful technology for potato cultivation.

Agricultural Biotechnology and Genetic Improvement of Crops

Considerable R&D work on biotechnology of several agricultural crops has been carried Out. These include establishment of tissue cultures and plant regeneration in rice, (Jour. Genet. Breed. 49, 9, 1995; Physiol. Mot. Biol. Plants 1, 81, 1995; Rice Genetics Newsletter n, 183, 1994, Cereal Res, Commun. 25, 27, 1997) **wheat** (Proc. Ind. Natn. Sci. Acad. 48, 371, 1982; Plant Cell Rep., 1, 215, 1992; Proc. Ind. Acad. Sci., 24, 33, 1985), **rye, triticale** (Plant Cell Tissue & Organ Culture 1, 221, 1982; Proc. Ind. Acad, Sci, 5Q, 431, 1984; Euphytica L4, 153, 1995), sorghum (Proc. Ind. Acad. Sci. 99 405, 1989), **peadmillet** (Plant Cell Tissue & Organ Culture 32, 91, 1993), **flaprmillet** (Biol. Plant, 3, 5, 1993), **groundnot,** (Curt. Sci. 54, 1052, 1985; Biol. Plant. 35, 499, 1993), **mothbean** (Proc. Ind. Acad. Sci. L6, 55, 1986), **mungbean,** Z.Pflanzenphysiol. ***IU, 325, 1984; Z. Pflanzenzuchtung, 96, 169,*** 1986), **and urdbeas** (J. Plant Physiol, 130, 15, 1987). Development of plant regeneration protocol in these strategic crops constitutes a crucial step in genetically manipulating them for desired traits.

Development of **haploids** (Plant Sci Lett 2~, 111, 1982) and yellow seeded somaclone of **mustrad** (Plant Sci Lett 30, 327, 1983) has been achieved. As a part of the national Network programme on the Development of transgenic **cotton** varieties improved for resistance to cotton bollworm, supported by the Department of Biotechnology, Govt. of India, regeneration of plants in six Indian **cotton** cultivars has been achieved (Asia Pacific Jour Mot Biol & Biotech *1,* 173, 1999),

Protoplast culture & Genetic transformation

Isolation of protoplasts and in some cases plant regeneration has been obtained in **Tylophore Indica** Q. Plant Physiol. 115, 23, 198 5), **PWXularia palUda (i Plant Pk)wiA 124, 4-13, 19,86), VA*pa** species (Theor, App. Genet. L4, 1052, 1985), **Catharanthus reseus** (Proc. Inct Acad Sci. %. 413, 1986), **Sesamunt indicum** (Ind, J. Exp. Biol. 27, 182, 1989) and **Arachis hypogata** (Curr. Sci. 54, 1052, 1995). Protoplasts from a forest tree, **Sandalwood,** were successfully isolated and regenerated into complete plants (Protoplasina, 124, 80, 1985). **This is the firs; report as far as tree protoplasts *am concerned* and would have implications in tree breeding programm's.** ***Studies*** On ***Agrobactenum*** mediated gone transfer to obtain **tranagenic plants** have been carried

out and successful transformation has been demonstrated in ***Brassicajunma*** (Plant Sci. Lett. 39, 49, 1985; Plant *Sci.* 72, 245, 1990), ***V19M*** **dcon&YO&** (Proc. Inct Acad. Sci. 98, 495, 1988) and **AiMPO beffad0fta** (I. Plant Physiol. 13 6, 404, 1990).

Tissue Culture of Medicinal and Aromatic Plants

Higher Plants represent a valuable resource for a great variety of speciality chemicals including Pharmaceuticals, flavours and fragrances, pigments and fine chemicals. Medicinal plants are the most exclusive source Of life saving drugs since early history. About 8000 herbal remedies have been codified in the Ayurveda. The continuous production of desired bioactive compounds requires repeated collection of the plant material and consequently this leads to the depletion of natural sources which has become a serious concern Mass cultivation of medicinal plants under natural conditions may not be possible due to ecological conditions, length of propagation or the need to use the land for producing one of the primary food crops.

The application *Of* tissue culture techniques for medicinal plants holds promise because of the following reasons: (1) plant cell cultures can be established from medicinal plants which can be used to isolate bioactive compounds which would form a reliable source for the production of natural products throughout the year without the destruction of the plant, and (2) higher quantities of desired compounds can be obtained through cell line selection or addition of precusor into the production medium and (3) (1) Clonal multiplication of medicinally important plants to increase the biomass production can be achieved. **Thus medicinal plant biotechnology assumes significance both in terms of preservation of medicinal plants and product synthesis.** Some of the important products under development include Taxol and Camptothecin (anti-cancer), Castenospermine and Hypericin (anti-AIDS), Artemesinin (anti-malarial) and Forskholin (cardiotonic).

Cell and tissue cultures for the production of bioactive products have been established in **Catkarantkus rosems** for ajmalacine, **Rauvoyia** *serpenfina* for ajmaline, **Castenospermum austmie** for tetra-hydroxy indolizidine and *Nothopodytes foefida* for camptothecin. Also bioreactors have been employed for large scale cultivation of plant cells of *Artemesia annua* for the production of terpenoids. (Jour. Biotech. 40, 139, 1995).

Bioencapsulation and Synthetic seeds

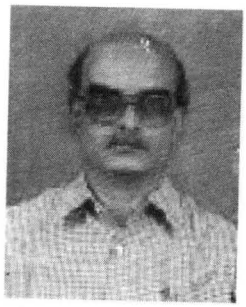
Conventional methods of plant propagation is through seeds and in plants which do not produce seeds it is accomplished through cuttings/grafts. However, recent advances in biotechnology have made it possible to produce 'synthetic seeds'. Synthetic seeds are prepared by encapsulating tissue culture derived somatic embryos which are produced in large numbers in a gel matrix. The encapsulated somatic embryos can be 'germinated' to produce plants. Likewise, growing shoot tips/axillary buds can also be encapsulated in a similar way and plants can be recovered.

Protocols for **bioencapsulation** of somatic embryos of **Sandalwood** (Plant Cell Rep. 7, 434, *1988)* , Rice (Asia Pacific J. Mol. Biol. & Biotech, 4, 90, 1996), and axillary buds of **Mulberry** (plant Cell Rep. ~, *393,* 1987), and shoot tips of **Banana** (Plant Cell Reports R, *571, 1992,* Informusa. Z, 4, *1993)* and **Cardamom** (Biotechnology Techniques a, *239, 1994),* and their conversion into plants has been Optimized, Production of synthetic seeds will have potential applications as novel delivery system for propagation. in addition to the above research programmes in which there was direct involvement, as Head of the Division, Dr. Rao was overseeing the work in other areas of Agriculture viz., crop improvement through mutation breeding, integrated pest management for crop protection, pesticide residue analysis using radioisotope techniques, increasing fertiliser use efficiency as well as Bioremediation of nuclear waste.

Golden Lecture 9

BIOINFORMATICS: THE POST GENOME ERA

Profile:



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Bioinformatics Centre
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Academic Qualification : Post doctoral work at Purdue University, USA

Work Experience : Structural biology
(enzymes, light harvesting systems)
Bioinformatics
Design of novel molecules
Protein folding problem
Understanding thermal stability of proteins

Awards : Won gold medal for best thesis.

Present Position : Chairman,
Bioinformatics Centre,
Indian Institute of Science, Bangalore.

Publications : More than 60 research publications in leading
National and International Journals

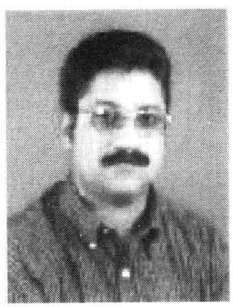
BIOINFORMATICS: THE POST GENOME ERA

Write up:

Bioinformatics is a field which encompasses many disciplines such as molecular biology, biochemistry, biophysics, and genetics on one hand and computer science on the other. Bioinformatics utilises methods from areas of computer science such as algorithms, optimisation, neural networks, motif recognition, database systems and database mining. Bioinformatics aims to transform the deluge of data in molecular biology into new understandings and useful knowledge. The genome of many organisms including several pathogens is now fully sequenced. Important milestones have been reached in the sequencing of the human genome. We have therefore moved from the pre-genome era to post-genome era. The emphasis in the post-genome era has shifted from sequencing more and more genomes to developing drugs which are marketable products. Bioinformatics can help in the drug discovery process in several stages such as identifying new genes in genomes, predicting the three dimensional structure of protein molecules say in pathogenic organisms, identifying the active site regions in proteins, identifying small molecules which can dock to the target protein and optimising the structure of small chemical molecules through design cycles. Bioinformatics can also provide methods for the insilico evaluation of bioavailability and toxicity of candidate drugs. Thus bioinformatics can improve the overall productivity and speed up the drug discovery process at reduced costs. Hence it is not surprising that pharmaceutical companies in both India and abroad are strenghthening their bioinformatics teams in a big way. It is suggested that bioinformatics, which is essentially computer based analysis in combination with modern experimental protocols will eventually lead to the availability of personalised medicine and transform the health care industry.

Golden Lecture 10

TOWARDS A PROTEASE INHIBITOR MEDIATED RESISTANCE TO WESTERN FLOWER THRIPS IN CHRYSANTHEMUM



Profile:

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Academic Qualificaion : Ph.D Biotechnology, 2001
M. Sc., Biotechnology 1997
B. Sc., Agriculture 1993

Work Experience : He has worked as Technical Consultant for Agri Horti Projects (Tissue Culture & 100 % EOU Floriculture, 1993 –95)
From 1st August 1999 to till date, he is Coordinator India for Wageningen University, Plant Research International and Keygene Genetics, The Netherlands.
Consultant to a Seed Company for establishment of Molecular Biology and Transformation Lab
Consultant to Academic Institutions (Public & Private) to develop course curriculum and conduct quality training Programs.

Awards : **3** Gold Medals from UAS, Dharwad for the Highest Academic merit in the outgoing batch of B.Sc., Agriculture
M. Sc., with **Distinction**

Present Position : Consulting Biotechnologist to seed companies and Academic Institutions
Program Co-ordinator for Center for Life Technologies.
Coordinator India for Wageningen University, Plant Research International and Keygene Genetics, The Netherlands.

Publications : Published over 6 papers in international journals

TOWARDS A PROTEASE INHIBITOR MEDIATED RESISTANCE TO WESTERN FLOWER THRIPS IN CHRYSANTHEMUM

Write up:

Dr. Seetharam Annadana, M.A. Jongsma, P.B. Visser, G. Kuijpers & M. Udayakumar.

Chrysanthemum history and status

Chrysanthemum is one of the few cutflowers which has been widely grown across the globe, finding different uses like floral arrangements, individual flowers in garlands and detached ray florets stuck on walls and paper for decoration etc. It is a unique cutflower in many different shapes, on the basis of which they are classified as single, spidery, intermediate, anemone-centred, incurved, reflexed, pompon and other types. The origin of this interesting cutflower is a little obscure, but it is known to be in cultivation for over 3000 years. The earliest records of chrysanthemum date back to 500 B.C. in Chinese scriptures, and it was described in the ninth moon by Confucius as the "chrysanthemum with it's yellow glory". France introduced chrysanthemum from China into Europe in 1789, reached the Kew garden in UK and was mentioned in the *Botanical Magazine* in 1796. Seeds were produced in Europe for the first time in 1827. Only by the middle of the nineteenth century chrysanthemum was also introduced to the USA, when cultivar William Penn was shown at the Pennsylvania Horticultural Society in 1841 (Smith, 1975). Since then chrysanthemums have undergone a huge rise in popularity leading to their present economic status as the second largest selling cutflower next to roses. Chrysanthemum is grown in greenhouses with commercial material being vegetatively multiplied and supplied as cuttings or plugs. The production of chrysanthemum under cover today is threatened by insect pests like leafminers, aphids, and western flower thrips (WFT) (van Dijken et al., 1992; Guldemond et al., 1994; Robb, 1989). WFT is the most predominant, mainly because chemical control of this species is difficult, due to their secretive habits and because of rapid development of resistance to chemicals (Broadsgaard, 1994). Biological control is mostly not effective enough. In chrysanthemum the flowers are preferred for feeding (De Jager, 1995), wherein the ray florets are most preferred (Dijken et al., 1995).

The western flower thrips *Frankliniella occidentalis*

The western flower thrips (WFT) *Frankliniella occidentalis* belongs to the insect order Thysanoptera, sub-order Terebrantia and family Thripidae. It is a haplo-diploid; females are diploid and males are haploid. Fertilised females produce large numbers of females from fertilised eggs and a few males, while unfertilised females produce only males (arrhenotoky). The life cycle consists of six stages comprising of an egg, 2 larval, 2 pupal and an adult stage. The eggs are laid in parenchymatous tissue of the host plant, in a pocket created by the ovipositor (Brodsgaard, 1989). WFT was till 1980 a local pest limited to the western USA (Brodsgaard, 1991). Since then it has become a major problem world-wide on many crops and wild plants. Its polyphagous nature is extraordinary as it can feed and propagate on at least 240 species from 62 different families of plants (Loomans et al., 1995). The rapid world-wide spread has mainly been attributed as an effect of the practice of long distance trade in glasshouse plants. WFT is extremely difficult to control using any single mechanism and causes damage to a wide range of cash crops like cotton and tea, vegetable crops like onion, tomato and cucumber, fruit crops like pear, apple and orange, and practically all cutflowers. WFT generally feed by sucking plant juices by way of tissue penetration causing discoloration, resulting in reduced market quality (Gullan & Cranston, 2000). Additionally WFT also cause indirect damage as transmission vectors for tospoviruses (Bunyaviridae, Wijkamp et al., 1993) wherein larvae acquire and multiply virus while adults only transmit (Sakimura, 1962). In the absence of visual perception WFT tend to prefer the fragrance of open buds rather than fully open flowers (Smits et al., 2000). On the contrary, visually, the fully open chrysanthemum is preferred over the open bud, but the attraction for colour also depends on other local conditions and stimuli. In the presence of only chrysanthemum flowers, the colour of the flower had no influence, but the plant stage influenced the feeding pattern (Dijken et al., 1993). A high variability in Western flower thrips populations has been reported in their response to natural resistance in chrysanthemum (de Kogel, 1997), insecticides (Immaraju et al., 1992; Brodsgaard, 1989; 1991; Mcdonald, 1995), and virus transmission (Van de Wetering et al., 1996). Some of the reasons for WFT to be so virulent are attributed to its unique characteristics: arrhenotoky of sexual differentiation, a polyphagous feeding habit, opportunistic herbivory, thigmotactic behaviour and tendency for cannibalism (Lewis, 1973). In the years 1988-90 Dutch growers spent 39.5 million euro per year on crop protection in the specialized horticultural segment. This was close to 30% of the total crop protection costs in the Netherlands (Brouwer et al., 1994). Cost of pestcides used by the Dutch to protect ornamental and cutflower crops during that period was 25 million euro, which was the highest in the European

Union for that category of crops (Brouwer et al., 1994). WFT control in 1996 in the Netherlands cost 2.4 million euro (Roosjen et al., 1998) indicating the importance of this pest and the need to control it.

WFT control strategies
1. chemical strategies (Helyer and Brobyn, 1992, Loomans et al., 1995, Agrimonitor, 1997 & Lewis, 1999)
2. biological strategies (Loomans et al., 1995, 1996)
3. host plant resistance through breeding (Smith, 1989, De Jager, 1995; De Kogel, 1997).

STEP I: Regeneration of Chrysanthemum
To develop WFT resistance using a transgene approach, the pre-requisite is an efficient regeneration system for *Agrobacterium*-mediated gene transfer. Chrysanthemum has been regenerated using different explants like flower receptacle shoot tips, pedicel, petal segments and leaf. However these protocols are cultivar specific, as a result of which regeneration of a new cultivar is by trial and error which can be a draw back to genetically engineer a large number of cultivars. Thirty five chrysanthemum cultivars were tested for regeneration using stem explants on a direct organogenesis protocol used for Agrobacterium-mediated gene transfer (De Jong et al., 1994). The cultivars were grouped into four groups based on the kind of growth put forth (response) on the stem explants namely Group 1: more than 1.6 shoots per explant, Group 2: less than 1.6 shoots per explant, Group 3: No shoots but only callus and lastly Group 4: no response. Hormone source and concentration were modified and alternate explants were tested based on the performance of cultivars to initial screening (grouping 2-4). The initial screening provided indirect information on the endogenous hormone levels which was the criterion for the direction of change. The principle behind this approach was exposure of explants to different sources and concentrations of hormones or use of different explants to make use of the differences in endogenous hormone levels to affect regeneration. Five different groups of media totalling 23 different combinations were tested in total. By systematically following this approach it was possible to successfully regenerate 23 of the 35 lines tested while standard protocols only allowed regeneration of 11 lines.

STEP II: Transgene expression in green and floral parts of Chrysanthemum
After having developed a systematic approach for regenerating chrysanthemum, the focus shifted towards the improvement of transgene expression in chrysanthemum, which had a unique problem. Various cauliflower mosaic 35S (CaMV) promoter variants have been used in chrysanthemum to drive different transgenes. The single CaMV-GUS construct showed bright blue coloration upon overnight X-gluc staining, but fluorometric analysis showed very low to undetectable expression. This is a problem not commonly observed in other plant species, which had to be solved to genetically engineer chrysanthemum with protease inhibitors. Thus, with the aim of improving transgene expression levels in green parts of chrysanthemum, two putatively stronger promoters, the doubled cauliflower mosaic virus 35S (dCaMV) promoter and the potato *Lhca3.St.1* promoter, were tested. In contrast to the low activity of the dCaMV promoter, it was observed that the potato *Lhca3.St.1* promoter gave up to 175-fold higher activities in chrysanthemum, while the same two promoters tested in tobacco resulted only in a 2-5 fold difference (Mlynarova et al., 1994, 1995 & 1996). Average GUS activity in chrysanthemum leaves is 44 pmol MU/min/microgram protein under the control of the *Lhca3.St.1* promoter, while the same promoter-GUS construct in tobacco was 4-fold higher (Mlynarova et al., 1994). Expression in chrysanthemum is somewhat higher in stems, but lowest in the ray florets (7.8 pmol MU/min/microgram protein). The *Lhca3.St.1* potato promoter is, therefore, a suitable promoter to obtain high expression levels in the green parts of chrysanthemum. It remains un-explained why the *Lhca3.St.1* promoter performs 4-fold higher in tobacco over chrysanthemum. This phenomenon is not specific to this foreign promoter as it is also observed with the chrysanthemum derived *Rbcs* promoter (Outchkourov et al, submitted). Secondly, it is also unclear why the CaMV based promoters are not active in chrysanthemum. Possibly, the necessary transcription factors commonly found in other plant species are present in lower concentrations, have diverged from ancestral specificities or are simply absent in chrysanthemum, resulting in the observed weak activity.

STEP III: Expression in the ray florets
WFT predominantly damages the ray florets. Hence, a promoter conferring high levels of transgene expression in the ray florets would be useful. Known heterologous promoters like, the chalcone synthase gene *chs-A* (van der Meer *et al.*, 1990), the zinc finger transcription factor *EPF*2-5 (Takatsuji *et al.*, 1994), promoter of the *Arabidopsis* eceriferum gene *CER6* involved in wax biosynthesis (Pereira, unpublished) and the wound-inducible promoter of potato multicystatin, *PMC* (Walsh *et al.*, 1993) were selected. Ubiquitin genes were chosen to contain potentially strong homologous promoters based on DNA microarray data which demonstrated that ubiquitin genes in *Arabidopsis* are among the most abundant messengers in most plant organs including flowers (Ruan *et al.*, 1998). Based on the activity of the GUS reporter gene the homologous ubiquitin extension protein (*UEP1*) promoter

conferred significantly higher GUS expression (8.5 pmol/min/µg protein) over the heterologous promoters tested (2-5.5 pmol/min/µg protein), and was comparable to the activity of *Lhca3.St.1* in the florets (7.8 pmol/min/µg protein). The activity of the *UEP1* promoter was, however, 3-fold lower in the disc florets and 9-fold lower in the leaves. The activity of *UEP*1 promoter was observed only histochemically in the pollen grains, hence, there is no quantitative expression data. As the *UEP1* promoter confers significantly higher levels of transgene expression in the petal tissue of the ray florets over the heterologous promoters they can be used to express genes to alter flower colour, vase life, fragrance etc. In this study the *UEP1* promoter was used to express the potato multicystatin gene in chrysanthemum. Thus, two promoters were identified one heterologous from potato and the other homologous from chrysanthemum whose activity are comparable in the ray florets. However, the *Lhca3.St.1* promoter confers GUS at 44 pmol/min/µg protein in the leaves, which is 50-fold higher than the *UEP1* promoter (0.9 pmol/min/µg protein), thus giving each of them unique potential for different applications.

STEP IV: MAR's flanking

Accurate characterisation of promoter activity has to be position effect independent. In order to remove position effects, resulting from the random place of integration of the transgene, the effect of flanking matrix-associated region (MAR) on either side of transgenes was followed (Mlynarova et al., 1994, 1995 & 1996). There was no difference in variance between the populations (different to the results presented by Mlynarova et al., 1994) generated with and without MAR flanking. These results imply that in chrysanthemum the chicken lysozyme MAR element is not effective and the action of a MAR element may depend on the host organism.

STEP V: Characterisation of WFT proteases *in vitro*

To select effective protease inhibitors against insect herbivores, it is essential to first determine the predominant protease activity and the pH optimum in the insect gut. On that basis proteinaceous protease inhibitors can be selected in a second step for optimal inhibition *in vitro*. In a third step, this is followed by studying effects on the insect in bioassays *in vivo*. To study the effects of protease inhibitors on WFT proteases, the whole Western flower thrips were ground (seperating guts is not practical due to size), with the presumption that the gut proteases would be dominant also in whole WFT extract. Over 90% of the WFT protease activity was inhibited when they were exposed to chemical and proteinaceous cysteine protease inhibitors. Thus, cysteine proteases were dominant in the whole WFT extract, and exhibited a relatively low pH activity optimum of 3.5. This pH optimum is low compared to other insects, which also utilise cysteine proteases for protein digestion in the gut.

STEP VI: Production of cysteine protease inhibitors in the yeast *Pichia pastoris*

To test *in vivo* effects substantial quantities of purified protein are required. Purifying the protein from its natural source has its limitations in terms of the heterogeneity of such samples, making a link with a specific candidate inhibitor gene rather dubious. These problems can be overcome by producing the recombinant proteins in microbial hosts. The yeast *Pichia pastoris* is a preferred expression system for protease inhibitors due to its high yield of protein secreted into the medium, which is relatively free of other proteins. Potato cystatin (PC) and potato multicystatin (PMC) were expressed in *Pichia pastoris*. PC was successfully produced in large quantities, purified by FPLC, and the apparent equilibrium dissociation constant was determined to be 0.6 nM. However, there was no success in large scale production of secreted PMC in spite of trying several media combinations and conditions for fermentation. Most of the little PMC that was expressed was retained in the pellet. Hence, we did not further purify. The reasons for lack of efficient secretion of this large 86 kDa protein is unclear. The cellular expression of PMC in the cytoplasm could be an alternative, to improve expression, which was not tried in this investigation. It is to be still clarified if PMC is also forming crystals in *Pichia* as it does in its native state in potato (Rodis and Hoff, 1984). Such crystals may get aggregated in the cytoplasm, and, hence, may not be secreted. PMC produced in *Pichia* was checked for size by western blot. This indicated that protein of the correct size of 85 kDa was produced, which implied the gene was maintained and stable. The *Sna*BI site used for the cloning may have affected the expression of PMC, as recently it was found that the *Sna*BI site is identical to an mRNA efficiency element and may cause strong 10-fold reductions of gene expression in Pichia pastoris using the pPIC9 vector (Outchkourov et al., in press). PMC expression could be tested without using the *Sna*BI site for cloning.a Alternatively, expression in the cytoplasm or the use of other strains of *Pichia* and other cloning vectors could be investigated.

STEP VII: Effects of cysteine protease inhibitors on WFT *in vivo*

Dietary protein ingested by adult thrips mainly serves to support the production of eggs as the adult insect is full grown already. Limiting the availability of protein by adding protease inhibitors (PIs) or reducing the protein content in a diet will, therefore, directly affect the number of viable eggs that can be produced. In case of larvae dietary protein serves mainly to support rapid larval growth. Limiting the available protein with protease inhibitors

affects larval growth and may result in death of the larvae if the inhibition is strong enough. Effects of cysteine PIs have been studied on other insects like oryzacystatin I on *Perillus bioculatus* (Ashouri et al., 1998), E-64, pHMB, cystatin, leupeptin on *Hypera postica*, E-64 and equistatin on *Leptinotarsa decemlineata*. There are no earlier reports, however, on testing of cysteine PIs against WFT and other members of the order of Thysanoptera, or the characterisation of dominant proteases present in WFT. In this study cysteine PIs, PC (potato cystatin) and EI (Equistatin) were tested for their effects separately and in combination on adult WFT in a bioassay cages made of transparent perspex tubes. The bottom of these cages were sealed with nylon gauze while the top was sealed with 2 sheets of stretched parafilm, with a liquid water sandwich in-between. PC and EI introduced into the liquid sandwich at 30 µM concentration, in the range of concentrations found in plant leaves (1.5-3% of total leaf protein), were provided to WFT for a period of five days. Both PC and PC+EI in combination reduced the oviposition rate by 50% compared to control. PC and EI were exposed to WFT for only 5 days, while the trend of reduction in oviposition rate was downward from day 3. If PC and EI were exposed for a longer period, there may have been a further decrease in egg production. Kirk (1985) reported that egg production by adult thrips would stop after 2-3 days of deprivation of nutrition. At the end of PI feeding, day-5 in the experiment, egg production was reduced by 50% relative to control, suggesting that five days were insufficient to fully deplete WFT from its protein reserves. This indicates that there may be a threshold level of protease activity.

The 50% reduction in oviposition rate achieved by exposure of WFT to PC and PC+EI could have a stronger effect on the size of the final WFT population after a certain period of time. As a consequence of a 50% reduction in oviposition rate, for example, a 92% reduction in the population of WFT, after a 90-day period, is predicted by a mathematical model based on the relevant life history parameters of WFT on chrysanthemum in greenhouses (18-day egg-egg, 3 eggs/female). Similar mathematical models for whitefly by Yano et al., (1989) predicted 75% reduction in population, over an 80-day period, resulting as a consequence of 50% reduction in oviposition rate. In addition to reducing the oviposition rate, PIs can also affect egg hatch which is functionally equivalent to further reducing the oviposition rate like shown in *Stomoxys calcitrans* by Spates & Harris (1994). However such effects of PC and EI on WFT, in addition to their effects on the different larval stages is yet to be investigated. EI has been shown to have an aspartic PI domain in addition to the cysteine PI domain (Strukelj et al., 2000). The combination of PC and EI did not result in further reduction in oviposition rate as compared to the reduction with PC alone. However in other insects having cysteine proteases as a dominant class of proteases, like *Leptinotarsa decemlineata* (Wolfson and Murdock, 1987; Gruden *et al.*, 1998) and *Diabrotica undecimpunctata* (Edmonds *et al.*, 1996) in addition have aspartic proteases. In WFT the aspartic protease inhibitor pepstatin showed 16% inhibition of proteolytic activity in total extract. This suggests that in WFT the contribution of aspartic proteases to total gut proteolytic activity may be limited despite the low pH optimum, which may be an explanation for the lack of additional effects with EI on WFT. Nevertheless serine and metallo PIs have not been tested in combination with PC, hence the possibility of generating a synergistic effect by combing inhibitors with PC is still possible. Considering the positive results reported by Thomas *et al.* (1994), and based on the serine PI elastatinal inhibiting 20% of WFT proteases *in vitro*, it may be worthwhile to test elastase and cysteine protease inhibitors in combination against WFT. In addition, the combination of cysteine PIs of different subclasses, with unique specificities from plant and non-plant sources may result in further improvements of the effects on WFT. Thus the *invivo* effects of the tested PIs provide a first proof of concept that cysteine protease inhibitors may be used to control the population of WFT. On this basis transgene-mediated resistance to WFT may be designed.

STEP VIII: Transgenic chrysanthemum expressing potato multicystatin

The homologous *UEP*1 promoter was used to drive in chrysanthemum the expression of a 3.5 kb PMC gene to generate an 85 kDa potato multicystatin protein. Transgenic lines were analysed by immuno-dotblot. However, as the protein used for the standard curve was the single domain PC while the 8 domain PMC was expressed in chrysanthemum, reliable quantitation of the absolute levels was not possible. The highest expression of PMC was estimated at 2.2 units/µg protein which conferred 0.28 pmol/µg protein of papain inhibitory activity (PIA). After subtraction of the background activity (0.15 pmol/µg protein) the expression was estimated to be 0.13% of total protein. This was rather low as protease inhibitors should be 0.5-1.0% of the total protein to effectively inhibit insect gut proteases of WFT in this case. On the artificial diets WFT were exposed to PC and EI at 30 µM concentration, which is equivalent to 0.03-0.06 % (w/v). Assuming the protein content in plant leaves to be 2%, than it is necessary to obtain a transgene expression level in the range of 1.5-3.0% of total protein. As a consequence of low PMC expression (based on the analysis hitherto) driven by the *UEP1* promoter, we observe 10-20 fold lower levels of expression than required. Hence, no correlation between oviposition rate and PMC expression or PIA (papain inhibitory activity) is observed. It will be necessary to generate plants with higher levels of transgene expression to obtain resistance. By increasing the level of expression one would be able to significantly reduce the WFT population on chrysanthemum based on the proof of concept. By changing the

promoter driving the PMC gene we not only enhance the expression by 5-fold but also spatially change expression to leaf. As leaves of chrysanthemum in the greenhouse are exposed to WFT for a longer duration over the flowers, the building up of the population will normally be on leaves. Expression of PMC at 5-fold higher levels in the leaves of chrysanthemum, may extend the proof of concept provided in this study on the use of cysteine inhibitors to control WFT. Alternatively the cysteine protease inhibitory activity (CPIA) can be enhanced by identifying the natural variation for CPIA among the breeding lines of chrysanthemum, based on PIA. We observe 0.15 pmol MU/min/µg protein of PIA in the control ray florets and leaves of chrysanthemums, which is relatively high as compared to similar tissues from other plants. When this variation is not available than the CPIA can be enhanced by mutation breeding to achieve the same

Thoughts for the future

Though difference in regeneration efficiency related to quality of light incident is shown, it still remains to be seen how microshoots from the different coloured boxes would perform in the greenhouse, under standard light conditions. In addition it remains to be seen if light quality has any influence on the generation of somaclonal variants and transformation efficiency. Studies on these aspects would be interesting and also broaden the influences of light quality. It will be relevant to study the effect of PI on the different larval stages of WFT and on egg hatch. Bioassays with pure protein on different larval stages are not practical, hence these proteins have to be tested in transgenic plants. To further improve the effects of PI observed on WFT, testing a combination of PIs of different classes and PIs within the cysteine PI family with different protease specificites may generate stronger effects than by using a single inhibitor alone.

CONCLUSION

Dendranthema grandiflora or chrysanthemum, as it is better known, is after roses worldwide the second largest cutflower crop. *Frankliniella occidentalis,* commonly known as western flower thrips (WFT) is its most threatened pest both under cover and in field cultivation. With the aim of developing transgene-mediated WFT resistance in chrysanthemum, efficient regeneration techniques were developed, along with the identification of effective promoters which confer high levels of expression in chrysanthemum. Recombinant cysteine protease inhibitors effective against WFT were identified based on the inhibition of the predominant group of proteases *in vitro* and reduction in oviposition rate (OR) *in vivo*. Potato multicystatin (PMC) an eight domain cysteine protease inhibitor was expressed in chrysanthemum in an attempt to endow chrysanthemum with thrips resistance. Plants with levels of expression ranging at maximum 0.1-0.13% of total protein were generated. A correlation with thrips resistance could not be established at these relatively low levels of expression, but the results provide evidence that with higher expression levels the control of thrips is feasible.

Golden Lecture 11

APPLICATIONS OF MOLECULAR MARKERS IN THE IMPROVEMENT OF HORTICULTURAL CROPS

Profile:

Name : Dr. Lalitha Anand

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Academic qualifications :

M.Sc. Biochemistry, Kerala University - 1976
Ph. D. Department of Biochemistry,Indian Institute of Science– 1988

Work Experience :Working in the areas of

(a) molecular basis of plant defence responses
(b) molecular events during fruit ripening
(c) molecular markers
(d) banana genomics

Present Position : Principal Scientist and Head, Division of Biotechnology, IIHR, Bangalore.

APPLICATIONS OF MOLECULAR MARKERS IN THE IMPROVEMENT OF HORTICULTURAL CROPS

Write up:

The process of developing new crop varieties involves many steps and takes several years. In recent times, biotechnological advances have helped in reducing these considerably. Apart from the techniques of haploid culture and genetic engineering, one of the tools which is making it easier for scientists to select plant traits and develop new varieties is the use of molecular markers. Polygenic characters which are very difficult to analyse using traditional plant breeding methods can now be easily tagged using molecular markers.

Molecular markers can be used for (a) characterization of germplasm (b) varietal identification and clonal fidelity testing (c) assessment of genetic diversity (d) validation of genetic relationships and (e) marker-assisted selection. These have widespread applications in management of genetic resources as well as in breeding programmes. Marker-assisted selection has practically revolutionised breeding programmes. Traditionally, plant breeders have selected plants based on their visible or measurable traits called the phenotype. But this process can be difficult especially in the case of multigenic or quantitative traits. Molecular markers can be used to trace linkages with traits of importance.

Molecular markers are a string or sequence of nucleic acids which makes up a segment of DNA and are near the DNA sequence of the desired gene. Since the markers and the genes are also close together on the same chromosome, they tend to stay together as each generation of plants is produced. This is called genetic linkage which helps scientists to predict whether a plant will have the desired gene or not. If the marker for the gene can be located, it would indicate the presence of the gene itself. This approach permits the breeder to make earlier decisions about his selections while examining fewer plants. Molecular markers have been used to develop linkage maps for many important crop species. These genetic linkage maps show the location of markers and genes and show the distance from other genes.

Marker systems being used are isozyme markers and DNA-based markers. Of the several DNA-based markers, Restriction Fragment Length Polymorphism (RFLP), Random Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP) markers and microsatellite (SSR -single sequence repeat) markers are generally the markers of choice. The two applications of molecular markers in the area of horticultural crop improvement are assessment of genetic relatedness and marker-assisted breeding.

Assessment of genetic diversity

A number of reports are available on the use of DNA markers to assess genetic diversity among species of several horticultural crops as well as validation of genetic relatedness among them. This has significant application especially in difficult-to-breed woody perennials.

Genetic diversity has been assessed using DNA markers in several fruit crops. These include avocado, banana, blueberry, citrus, grapes, mango, strawberry and walnut. Vegetable crops in which DNA markers have been developed for assessment of genetic diversity and construction of linkage maps are *Allium* spp., *Amaranthus* spp, common beans, crucifers, eggplant, melon , onion, pea, pepper, sugarbeet, sweet potato, tomato and watermelon. Tomato has been extensively studied and a high-density saturated linkage map is now available. In ornamental crops, genetic diversity assessment using DNA markers have been carried out in petunia and rose. Most of the reports are on varietal identification.

Marker-assisted selection (MAS)

This is one of the most important applications of molecular markers. Molecular markers can potentially increase the importance and usefulness of indirect selection in plant breeding. MAS is being increasingly adopted in breeding programmes since this approach permits the breeder to make earlier decisions about his selections while examining fewer plants. An added advantage in disease-resistance-breeding is that this could be done in the absence of pathogen once marker-information is available. This opens new possibilities to breeders since quarantine regulations restricting pathogen entry is a major obstacle in disease-resistance breeding.

Initially markers were being developed only for monogenic traits. Several disease resistance genes have been identified in horticultural crops. However, in recent years, markers have been developed for several quantitative traits as well which are governed by multigenes. Most of the important agronomic characters like yield and yield components, plant height and days to flowering are controlled by several genes. One of the first reports on the development of markers for QTL (Quantitative Trait Loci) in horticultural crops has been on development of

RFLP markers for soluble solid content in tomato. Molecular marker studies using bulked segregant analysis of near-isogenic lines or recombinant inbred lines have accelerated the mapping of many genes in different horticultural crops. Illustrative examples are given in Table-1.

Table 1. Illustrative examples of association of molecular markers with traits of interest in different horticultural crops

Crop	Trait of interest	Crop	Trait of interest
Apple	Columnar growth habit Scab resistance Morphological and developmental traits	Pea	Green seed colour Resistance to Pea mosaic virus, Fusarium wilt and Powdery mildew
Avocado	Skin colour	Papaya	Sex determination
Capsicum	Stunted growth	Potato	Resistance to Potato virus Y *Phytophthora infestans* Cyst nematode H 1 gene
Citrus	Differentiate zygotic from nucellar progeny Dwarfing; CTV resistance	Tomato	Pest resistance in *L. pinnelli* Resistance against *F.oxysporum* f.sp. *lycopersici* race I, Spotted wilt virus;
Common bean	Rust resistance		*Pseudomonas* , insect, nematode,
Crucifers	Flowering time in *Brassica oleraceae*		*Cladosporium fulvum,* powdery mildew;
Lettuce	Downy mildew resistance		Tm-1 locus, Tm-2 locus *Jointless* Soluble solid content

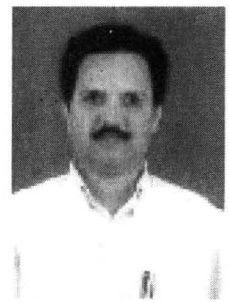
In India, work on these lines in horticultural crops is going on in IIHR, Bangalore, NRC for DNA Fingerprinting, New Delhi, CPCRI, Kasargod, IISR, Calicut, NCL Pune and TERI, New Delhi. Action has been initiated to start DNA fingerprinting facilities at State Department Biotechnology Centre, Hulimavu, Bangalore, Govt. of Karnataka. National Facilities have been set up by DBT, GOI at NCL, Pune and TERI, New Delhi for testing clonal fidelity and certifying a few horticultural crops raised through tissue culture.

DNA marker technology has had great impact on plant breeding programmes as well as in germplasm management. Research on requirements in the techniques employed and development of newer methods is making rapid progress. A number of markers closely linked to desirable traits in horticultural crops have already been developed. Identifying gene sequences responsible for conferring a particular trait has always been a formidable task. However, with the advent of molecular marker techniques, more and more such genes are being identified. It is not difficult to visualize the identification of several more genes in the near future. More information on linkage maps in several horticultural important crops using markers would also be made available. Molecular marker technology is being integrated into existing plant breeding programmes all over the world in order to allow researchers to access, transfer and combine genes at a rate and with a precision hitherto not possible. DNA fingerprinting of varieties also assumes importance in the years to come in the Indian scenario since legislation is now in place in the country on plant variety protection. The success of DNA marker technology would depend on close interaction between plant breeders and biotechnologists, availability of skilled manpower and substantial financial investment in research. However, the usefulness of these techniques and their relevance in applied research would far outweigh the investments made in terms of useful and far-reaching applications.

Golden Lecture 12

BIOPHARMING

Profile:



Name :Dr.P.H.RAMANJIM.GONMA

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Academic Qualification :B.Sc.(Agri) 1978,
M.Sc. (Agri) in Horticulture 1980,
Ph.D. in Horticulture 1984, from the University of Agricultural Sciences, GKVK, Bangalore

Work Experience :Indian National Science Academy Fellowship was awarded to undergo training in Biotechnology in U.K. Netherlands
Government Fellowship was awarded to undergo training in "Hitech Vegetable Production.
UNESCO fellowship was granted to undertake training in Biotechnology at University of Tuskegee, USA.
Served as a member in the advisory committees of MS and Ph.D.
Students of different departments

Awards :Merit Scholarship and United Nation Developmental Programme Fellowships during M.Sc. and Ph.D. degrees respectively.
In 1997 Awarded Sir C.V. Raman Award from the Govt. of Karnataka, India for the best research work.
The Tuskegee University, USA, has awarded best research worker award during 1996

Present Position :At present, working in the Department of Biotechnology, UAS, and Bangalore as an Associate professor.

Publications : Published 50 research papers and 25 popular articles in the national and international journals.

BIOPHARMING

Write up:

Novel products from plants:

Higher plants synthesize and enormous spectrum of chemical constituents. Many have known value as drugs (eg.taxol) biomaterials (e.g. rubber), solvents, flavorings, fragrances or coloring agents. Through development of transgenic plants, the purity of many plantderived chemicals can be enhanced and the range of chemicals expanded. One example is you pending development of plants that produce hydroxylated fatty acids. These oils can be used in applications ranging from hydraulic fluids to nylon synthesis. Similarly, the recent development of plants that accumulate biodegradable thermoplastics illustrates the potential for producing completely new compounds in plants. Many natural chemicals are important in plant defense mechanisms or in biotic stress responses. Some chemicals confer important horticultural qualities to plants. Thorough studies have been conducted on the biosynthesis of certain classes of these compounds such as flavonoids, cynogenic glycosides and certain alkaloids (eg.nicotine). Other classes of compounds, such as terpenoids, have received relatively little attention. Terpenoids are important for plant growth and can be used as essential oils, resin acids and pigments. An efficient mechanism must be developed to obtain specific biochemical information about these classes of compounds, to complement the extensive ongoing efforts to identify them and elucidate their structures. Biotechnology offers promise for accelerating the characterization of these substances as well as facilitating their production for commercial use (Anon., 1995). Plant based edible vaccines Vaccines are important in human health care as preventive medicines against infectious diseases. Infectious diseases are a major cause for concern in developing countries. In India infectious diseases like measles, pertussiis, tetanus, polio and diphtheria which can be prevented by vaccination is not effectively prevented due to high cost of vaccines and poor storage facilities. The field of biotechnology has provided new strategies for vaccine design based on techniques that make it possible to isolate genes from pathogenic viruses or bacteria. The subunit vaccine production is an important strategy for the large-scale production. The subunit vaccine has only a small part of the e pathogen and lack the virulence but it mimics the pathogen, there by the body will produce large amount of antibodies and prevent the occurrence of the disease. The production of subunit vaccine in yeast by fermentation is extremely safe and effective but relatively expensive and need refrigeration. Hence transgenic plants that express antigens in their edible tissue might be used as an inexpensive oral-vaccine production and delivery system; therefore, immunization might be possible simply through consumption of an 'edible vaccine'. Methods of transformation of plants: There are various methods for the transformation of plants. The most favorable method is Agrobacterium transformation followed by Gene gun, the various other methods include electroporation, silicon carbide methods and using viral vectors. The use of viral vectors is not very feasible since it is only a transient expression. Stable expression for the advantage of the subsequent generation of large number of transgenic plants either by vegetative or sexual means. Mucosal response: Disease causing agents enter the human body by several ways. An immune response is triggered by the entry of the antigen specialized cells of the immune system (macrophages, lymphocytes, B and T cells) migrate to the point of invasion to block the spread of the infectious agent. Vaccines try to mimic the antigen, there by stimulate the protective immunity. Lymphoid cells that are responding to antigens located at the mucous surfaces initiate the mucosal immune response.

E.coli heat labile enterotoxin B subunit and Cholera toxin B subunit. In developing countries, diarrhea disease is an important cause of mortality in children. Bacteria which cause diarrhea include vibrio cholera and he related enterotoxigenic Ecoli. An oral vaccine composed of the cholera- toxin B subunit (CT-B) with killed V.Cholerae cells gives protection against cholera and enterotoxigenic E.Coli LT-B has been expressed in transgenic plants. The oral immunogenicity of rLT-B was tested in mice and compared with bacterial LT-B when given orally to mice by gastic intubation, the plant-derived antigen stimulated humoral and mucosal immune responses. This demonstrates that a food source containing a foreign antigen can induce oral immunization (Mason et al., 1992). Hepatitis B surface antigen (HBs Ag): The expression of Hbs Ag at levels equal to 0.01% of total soluble protein in tobacco has been demonstrated. A crude extract of rBBsAg from plants was used parenteral immunization studies with mice. The extract used an immune response that was similar to the one achieved with Recombiivax@ i.e., commercial vaccine used to stimulate immune response. Rabies vaccine in Tobacco and Muskmelon The recombinant plasmid pRGRgp containing the rabies glycoprotein was cocultivated with Tobacco and Muskmelon (Ramanjini Gowda. etal 200 1). The presence of proteins was detected by western blot. The antigenic property of the cloned antigen was also determined by feeding mice and testing its antisera for the presence of glycoprotein specific antibody by ELISA. This is the first report on the production of immunogenic rabies vaccine produced in plant system.

Golden Lecture 13

NOVEL TECHNOLOGY IN AGRICULTURE

Profile:

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Academic Qualification : Ph.D. Biotechnology (Plant) 1995 - 2001
M. Sc. Biotechnology 1989 – 1991
B. Sc. Botany (Honours) 1985 – 1988

Work Experience : Worked as a Research Associate on a project involving clonal homogeneity testing and typification of tissue cultured plants of several genotypes of *Eucalyptus tereticornis*, *E. camaldulensis* and *Musa* spp., and also a number of ornamentals and cut-flowers, using ISSR/SSR and AFLP marker systems. The aim is to develop a 'kit' that would be useful in testing the tissue cultured plants being supplied farmers/forest department for uniformity and also would aid in fingerprinting of the product.

Current Position : Head - Plant Transformation Research and Services:

Publications : Published over 12 papers

NOVEL TECNOLOGY IN AGRICULTURE

Transgenic Research at Avesthagen

Genesis of the company

Avestha Gengraine Technologies Pvt. Ltd. "*Avesthagen*" was founded in 1998 by Dr. Villoo Morawala-Patell, a molecular biologist with a Ph.D. From University Louis Pasteur in Strasbourg, France. It is one of the very first plant biotech companies in India. From a small academic start up with 5 people, operating from the laboratories in the University of Agricultural Sciences in Bangalore, it has grown to more than 70 people in 2002. In October 2000 the company moved to its present location, in the International Technology Park, and is equipped with state-of-art equipment for genomics, proteomics and bioinformatics activities.

The three main activities or the research platform of the company are:

Seed for Food

This activity focuses on gene discovery and development of plant prototypes that are genetically engineered for traits like environmental stress tolerance and quality. The initial focus has been on rice, but subsequently other crops, pearl millet, sorghum and tomato to name a few, are being worked on as well. The Seed for Food activity is an in-house R&D activity to develop varieties with value-added traits, capable of being cultivated under adverse conditions.

Food for Medicine

The focus of this activity is on Indian medicinal plant species. Work is in progress to identify the genes responsible for the production of secondary metabolites of medicinal value, with the ultimate goal of increasing the yield of the active compounds though genetic engineering.

R & D Services

Avesthagen provides a range of services to the seed, pharmaceutical, biotech and food industries. Transformation is one of the areas in which we provide service and for which in-house capabilities have been developed over the last two years.

A dedicated team effort by the "Seed for Food" group at Avesthagen utilizing state-of-art technology for the improvement and increase in the agricultural produce. The thrust of the company lies in developing novel plant varieties that are not only tolerant to environmental and biotic stress, but also plant products with improved taste, nutrition and longer shelf life.

Plant breeding

For several thousand years, man has been altering the genetic makeup of the crops they had selected and grown. Human selection for features such as faster growth, larger seeds or sweeter fruits has dramatically changed domesticated plant species compared to their wild relatives. Remarkably, many of our modern crops have been developed by people who lacked an understanding of the scientific basis of plant breeding. The genetic basis of improvement of a particular variety were totally unknown until the remarkable studies on peas by Gregor Mendel were revealed and understood.

Transgenic technology - is a very powerful tool to complement conventional plant breeding by cutting down selection time and introduction of novel traits across the species and even genera. Transgenic technology provides the means to make even more distant "crosses" than were previously possible. Organisms that have until now been completely outside the realm of possibility as gene donors can be used to donate desirable traits to crop plants. These organisms do not provide their complete set of genes, but rather donate only one or a few genes to the recipient plant. For example, a single insect-resistance gene from the bacterium Bacillus thurengenesis can be transferred to a corn or cotton plant to make Bt corn/Bt cotton.

This knowledge ot technology should not be considered as a panacea to solve all food problems of the world but, only something that has to be utilised carefully as a new tool for the betterment of life.

What is a transgenic crop?

A transgenic plant is a plant containing a gene or a set of genes that have been artificially inserted into it, instead of the plant acquiring them through natural pollination. This inserted gene sequence (known as the transgene) may come from another unrelated plant, or from a completely different species

Why transgenics?

Traditional plant breeding has been limited to artificially crossing the plants within the same species or with closely related species to bring different genes together (gene pyramiding). This transgenic technology provides the means for identifying and isolating genes controlling specific characteristics in one kind of organism, and for moving copies of those genes into another quite different organism, which will then also have those characteristics. Thus enabling the plant breeders to do what they have always done - generate more useful and productive crop varieties containing new combinations of genes - but it expands the possibilities beyond the limitations imposed by traditional cross pollination.

Steps involved in creating a transgenic crop

1. Identifying gene(s) of interest
2. Designing (cloning) genes for insertion or transformation
3. Plant transformation (Agrobacterium/Gene gun mediated)
4. Selection and regeneration of transgenic plant
5. Transgene stabilization in the trangenic plant in containment
6. Testing the transgenic product for toxicity etc.
7. Release of new variety

Transformation methods

Transformation is the heritable change in a cell or organism brought about by the uptake and establishment of introduced DNA. There are two main methods of transforming plant cells and tissues:

1. The "gene gun" method (also known as microprojectile bombardment or biolistics). This method has been especially useful in transforming monocot species like corn and rice. In this method the DNA is coated on gold/tungsten particles and 'bombarded' in to the plant tissue/callus under pressure. The gene insertion is random and generally results in a number of copies of the same gene being inserted in one plant.

2. The *Agrobacterium*-mediated method: Plant transformation via *Agrobacterium* has been successfully practiced in dicots (broadleaf plants like soybeans and tomatoes) for many years, but only recently has it been effective in monocots (grasses and their relatives). This method utilizes the natural method of DNA transfer used by *Agrobacterium*, which is engineered to carry the gene of interest in the host plant. This method is more reliable and one can be assured of single copy insertions of the transgene.

In general, the *agrobacterium* method is considered preferable to the gene gun, because of the greater frequency of single-site insertions of the foreign DNA, making it easier to monitor.

Transgenic research at Avesthagen

Avestha Gengraine Technologies Pvt. Ltd has perfected plant transformation technology and utilized it in developing novel crops with novel characters or traits, mainly generating crops having tolerance to abiotic stresses like drought, salinity, etc. Avesthagen boasts of a complete lab to land set-up and also is involved in fundamental research in identification and isolation of useful genes from its various DNA libraries. Presently Avesthagen is working with a number of crops viz. rice, millet, sorghum, tomato, cotton, chickpea, pigeonpea, sesame and

mustard. One of the technologies developed by Avesthagen for generating Cytoplasmic Male Sterile lines has been described below.

Avesthagen Hybrid-Fit™ Technology

RNA editing - a tool to generate male sterile lines in agronomically important crops

- *Generating male sterile lines by inducing mitochondrial dysfunction using a novel gene product by plant transformation*
- *Powerful tool enabling the breeders to shorten the laborious emasculation and selection procedure*
- *A species/variety independent protocol that can be extended to a number of important crop varieties for F 1 hybrid seed production*

The utility of **Avesthagen Hybrid-Fit™** lies in its economic importance for the production of hybrid seeds. **Cytoplasmic male sterility** is a maternally inherited trait in plant mitochondria resulting in the abortion of pollen grains after meiosis during microsporogenesis. **'RNA-editing'** in plant mitochondria is a process that changes the nucleotide sequence of the RNA molecule from that of the DNA template encoding it. As mRNA editing normally occurs in most plants, the transgenic plants containing genomic (unedited) sequences will therefore, synthesize the mitichondrial protein with a modified (inactive / less active) function, thus producing sterile plants.

Avesthagen Hybrid-Fit™ strategy has been successfully utilized in producing male sterile lines of indica rice (variety - basmati). Two forms (unedited and edited) of one of the multi-protein complexes of the mitochondrial inner membrane protein encoding gene were cloned in plasmid vectors and bombarded in to the indica rice calli. Transgenic calli have been selected and regenerated in to complete plantlets in containment.

Avesthagen Hybrid-Fit™ can be extended to a number of agronomically important crops and vegetables. Currently ***Avesthagen*** is proving the utillity of this technique in oilseeds (sesame and mustard), cereals (sorghum and millet), vegetables (tomato and okra) and cotton.

Abiotic stess tolerance

Avesthagen has been instrumental in the Identification of novel stress tolerant genes by the analysis of stress proteins from expressed sequence tags (EST) database and incorporate them in a number of agronomically important crops. The information for the stress genes has been compiled in Avesthagen proprietary database known as **Plant stress information resource (PSIR)**. PSIR is a comprehensive collection of information on abiotic stress in plants, giving an overview on the concept of plants under aboitic and biotic stress and, an insight into different classes of stress encountered by plants. It includes the information on genes, regulatory elements, markers and proteins related to plant stress. We believe that PSIR will be a useful source of information for researchers working in this field and, provide as a source to identify the conserved motifs of stress related proteins from all biological systems. This will be used to identify and isolate novel genes associated with the regulation of abiotic stress from plants. Some of the genes isolated by Avesthagen are the MnSOD (superoxide dismutase family gene), BCP (blue copper protein gene), AGTSal11 (gene for salinity tolerance) and various protease inhibitors. These genes are being transformed into a number of crops and some of them are now at the greenhouse level of selection and propagation.

Risks and concerns.....?

With every new and powerful technology comes a whole lot of risks and concerns. Some of the concerns and questions raised by the common man for the transgenic researchers are given below. But the answers to al these queries will be fully understood only after thorough analysis of the transgenics has been taken place. It is still too early to go into these questions and try to answer them. But, all transgenic researchers have to take into consideration these important queries and try to address and minimize the risks involved in this kind of research activities

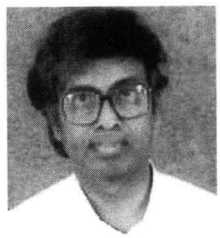
- Is eating food from transgenic crops a health hazard?
- Can antibiotic resistance genes used as markers in transgenic crops be transferred to pathogenic bacteria?

- Are transgenic crops a threat to other organisms in the environment?
- Will pollen from transgenic crops contaminate non-transgenic crop varieties?
- Will herbicide-resistant transgenic crops create "superweeds"?
- Do transgenic crops reduce biodiversity?
- Will the widespread adoption of transgenic crops lead to increased corporate control of the world's food supply?

Although some of the questions can be partially answered at the moment but I would like to leave the discussion open at this juncture for people to understand and think over the benefits that we can get from this powerful transgenic technology and weigh them against the queries raised above.

Golden Lecture 14

BIOTECHNOLOGY IN DEVELOPING ABIOTIC STRESS RESISTANCE



Profile:

Name : Dr. M. Udaya Kumar

Address : Department of Crop Physiology
University of Agricultural Sciences
GKVK, Bangalore – 560 065

Academic Qualification : M.Sc. (Agri)., Ph.D., Academy of Sciences, USSR.
Proficiency in Russian language (Diploma in Russian language and Russian to English translation Diploma)

Work Experience :

Pool Officer	1971-72	UAS, Bangalore
Jr. Plant Physiology	1972-74	UAS, Bangalore
Plant Physiologist (Assoc. Professor)	1974-83	UAS, Bangalore
Professor and Head Department Of Crop Physiology	1983-to date	UAS, Bangalore
Professor and Head Department of Crop physiology and Head & Division of Plant and Soil Sciences	1997-to date	UAS, Bangalore

Awards :
Received Professor R.D. Asana Medal (1980)
Received meritorious invention award from NRDC (1985) for the development of a scientific instrument in collaboration with ISRO
Rotary gold medal for distinguished scientific contribution 1994, Rotary International Club, Bangalore.
Best Teacher award by ICAR in the year 1997
Best Scientist award –by ITC – Golden Jubilee celebrations of ILTD, ITC

Present Position : Co-ordinator for Biotechnology Department, UAS, Bangalore

Publications : More than 120 research articles
(52 in International Journals)
Papers presented at various National and International Symposia -14
Book/Chapters - 9

BIOTECHNOLOGY IN DEVELOPING ABIOTIC STRESS RESISTANCE

Write up:

Biotechnological Interventions for Developing Abiotic stress Resistance in Agricultural Crops.

Srikanth Babu, V, Senthil Kumar, M and Dr. M. Udaya Kumar,
Dept. of Crop Physiology, UAS, GKVK, Bangalore-560 065.

Abiotic stresses especially salinity, temperature and moisture stress adversely affect the plant growth and development and are the major constraints to realize the potential yields of our crop plants. Less than 30% of the world's cultivated area is problem free. Whereas the crops grown in 70% of the remaining area experience one or the other abiotic stresses. Unlike the biotic stresses, we have few options to manage the abiotic stresses that are multigenic and quantitative in nature. Amongst the abiotic stresses, drought is the most widespread at the same time complex. Drought directly affects the water use of the crops, which in turn affects the carbon gain and hence affects the productivity drastically in most of the semi-arid tracts. Drought associated with highlight is the major constraint to achieve the potential yield of our crop plants.

Phenomenal progress has been made in recent years in characterizing stress and stress responses. The very concept of drought has been redefined from the agronomic prospective and we look for the crops to yield satisfactorily under water deficit conditions.

It is now evident that cellular response to drought is of complex nature involving simultaneous interplay of several mechanisms. Although, there is a great deal of progress in categorizing biochemical processes that are associated with plant abiotic stress responses, precise understanding of adaptive mechanisms leading to acquisition of stress tolerance remains a challenge. More recently the stress resistance mechanisms are being examined at molecular level. The molecular analysis of stress responses has been carried out at the level of stress proteins, stress genes, stress promoters and signal transduction components involved in mediation of stress response. The functional relevance of diverse stress genes is being tested in different systems ultimately with an aim to identify the relevant genes that are likely to bring in requisite adaptation to achieve higher growth rates under stress. The significance and importance of identifying the novel stress responsive gene from highly tolerant with phenomenal adaptation to extreme conditions has been one of the approaches.

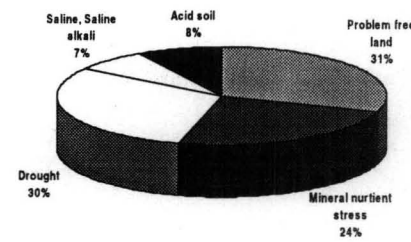
It is now realized that a mere tolerance at cellular level is inadequate for growth unless there is water uptake and it is efficiently utilized. Further, growth without yield potential may not be rewarding. For a target environment, which varies in terms of the nature of stress and its magnitude, the crucial aspect is identifying the choice mechanisms to achieve the desired levels of productivity.

In this presentation an attempt was made to review the diverse plant mechanism to drought, highlighting the molecular mechanisms identified. A few projections have been made to achieve tolerance of our crop plants.

Abiotic Stresses are the Major Constraints to Realize Potential Yields of our Crop Plants

Estimated Losses
Quite Phenomenal

Management options
Are few – Biotic Yes



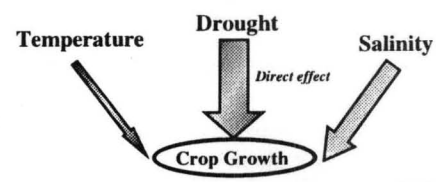
Area under water logging, Heat stress, cold stress are not included

Situation in India

	Mha	%
Humid →	39.7	12.1
Sub humid →	124.0	37.3
Semi Arid →	109.2	32.8
Arid →	49.5	14.7

Rainfed area (1996-97) – 61.3%

99 districts are drought prone
8 in AP, 14 in Karnataka



Water use by Crops Decreases Transpiration ↓; Carbon Gain ↓

Plant Growth is an Interplay of Transpiration and Photosynthesis

Drought & Highlight - *Problem*

Phenomenal Progress has been made !

- **In characterizing stress and stress responses**
- **Redefining the drought resistance and**
- **Drought Resistant crop / genotype**

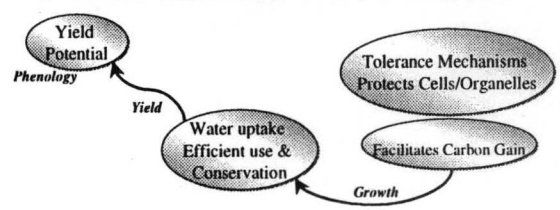
from Agronomic Perspective

Ability of a crop / genotype to yield Satisfactorily under water deficit conditions

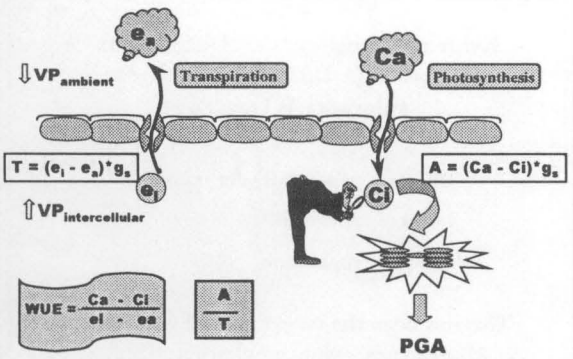
Stress resistant mechanisms are being examined at molecular level

140 reviews – 1999 to 2002

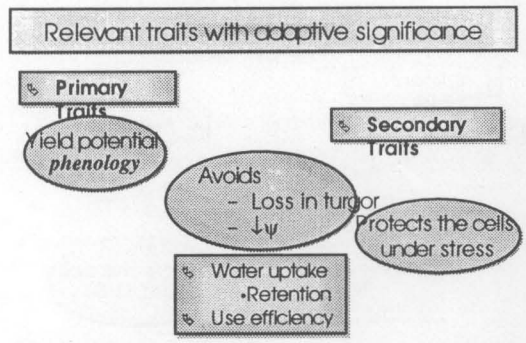
Several drought resistant traits with adaptive significance have been well characterized



- **Cell protection alone is inadequate for growth unless there is water uptake and is efficiently used**
- **Growth without yield potential – not rewarding**



Plant growth depends on the T and the carbon gain / T (WUE)



Exploitation of short duration cultivars for end season stress

Phenology, partitioning, root, WUE, etc. are highly relevant in Crop Improvement

Significant Genetic Variability exists

In fact, water mining, water use efficiency and partitioning determines growth rates and productivity

Yield = T x WUE x HI

(We now have Isotope signatures as potential tools to quantify T and WUE)

Did we exploit these traits?

- ✓ **These are multigene regulated**
- ✓ **Biochemical basis for variability for these traits – not understood**
- ✓ **Still too far to clone the candidate genes regulating these traits**

Stomatal regulation – molecular mechanisms fairly well elucidated

There are only few instances where these aspects are addressed at molecular level

Tobacco transgenics (Cytokinin Oxidase)

Genetic Control

Root hair Development

Well Understood

Molecular markers – has been the thrust

QTL have been identified for

Root Traits

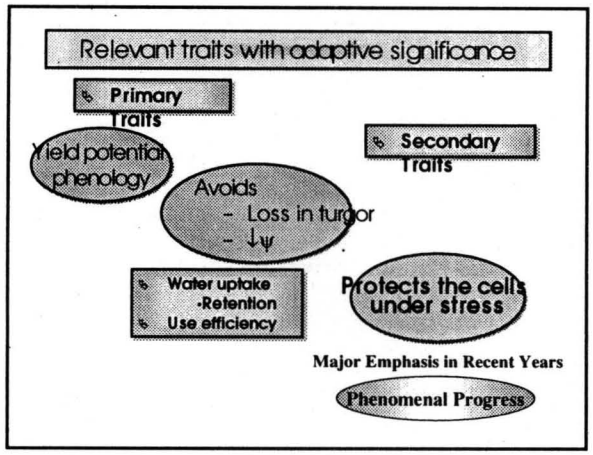
Stomatal Factors

Water Use Efficiency

Osmotic Adjustment

Phenology

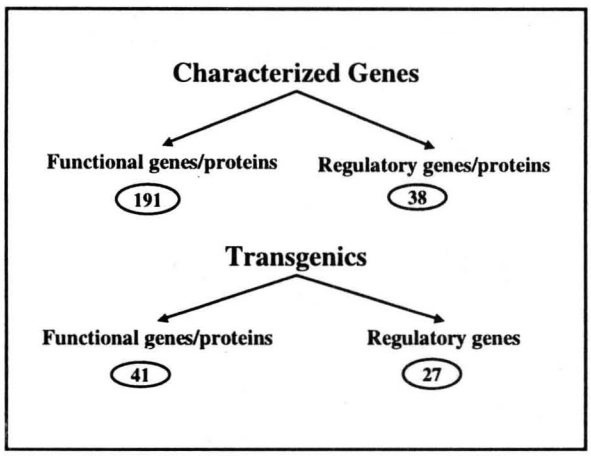
Progress is still slow – phenotyping for these traits is the lacuna

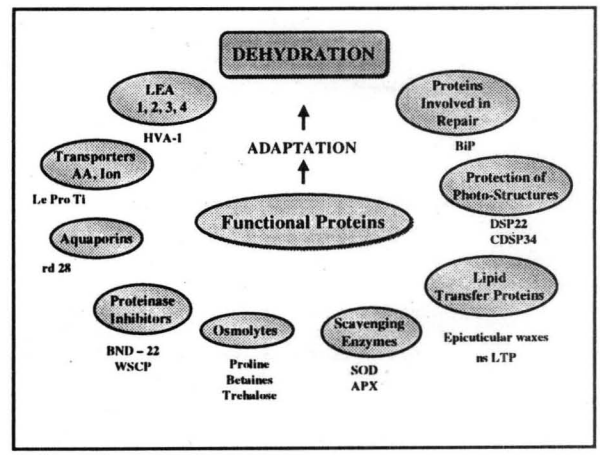


Evidences Suggests that Differential Adaptation or Differences in Stress Tolerance is Due To

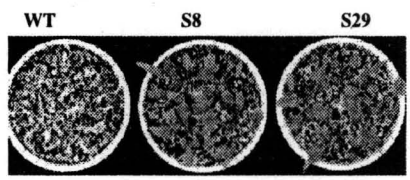
- Different genes that gets expressed
- Level of expression
- and their regulation

This has been the target area of research
Many genes – cloned / characterized





Enhanced drought tolerance of Glos2 transgenics (Trehalose)



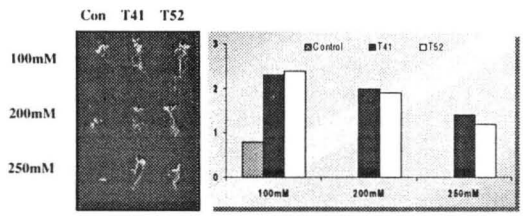
The Plant Journal (2002) 29(4), 417±426

Antisense expression of BIP gene disrupts water stress tolerance

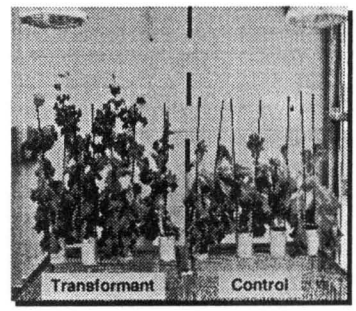
BIP-Sense WT BIP- Antisense

Alvim, et. al., 2002, Pl. Physiol. 126, 1042

Transgenic lines overexpressing Sdi – 8
(LEA – 2)



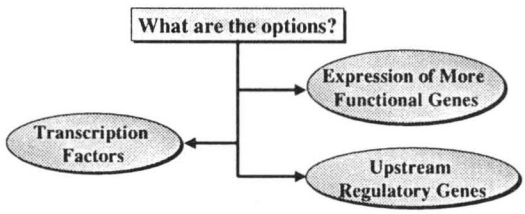
UAS, Bangalore



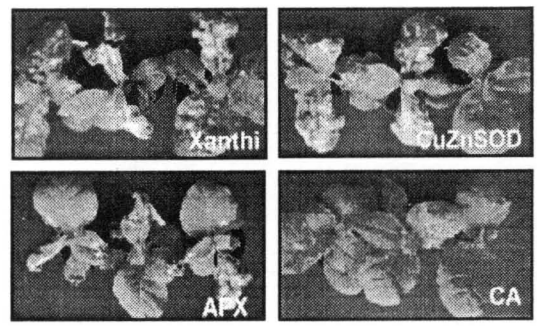
Transgenics with ferritin show increased growth rates

Goto et. al., 2000

Stress adaptation is optimum only when co-coordinated expression of many stress genes occurs



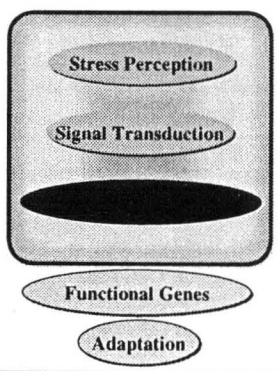
Some clues that a group of stress related genes can be expressed

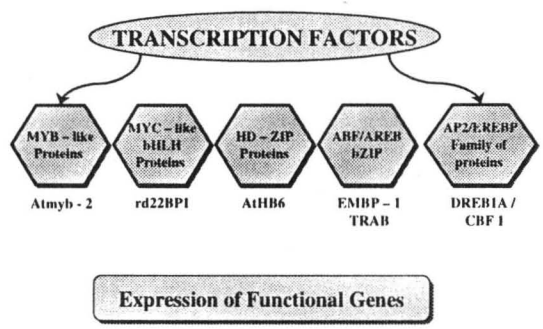


Tobacco plants expressing both SOD and APX showed enhanced tolerance to oxidative stress

Kwon et. al., PCE, 25, 873; 2002

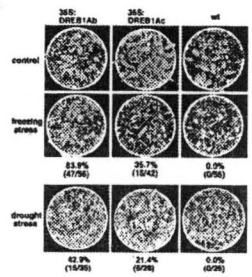
Stress responses leading to adaptation





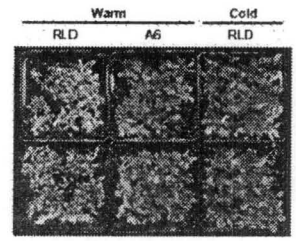
Early response genes – Zhu, 2002

Freezing and drought tolerance of the DREB1Ab and DREB1Ac transgenic plants



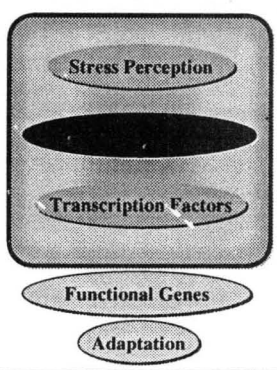
Plant Cell, Vol. 10, 1391-1406, August 1998

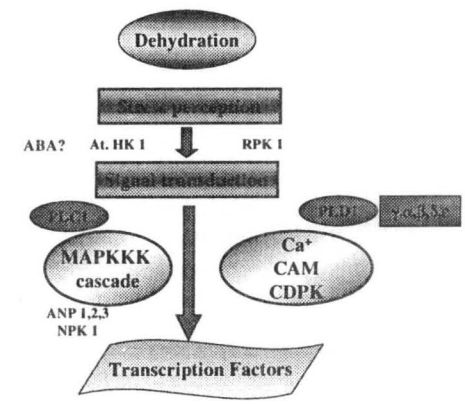
Enhanced freezing tolerance of CBF1 over-expressing plants

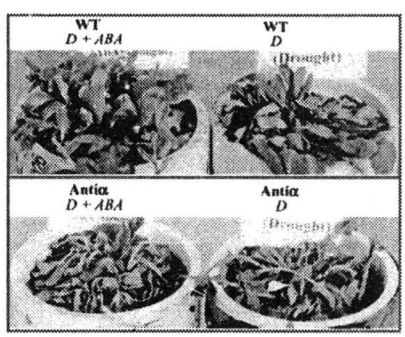


SCIENCE VOL. 280 3 APRIL 1998

Stress responses leading to adaptation

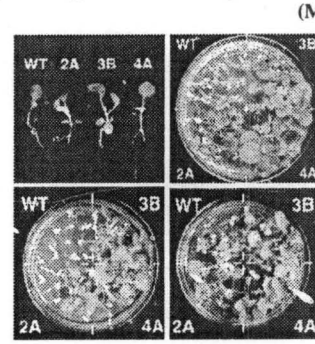






Antisense PLDα plants show more wilting under moisture stress

Stress tolerance of tobacco plants expressing constitutively active NPK1 (MAPKKK)



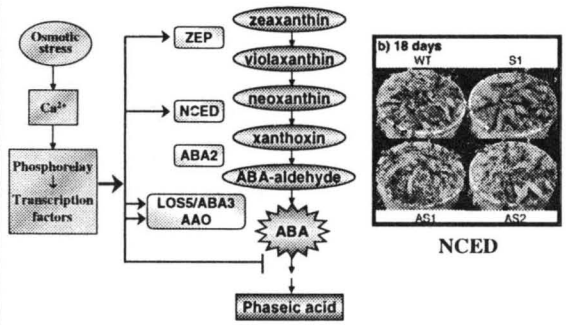
Freezing stress (-10° – 30')

Heat shock (48° – 45')

Salinity (300mM – 3days)

PNAS March 14, 2000 vol. 97 no. 6 P: 2945

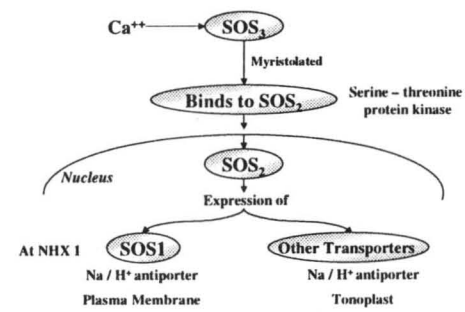
NCED rate limiting step in ABA biosyntheis
Conversion of neoxanthine to xanthoxin



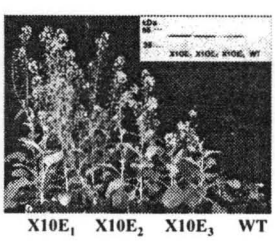
NCED

Upstream Regulatory Elements

SALT

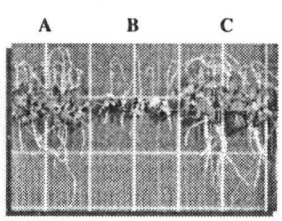


AtNHX1 over-expression in Brassica (Salinity tolerance)



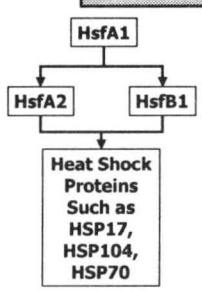
$X10E_1$ $X10E_2$ $X10E_3$ WT

Role of SOS3 in salinity tolerance

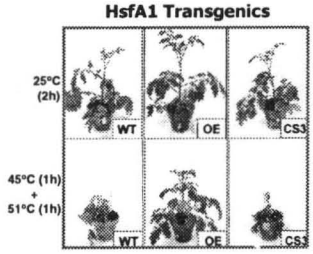


SCIENCE VOL. 280 19 JUNE 1998

HsfA1 – Master Regulator of thermotolerance



HsfA1 Transgenics



OverExpressing Co Suppressed

Genes &Development, Shravankumar Mishra et. al. 2002

Recent Findings
Did Provide LEADS
Not the Solutions

Non - lab performance is still inadequate

We could not increase the threshold levels substantially

WHY ?

This has been our major concern

- Choice of mechanisms needs review
- Difference in threshold levels is marginal within species
- Relevant throughput screens

  Germplasm – Transgenics - Lacking

Choice of Mechanisms

- **Target Environment**
  - Yield Potential / Phenology
  - Water use and its efficiency
  - Light management under stress
  - Chloroplast stability

Light Management Under Stress

- Scavenging Systems
- Osmolytes
- Xanthophyll
- Lipid Transfer Protein
- Chloroplast Stability

Only field trail of cotton transformed with APX

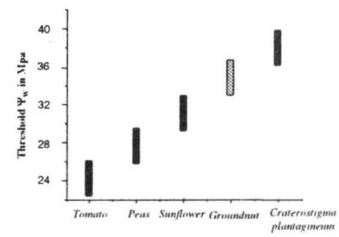
Photochem Quenching		Yield Kgs	
C	APX	C	APX
0.176	0.306	168	280

Yield Potential/ Phenology

Water Use – Water Use Efficiency

- Yield advantage was shown when selected for high Water Use Efficiency
  Richards et al., (Winter Wheat), 2002. Crop Science
- Selection for WUE and T improved productivity
  Groundnut (2001). ICAR – ACIAR PN – 9276
- Selection for WUE (Δ) improved productivity
  Matues et al. (cannola), 1996. Crop Sci.
  Ismail et al. (Cowpea), 1993. Crop Sci.
  Merah et. al. 1996. (Durum wheat). Physiol. Plant.

Differences in threshold is marginal



The choice is to transfer specific genes from highly tolerant species

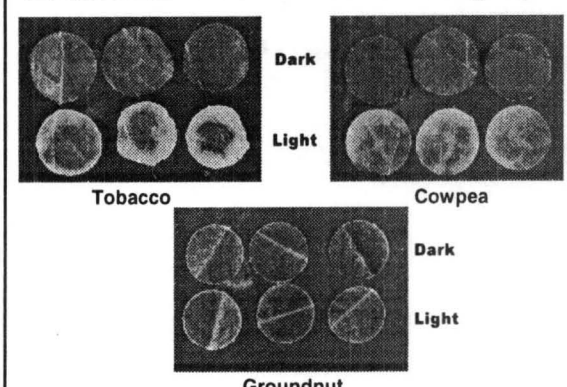
These genes could be functionally efficient or there should be specific genes with different metabolism

Just 6 or 7 years ago, given the then capacity to control transformation, I would have scoffed at the [value of] 'weird and wonderful' genes from resurrection grasses or mosses. says Rockefeller Foundation scientist John O' Toole

'Identification of such genes is a promising next step'

Science_5_2002

MV induced Oxidative Stress Tolerance under High Light



To increase threshold levels

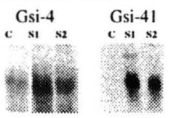
Look for genes from tolerant source

Groundnut Stress Substractive Clones

Twenty three genes were sequenced (92) (85)

Which have homology (17)

Which do not have homology (6)



⇒ Even in *craterostigma* – 40% of stress genes without known homology

⇒ Eleusine corocana another choice species [71]

Throughput screens is the emphasis

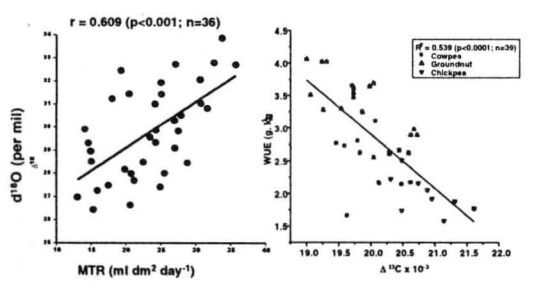
- ✓ Stress induction response technique
- ✓ Methyl viologen / Menadione – screen for oxidative stress
- ✓ Stable isotopes ^{13}C, ^{15}N, ^{18}O

Emerging as a potential surrogate

Transpiration ^{18}O → WUE ^{13}C

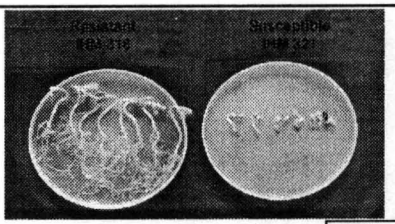
Carbon gain under stress

Chloroplast stability



Mean transpiration and ^{18}O in biomass are related (Rice)

Relationship between Carbon isotope discrimination and WUE in a few grain legumes



Stress protocol
Induction – Lethal - Recovery

TAG, 1999.
J Pl Physiol, 2002

- ✍ Genetic variability in stress response is seen only on pre-exposure to induction stress
- ✍ Directly exposing to lethal stress do not show genetic variability

CONCLUSIONS

There are exciting options to increase tolerance

Success stories are quite encouraging

So far the emphasis has been

- ✓ Tolerance mechanisms at cellular level
- ✓ Factors involving productivity under stress still less amenable for molecular analysis – A concern

The major thrust in years to come

- ✓ Emphasis on choice mechanisms
- ✓ Search for novel genes from adaptive species
- ✓ Cloning and throughput screens for transgenics

THANK YOU

Golden Lecture 15

APPLICATIONS OF BIOTECHNOLOGY IN ENVIRONMENTAL POLLUTION PREVENTION AND CONTROL

Profile:

Name : Dr. Kaggere Shivananjaiah Lokesh

Address :Assistant Professor
Department of Environmental Engg.
S.J. College of Engineering,
Mysore - 570 006

Academic Qualification :

Ph.D. (Env. Engg.) 1996 University of Roorkee, INDIA (Now IIT, Roorkee)
M.Tech. (Env. Engg.) 1987 IIT, Kanpur, INDIA
B.E. (Civil Engg.) 1982 Bangalore University, INDIA

Work Experience :

Teaching : Total : 20 Years
Undergraduate : 20 Years
Postgraduate : 15 Years

Research : 10 Years

Present Position : Assistant Professor,
Department of Environmental Engg.
S.J. College of Engineering, Mysore - 570 006

Publications : 36 (National & International Conferences & Journals)
10 Technical Reports

APPLICATIONS OF BIOTECHNOLOGY IN ENVIRONMENTAL POLLUTION PREVENTION AND CONTROL

Write up:

The ancient scriptures 'vedas' have shown reverence towards the Mother Earth and natural water sources such as rivers, streams, lakes and oceans. If they are pure, pristine and protected, from pollution, then they are sacred and beneficial to the mankind.

Life owes its existence and obtains its subsistence, growth and fulfillment from the environment, a product of complex and dynamic interactions of physical, chemical, biological and social systems. Since, the modern days, a great threat has been imposed on the environment we line in, causing serious deterioration. The environmental stress due to explosive population growth, modern agricultural practices, rapid urbanization and industrialization are well recognized. Some of the major ways in which modern technology has contributed to environmental alteration, deterioration of natural riverine systems and pollution are;

⇒ agricultural practices that have resulted in intensive cultivation of land, drainage of wetlands, irrigation of arid lands and application of herbicides, insecticides and pesticides.

⇒ manufacturing of huge quantities of industrial products that consumes vast amounts of raw materials and produces large quantities of air and water pollutants and toxic / hazardous waste by-products.

Lecture delivered at Golden Lecture Series on "Emerging Trends in Biotechnology" on 7th September 2002, at the Institution of Engineers (India), Bangalore.

⇒ extraction and production of minerals and other raw materials with ***accompanying environmental disruption and pollution.***

⇒ energy production and utilization.

⇒ modern transport and communication practices

Also the hazardous substances often released, adversely affect the entire ecosystem of a receiving body.

All the above are posing a constant threat to the clean environment and depleting natural resources. This is leading to a wide gap between increasing problem of control of environmental pollution and problems related to conservation. Both the problems are receiving constant attention by government and non-governmental agencies. In this direction, efforts are being made to achieve the objective of pollution prevention and conservation through a variety of approaches and Biotechnology is certainly one of them.

Environmental Biotechnology is concerned with the implications and applications of biotechnology in the wider context of environment.

Among implications, there is hue and cry for the genetically engineered organisms release and the effluents from biotechnology labs and industries. Also there is a lot of concern on the usage of biotechnology products bringing in Bio-safety standards.

Among applications, efforts are being made to protect the environment from pollution and to conserve natural resources, using environmental biotechnology aspects.

Towards a cleaner and sustainable environment, the applications of biotechnology have a major role to play. Following are the areas where the environmental biotechnology is effectively used to control pollution and to reduce damage to the environment.

- Industrial Effluents Treatment using Cleaner Technologies
- Toxic and Hazardous Waste Treatment
- Contaminated Site Remediation
- Oil Spills Treatment
- Grease Deposits Treatment
- Pests Control (Biopesticides)
- Land restoration and soil fertility improvement

Biotechnology is a promising area to:

- To produce bioplastics or green plastics which are of biodegradable in nature.
- To produce biofertilizers to be used in organic forming.
- To develop renewable sources of energy
- To produce biofuels as an alternative to conventional fuels to reduce significantly the serious problems of air pollution
- To conserve biodiversity to sustain the future environment.

The complex relationship and its major impact on human life, it is believed that biotechnology may become a vital force for human existence. The products of biotechnology including medical diagnosis, prevention and cure of diseases, new and cheaper products, new food sources, devices for environment protection and energy conservation are playing a very important role in employment, production, trade, economics and quality of life (QoL). Since, the scope of Environmental Biotechnology is little known to potential users as well as authorities, efforts must be made in this direction to popularise and use it effectively and efficiently in pollution prevention and control with adequate safety measures and standards.

Golden Lecture 16

IT PERSPECTIVE OF BIOINFORMATICS : INFORMATICS FOR STUDYING BIOMOLECULAR STRUCTURES

Profile:

Name : Dr. Ramanathan Sowdhamini

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Academic Qualification :B.Sc in Chemistry, *University of Madras*, India.
M.Sc in Chemistry at the *Indian Institute of Technology, Madras*, India.
Ph.D in Molecular Biophysics Unit, *Indian Institute of Science*, Bangalore.
Thesis: Motifs in proteins: Disulfide constraints and their applications to Protein engineering and peptide modeling

Work Experience : Worked for two years on the solution phase peptide synthesis, NMR and CD spectroscopic studies of peptides. Gained experience for over three years in protein and peptide modelling and structure analysis; worked for over nine years in protein sequence analysis, studying protein folds and structure prediction. Also learnt protein crystallisation and expression.

Present Position : Sr. Scientist, NCBS

IT PERSPECTIVE OF BIOINFORMATICS : INFORMATICS FOR STUDYING BIOMOLECULAR STRUCTURES

Write up:

Biomolecules play important roles in various cellular processes like metabolism, energetics, feedback controls, signaling and differentiation. Systematic studies of the physico-chemical properties of biomolecules can permit the analysis and prediction of such regulated events.

DNA, the basic material containing information in a biological cell, contains carbon, nitrogen, oxygen, phosphorus and hydrogen. The building blocks of proteins are 20 different amino acids that are themselves made up of carbon, nitrogen, oxygen, sulphur and hydrogen. Free amino acids contain an amino group, a carboxyl group, a side chain and a hydrogen attached to the C-alpha atom. The 20 amino acids differ from each other in their side chains. Adjacent amino acids in a protein are joined together by a peptide bond and hence proteins can be termed as polypeptides. Usually, a globular protein adopts the physiological or native conformation in a spontaneous manner; however, such structures are only marginally stable compared to the unfolded form of the protein. The structural information of proteins, both at the secondary and tertiary level, are determined by a multitude of factors like steric, hydrophobic, electrostatic and hydrogen bonding interactions. Preferred backbone torsion angles occurring at consecutive residues in the polypeptide can give rise to stabilizing units called secondary structures, that are either a-helix, b-strand or chain reversing units. Several combinations of dihedral angles are not permitted due to the possibility of short contacts of non-bonded atoms that is energetically highly unfavourable. The tertiary structure (or the three-dimensional structure or fold) of a protein is determined by the order in which the 20 amino acids are present in the sequence of a protein. The central dogma of the 'protein folding problem' relates to the fold prediction of proteins given only their amino acid sequence.

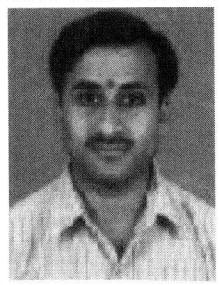
Computational attempts to predict protein structures are usually knowledge-based approaches. Databases are integral components of knowledge-based structure prediction and care must be taken for the creation, storage, maintenance and update of such databases. Since biological data, that record sequence or structural or functional information, are voluminous, it is advantageous to automate most of these steps. Sub-derived databases are queried using specific search procedures that allow users to query a sequence for possible homologues or search for patterns. Despite the availability of several secondary structure prediction and fold recognition methods, there is a growing need for developing more robust algorithms.

Proteins within the cell are constantly interacting with small molecules and other proteins. Protein-ligand interactions are usually high affinity interactions; protein-protein interactions, on the other hand, involve transcient and low-affinity binding that are still very specific. Docking is an informatics technique that examines the feasibility of such interactions by measuring shape-charge complimentarity and calculation of energies. They are quite powerful in suggesting novel pairs and modes of interacting partners and to perform 'virtual screening' of small molecules to act as drug targets.

Golden Lecture 17

STRUCTURAL BIOLOGY & BIOINFORMATICS

Profile:



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Academic Qualification : B.Sc. (Physics, Electronics, Mathematics) 1987
M.Sc. (Physics) 1990
Ph.D. (Molecular Biophysics Unit Crystallography)

Work Experience :

Lecturer, Department of Physics	June 1990 - Nov1990	BMS College
Lecturer, Department of Physics	July 1998 - Oct 1998	SBM Jain College
Lecturer, Department of Physics	Nov 1998 - till date	Sir MVIT

Present Position : Assc. Professor, Sir MVIT

Publications : Published 7 papers

STRUCTURAL BIOLOGY & BIOINFORMATICS

Write up:

INTRODUCTION

Structural Biology is defined as research on the structure and function of biological molecules, biomolecular assemblies and complexes using physico-chemical, molecular biological and computational methods. Structural Biology now underpins almost every aspect of cell biology and is revolutionizing approaches to many broader biological questions. Increasing numbers of biological researchers are integrating structural analysis as one component of their scientific approach. However, such analyses require access to both a range of expensive equipment and considerable expertise across a large number of methods. Structural Biology also underpins many of the industrial and Technology Foresight priorities. It is central in many areas of research and development in the pharmaceutical sector – and will play an increasing role in the food, agricultural and other biotechnology industries.

Bioinformatics, on the other hand, can be described as the science of collecting, storing, searching, annotating, modeling, and analyzing biological information. It involves a range of activities from data handling, publication, to data mining and analysis. An essential part of bioinformatics is to create new algorithms for the analysis of complex and/or large data sets.

Bioinformatics, now an integral component of Biotechnology, deals with the issues created by the massive amounts of new types of data obtained through novel biological experiments. The most well-known example is, of course, the determination of the complete nucleotide sequence of the human genome, which has to a large extent been accomplished, although not yet finished. The basic data has so far usually been the sequence information (nucleotide or protein), but other types of data (microarray, functional analysis, interactions) are now rapidly coming into focus.

The feature of Structural Biology that deals with the elucidation of atomic details of biological molecules has indeed proved to be a starting point for much bioinformatics work especially related to the flourishing pharmaceutical industry as, the information from structural biology act as a knowledge database and prime input for problems related to molecular modeling and structure based drug design. The character of biology that is thoroughly exploited in most bioinformatics work is of course the strong correlation that exists between the structural conformation of a biomolecule and its associated specific function. The 1D/2D/3D descriptions of biomolecule provided by the various techniques adopted in structural biology will necessarily help in detailed understanding of several facets of biological problems such as biomolecular functions, deducing rules that govern protein folding, inferring patterns of molecular evolution, and engineering novel molecules which could become potential drugs.

While Structural Biology helps in elucidation of the various interactions between molecules such as nucleic acids (DNA/RNA), proteins, enzymes, hormones, membranes, vitamins, carbohydrates, lipids, viruses, metal ions and many others small molecules, Bioinformatics approaches integrates the knowledge database necessary for large scale analyses. The structural description and nature of various interactions that can be deciphered by a number of biophysical techniques like X-ray/Neutron Crystallography, Nuclear Magnetic Resonance (NMR), Electron Spin Resonance (EPR/ESR), IR/Raman/Laser Raman, Electron Micrographs, Circular Dichroism / UV / Fluorescence spectroscopy etc. help in generating a good knowledge database to aid bioinformatics research. Bioinformatics, though a rather a young discipline that bridges the fields of life science and computer science, reiterates the need for such a interdisciplinary approach to handling biological knowledge, as it underscores the radical changes in quantitative and qualitative terms that the biosciences have seen in the last two decades. We realize today,

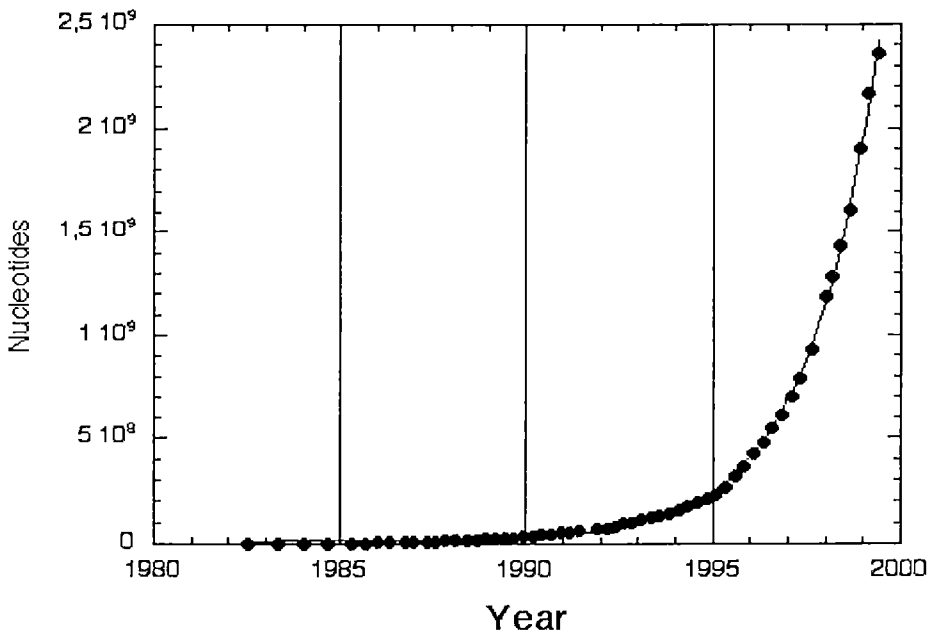
- That the knowledge database of biology has exploded in such a way, that we need powerful tools to organize that knowledge itself and

- That the questions we ask of biological systems today may be so complex, that we cannot hope for answers if we limit ourselves to the capabilities of our natural brains.

Thus, the two roles of current activities in bioinformatics are mainly in the organization and the analysis of biological data. Bioinformatics, though commonly is perceived as the handling of sequence data in large databases, activities such as algorithm development, maintain and accession of the sequence databases are among the most important contributions that bioinformatics has made for the life sciences. But there is important information other than biological sequences available, like citation databases, and in the end it takes a lot more than collecting and storing information to progress from data to knowledge.

Nonetheless, the explosive growth of biological sequence information has underscored the vital importance of integrating the computer sciences and life sciences. Sequencing of biomolecules began with the insulin B-chain - a thirty-residue peptide - which Saenger and Tuppy deduced through a combination of limited proteolysis and chemical analysis in 1951. It was a full 14 years later, until Holley et al. determined the sequence of alanine tRNA from yeast. And it took another 12 years, until "real" DNA sequencing was developed by Maxam & Gilbert and Saenger *et al* in 1977. Today we are sequencing tens of millions of bases a year and undertaking to sequence whole organism genomes. The growth of the sequence databases is an exponential function. The size of the EMBL nucleotide database is about a staggering 2.5 billion bases (refer to the figure below).

There is an interesting aside to this: the question of whether we may rapidly be approaching a limit to how much significant biological information we can turn into knowledge. It is becoming apparent, that the interplay of signals and pathways in complex biological systems - such as the regulation of transcription, cellular activation, developmental organization or cellular communication - may be so complex and sensitive to slight variations of interaction energies, that it may be impossible *in principle* for our human brains to understand such phenomena quantitatively. As ever more biological phenomena are being described, our concepts of biological knowledge and understanding will change, and we will need to recruit ever more computer tools to organize such knowledge and to extract and present the relevant information to us in a comprehensible way. The development of concepts and models that integrate such complex knowledge and allow its visualization to make it accessible is the grand future challenge of bioinformatics.



Growth of the EMBL nucleotide database

The three levels of bioinformatics are :

Analysis of a single gene (protein) sequence. For example:

- Similarity with other known genes
- Phylogenetic trees; evolutionary relationships
- Identification of well-defined domains in the sequence
- Sequence features (physical properties, binding sites, modification sites...)
- Prediction of subcellular localization
- Prediction of secondary and tertiary structure

Analysis of complete genomes. For example:

- Which gene families are present, which missing?
- Location of genes on the chromosomes, correlation with function or evolution
- Expansion/duplication of gene families
- Presence or absence of biochemical pathways
- Identification of "missing" enzymes
- Large-scale events in the evolution of organisms

Analysis of genes and genomes with respect to functional data. For example:

- Expression analysis; microarray data; mRNA conc. measurements
- Proteomics; protein conc. measurements, covalent modifications
- Comparison and analysis of biochemical pathways
- Deletion or mutant genotypes vs. phenotypes
- Identification of essential genes, or genes involved in specific processes

Technical issues associated

Collecting, storing, searching and using biological information entails a number of technical problems, ranging from the trivial but important (file formats, database interactions) to the sophisticated (algorithm design, data modeling, anthologies).
For example:

- Algorithms for analysis: properties, implementation
- Software libraries implementing analysis or access methods
- Data modeling: optimal ways to represent heterogeneous data
- Object-oriented vs. relational databases
- Database technologies, implementations
- Centralized database systems, or distributed data networks?
- Update policies, data tracking (who modifies what)
- Sharing of software: languages, licenses, machine independence

Legal issues associated

Bioinformatics databases, algorithms and software have wildly different levels of copyright, occasionally patents, and license conditions attached to them. This sometimes causes considerable problems for academic research. For example, a novel data-modeling scheme may use data from several sources and combine them into an entire novel framework. This new database becomes difficult to distribute, since it builds on other databases. Of course, such legal protection may also be very important for the academic researcher.

A current trend is to distribute software (and also some databases) under some variant of the GNU General Public License (http://www.gnu.org/). Software patents in bioinformatics are not very common, so far. The large sequence databases and a few others are also publicly accessible and redistributable. But most other databases have conditions that make development and subsequent redistribution problematic.

Commercial uses of bioinformatics

Bioinformatics is of direct relevance for the pharmaceutical and agricultural industries. It is necessary for analysis of the experimental data. It can suggest hypotheses to be tested by experiment. There is a very close connection between basic bioinformatics science and its technological applications.

Most large pharmaceutical companies do not have any large groups doing original science in bioinformatics. They usually have enough expertise to use and apply academic software and commercial products, but not much more. Most bioinformatics efforts in industry are directed at finding interesting genes, or helping experimental scientists with handling and analysis of their data.

The relationship between academia and companies are basically good. There is much collaboration and communication. However, the very fast progress and movement of the field makes some kinds of long-term collaborations, such as PhD projects, difficult. A company (especially a small company) is reluctant to commit to a four-year project; much can happen during that time.

It must be noted that different parts of industry have different interests: Some companies sell information (databases), algorithms or analysis tools, while other companies use these tools in pharmaceutical and agricultural research. For example, the large pharmaceutical companies have no strong interest in software patents, while a small bioinformatics company may build its entire business case on proprietary software.

Challenges ahead

An important issue that will probably be with us for a long time is the integration of the large (and increasing) number of biological databases. Although some technology developments will help (XML), the fragmentation is more fundamental than that. It is due to different ways of viewing the data, and this is a genuine scientific problem.

The complete genomes for several important organisms are basically in hand, and we know (in principle) how to deal with data of this type. Of course, many improvements must be made.

Much current work focuses on how to interpret micro-array expression data. Which genes are active or inactive during different states of the cell? How is the regulation carried out. There are fundamental research issues here, as well as obvious direct applications in diagnosis.

However, expression experiments on their own do not unveil the mechanisms underlying the various processes in the cell. New approaches, both experimental and theoretical/algorithmic, must be developed. This is where structural biology will contribute continuously.

There are many signs that bioinformatics will focus on the issues of how to describe and analyze the fundamental life processes in cells and organisms. Understanding the function of genes and proteins cannot be achieved without understanding the systems in which they work. This is needed for the fundamental understanding of living systems, as well as for successful technological application of the new biology in the agricultural and pharmaceutical industry.

This is indeed the fundamental challenges of structural biology and bioinformatics: How do we describe, analyze, simulate and predict the dynamics of life processes?

Golden Lecture 18

BIOINFORMATICS- AN ENABLING TOOL

Profile:

Name : G.RAMAMOHAN

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Academic Qualification : B.Sc (Horticulture)
M.Sc (Horticulture) from UAS
Master (Agriculture) from Purdue University, USA.
Ph.D (Biochemistry) from University of Mysore, Mysore.

Work Experience : Specializing in Plant Cell Physiology and Molecular Biology
Worked on the molecular aspects of fruit ripening in tomato
LEA proteins- a group of stress responsive proteins and their regulation and relevance in stress tolerance
Worked in Indo-American Hybrid Seeds in different capacity
Worked as Research associate in the Dept of Crop Physiology at UAS, Bangalore

Awards : Awarded University Merit Scholarship during B.Sc (Hort)
Awarded ICAR Junior Fellowship during M.Sc (Hort)
Awarded Research Assistantship in the Department of Horticulture, Purdue University, West Lafayette, USA

Present Position : Working as senior scientist at Monsanto Research Centre, Bangalore.

Publications : Published 4 Research Papers

BIOINFORMATICS - AN ENABLING TOOL

Write up:

Advances in the fields of Genetics, Microbiology, Biochemistry and Molecular biology and improvised techniques have led to the generation of vast amount of literature information on genes/proteins and their regulation of different processes and pathways. In addition, in the last two decades owing to the spectacular progress in the Genomics area, the genetic code of more than 25 organisms have been deciphered generating enormous sequence information. Under this challenging circumstances bioinformatics can be an enabling tool to decipher useful knowledge out of the enormous amount of information. Bioinformatics can be visualized as a set of tools to collect, analyze, evaluate, organize and share information on sequence, structure and function of biological molecules. Bioinformatics has evolved to serve as a bridge between the observations (data) in diverse, but biologically related disciplines and understanding how the systems or processes function.

Bioinformatics involves the creation of computer databases on genome and protein sequences; analysis of the genomic data & protein data in these databases by using algorithms; assigning functions to the genes and proteins in these databases for the purpose of accelerating biological research. Bioinformatics allows us to catalogue the information and identifies matching pattern from different branches such as - Structural genomics (including mapping, sequencing, gene finding), Functional genomics, Proteomics, Expression profiling and Metabolic profiling. A holistic approach would attempt at the deduction and understanding of the function of each gene or groups of genes though comparative studies of large data sets.

Many biological databases are available in the public domain, which can be made use for literature searching, nucleotide searching / protein sequence searching, which includes finding homologs, finding patterns-domain & motifs, phylogenetic analysis etc. In addition these tools can be used to predict the functions of unknown proteins, derive structures, manipulate structures based on the domain analysis. Some of the useful sites for researchers are given below:

http://www.ncbi.nlm.nih.gov/

http://www.ddbj.nig.ac.jp/

http://www.embl-heidelberg.de/

http://www.tigr.org/

http://www.genome.ad.jp/dbget/dbget.links.html

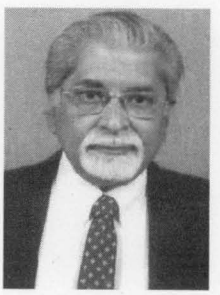
http://www/rcsb.org/pdb/

In conclusion Bioinformatics is a set of tools which can be used successfully to decipher knowledge out of enormous amount of data and this has created a platform for an inter-disciplinary collaboration between molecular biologists, physiologists, biochemists, molecular modelers, drug chemists, healthcare providers and computer scientists.

Golden Lecture 19

THE PLACE OF BIOINFORMATICS IN BIOTECHNOLOGY EDUCATION

Profile:



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Academic Qualification :B.Sc, (Hons.)
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Ph.D., from the Andhra University, Waltair

Work Experience :Started teaching career in 1965 as Lecturer in Botany, Andhra University. Served the Bangalore University from June 1967 to April 1998. Was a Professor of Botany, Chairman, Department of Botany, and Chairman, Department of Sericulture.He was partly or wholly responsible for the introduction of PG courses in Applied
Botany, Environmental science, Microbiology and Biotechnology in the University. Was a Honorary Professor at the Postgraduate Department of Applied Botany,
SSMRV College, Bangalore, for three years.

Awards :Awarded honorary D.Sc., by the Open International University for Alternative Medicine, Colombo
Certificate of Merit from the Lama Gangchen World Peace Foundation affiliated to the UN

Present Position :He is the Executive Secretary, Foundation for Biotechnology Awareness and Education, a non-profit organisation that works to spread biotechnology awareness and to promote sustainable development through safe biotechnologies

Publications :He compiled a database of medicinal plants and studied lectins and saponins in them, for over 15 years. His book 'Database of Medicinal Plants' was published by the Government of Karnataka and distributed free of cost. Now the book is placed with a lot of additional material and photographs of 90 medicinal plant species, on the internet in the public domain, at www.indmedplants-kr.org

THE PLACE OF BIOINFORMATICS IN BIOTECHNOLOGY EDUCATION

Write up:

1. INTRODUCTION

Biotechnology is the buzzword of the current times. If biotechnology is hot, bioinformatics is its hottest arm, shrouded in a lot of hype, with conveniently concealed ground realities. It is necessary that bioinformatics is viewed in the proper perspective in order to reap the rich benefits that accrue out of this area. In fact, serious efforts should be made to place even biotechnology in a rational perspective. Awareness is the key to a successful deployment of both bioinformatics and biotechnology, in enhancing the well being of people, animals and the environment. This effort should essentially begin with biotechnology education.

The term 'bioinformatics' is the short form of '*bio*logical *informatics*', just as biotechnology is the short form of '*bio*logical *technology*'. There are several definitions of bioinformatics, as there are for biotechnology, often depending upon 'whom, are you taking to?' Anthony Kerlavage, of the Celera Genomics, defined bioinformatics as 'any application of computation to the field of biology, including data management, algorithm development, and data mining'. Clearly, a number of divergent areas, many of them outside biotechnology, come under bioinformatics.

The concept of a computer database came into practice by 1948, through US Defence initiative. A database is meant to store voluminous information in an orderly fashion, to facilitate addition and/or deletion of information and to provide for its retrieval in any one or more of several different permutations and combinations as desired by the user. Biologists have taken advantage of this facility from the very early stages, and used it in different contexts. What is considered as bioinformatics today, by general consent (or silence), is actually a much later development, from the concept of the database.

2. WHAT IS BIOINFORMATICS?

Bioinformatics has emerged out of the inputs of specialists from several different areas such as biology, biochemistry, biophysics, molecular biology, biostatistics and computer science. Specially designed algorithms and organised computer databases are at the core of all bioinformatic operations. Algorithms, that are necessarily complex, make voluminous data easy to handle for defined purposes, in an amazingly short time, a process that is humanly impossible. The requirements of such an activity make heavy and high level demands on both the hardware and the software capabilities of computers.

With several divergent claimants, it is rather difficult to decide which areas of knowledge and information genuinely constitute bioinformatics. It may be helpful to identify areas that are not normally considered as bioinformatics, as for example,

a) structure determination by crystallography and NMR,
b) ecological modelling of populations of organisms,
c) genome sequencing methods (genetic mapping),
d) radiological image processing (human structure scans),
e) artificial life simulation such as artificial immunology and life security,
f) organism phylogenies based on non-molecular data,
g) computerised diagnosis based on genetic analysis (pedigrees), and

a few others, though all these constitute computer processing of biological data.

By convention, which no one explains why so, only genomics (study of the total molecular sequencing of one set of all genes of an organism) and proteomics (amino acid sequences and the three dimensional structure related to function of proteins) constitute bioinformatics. Thus, bioinformatics is concerned with compounds of high molecular weight (HMW), particularly the nucleic acids and proteins. In recent times, cheminformatics (study of low molecular weight, LMW, compounds), glycomics (study of carbohydrates), metabolomics (study of metabolic

pathways in organisms) and drug design through bioinformatics, are also being projected as legitimate areas of bioinformatics.

3. THE GREAT HIJACK

Organisms can be interpreted as variously ordained and organised packages of chemical compounds. The biological processes, susceptibility or resistance to pests and diseases and all other aspects of life, are all interactions of chemical compounds. Big or small, all biological molecules (biomolecules) have biological activity (bioactivity), either promontory or inhibitory, be it nutritional, enzymatic or therapeutic, in the organisms in which they occur and/or on other organisms. The biology of all molecules of biological origin should constitute molecular biology. However, the platform was hijacked a long time ago, for molecular biology to mean only the study of the chemistry and biology of the nucleic acids. Because of the importance of particularly the enzymes, proteins also came to be regarded as a legitimate area of molecular biology. The second hijack, that regards only genomics and proteomics as bioinformatics, was much more imperceptible.

Biomolecules, other than nucleic acids and proteins, are equally complex and important in the organisms' metabolism, have much wider applications and they had a much longer history. For example, it took 150 years to synthesise the stereo-conformational molecule of quinine, a LMW plant product, which has been the most important drug in the control of malaria for over one and a half centuries. Early attempts to synthesise quinine have opened up a whole new industry, that of the manufacture of synthetic dyes.

A lot of the bias in favour of nucleic acids and proteins arises out of the distinction of micro- (LMW) and macro- (HMW) compounds. The distinction, usually based on the MW of a compound, is quite arbitrarily placed at 10 kDa (kilo Daltons). Several polysaccharides have MWs of well over 10 kDa (galactomannan 310 kDa), while some proteins have LMWs, as for example the lectin (a protein) of the stinging nettle is 8.5 kDa and the melanin controlling peptide hormone is only 2.39 kDa.

In certain situations, the distinction between peptides and proteins also seems to be ambiguous. A structure with less than a dozen amino acids is usually considered as a peptide. Peptides too have a tertiary structure. However, structures with even 40 amino acids are sometimes regarded as (poly)peptides. Generally most proteins have MWs between 30 kDa and 120 kDa, though some like the cytochrome P can be over 900 kDa. Cytochrome C, the metabolic enzyme, is only 12.9 kDa. Insulin, with two chains of 5.8 kDa each, is often considered as a peptide.

4. GENOMICS

Genomics is an important area of modern biology, where the nucleotide sequences of all the chromosomes of an organism are mapped and thereby the location of different genes and their sequences are determined. Genomics involves extensive analysis of nucleic acids through molecular biological techniques, before the data are ready for processing by computers.

Entire genomes of several organisms such as *Escherichia coli,* yeast, the malarial parasite, *Arabidopsis thaliana*, etc., have now been unravelled. The most significant recent advancement in modern biology is the mapping of the entire genomes of man and the rice plant.

Estimating the number of genes in an organism basing on the number of nucleotide base pairs was not reliable, due to the presence of high numbers of redundant copies of many genes. Genomics has corrected this situation. It is now known that a human being has about 30,000 genes and not 1,00,000, as estimated earlier. The rice plant contains about 50,000 genes, many thousands more than in the human being. It is also clear that several thousands of genes are common to different organisms, irrespective of their taxonomic closeness or otherwise. Information derived from genome analysis not only tells us on which chromosome specific genes reside but also helps in determining their function. Such knowledge is necessary to improve the economic potential of organisms, reduce susceptibility to parasites and diseases, transfer genes from one organism to a totally unrelated organism to produce improved varieties, etc. Useful genes can be selected from a gene library thus constructed and inserted into other organisms for improvement or harmful genes can be silenced. Genomics is an area with

full of promise to greatly enhance the well being of humans, animals and the environment, in so many different ways.

4a. Structural Genomics is the area concerned with the identification of genes, their location, their nucleotide sequences and associated features, for which examples are already given. Many more of such studies to unravel entire genomes, of important crop plants, pathogens and others, are underway.

4b. Functional Genomics aims at determining the function of different genes. The insertion of the crystal protein genes for pest resistance from *Bacillus thuringiensis* in the genomes of several crop plants was the outcome of functional genomics. Very concerted efforts are on in understanding the function of human genes, genes of the rice plant and other organisms. Functional genomics also help us to identify genes responsible for the production of specific antibodies and to produce vaccines for mass inoculation purposes. It is now possible to identify the genes responsible for pathogenesis in the genomes of parasites and to produce DNA vaccines basing on this information.

4c. Nutritional Genomics, a rapidly emerging area, is the study and manipulation of genes responsible for the synthesis of nutritionally important enzymes or other molecules, often involving entire biosynthetic pathways. This will pave way for insertion of these genes into crop plants to enrich them in special ways. The first example of such a biofortified crop plant is Golden Rice, where the biosynthetic machinery for β-carotene (pro-vitamin A) is introduced into the rice genome to express in the rice grain, a feature that was not present there earlier. The genomes of the gene donors for Golden Rice, daffodil *(Narcissus pseudonarcissus)* and the bacterium *Erwinia uredovora,* have not been worked out. Nor the genome of the rice plant was available till the first successful product was generated.

5. PROTEOMICS

Proteomics involves the sequencing of amino acids in a protein, determining its three-dimensional structure and relating it to the function of the protein. Before computer processing comes into the picture, extensive data, particularly through crystallography and NMR, are required for this kind of a study. With such data on known proteins, the structure and its relationship to function of newly discovered proteins can be understood in a very short time. In such areas, bioinformatics has an enormous analytical and predictive potential. Metabolic proteins such as haemoglobin and insulin have been subjected to intensive proteomic investigation.

6. CHEMINFORMATICS AND DRUG DESIGN

Drug design through bioinformatics is one of the most actively pursued areas of research. Since a great majority of drugs are LMW compounds and since many of them are primarily derived from biological sources, there has always been a great interest in the study of LMW compounds of biological origin. Cheminformatics deals with such compounds, the products of secondary metabolism, often called natural products. Over one million products of secondary metabolism are known. The physico-chemical properties and chemical structures for over 100,000 natural products are available in different databases. For most of them, the biological role in the organisms in which they are synthesised is not known, but they have some kind of bioactivity against others. This bioactivity can be turned to advantage for therapeutic purposes. Here the expertise of a pharmacologist is required.

Several therapeutically active compounds are synthetic. Over a period of time, synthetic organic chemists have realised that it is no longer easy or possible, to continuously conceptualise new structures. The alternative is to use natural products with a desired and known activity and to use them directly or to structurally modify them for improved performance and lower levels of side effects. In this context, the natural products are of great importance to the field of drug design.

Whether synthetic or structurally modified natural products, drug development is a time consuming and expensive process. It would take any thing like 10 to 15 years and 100 to 150 million US dollars to develop a successful drug. At the end of this effort there is no guarantee that the drug would be as important as when it was conceived and/or that the market forces would accept it. It is now possible, through computer algorithm based bioinformatic procedures, to identify and structurally modify a natural product, to design a drug with the desired properties and to assess its therapeutic effects, theoretically. Such procedures, similar to an architect's on board plan before

construction, are described as *in silico* (in the computer, based on silicon chip technology), as opposed to the earlier *in vitro* (in experimental models) and *in vivo* (in clinical trials) methods. *In silico* procedures take a surprisingly short time, and provide the drug designers all the information they need before actually synthesising the drug.

Cheminformatics involves organisation of chemical data in a logical form to facilitate the process of understanding chemical properties, their relationship to structures and making inferences. Chemical structures are the input to identify similar compounds for screening for biological activity. It also helps to assess the properties of new compounds, by comparison with the known compounds.

The risk involved in the earlier random processes of drug discovery methods is largely removed by bioinformatics.

7. GLYCOMICS

Glycobiology is the study of carbohydrates of biological origin. Monosaccharides, the building blocks of complex polysaccharides, are LMW compounds like the nucleotides and amino acids. Polysaccharides are HMW compounds like nucleic acids and the proteins. There are iso- and heteropolymers of carbohydrates. Polysaccharides are involved in such biological functions as storage products (starch, glycans, arabans, galactans, mannans), structural components (cellulose, hemicellulose, pectin, chitin) and functional compounds (metabolic and nutritional). The structures of the monosaccharides, their number and sequences in polysaccharides, are all genetically determined, as for nucleic acids and proteins. While four nucleotides offer only 64 triplet codes, the carbohydrates offer 34,625 combinations. With ever continuously discovered numerous biological roles of carbohydrates, glycobiology is a rapidly expanding are of biological research. Glycomics, the application of bioinformatic procedures to carbohydrates research, is the future field of bioinformatics.

8. MOLECULAR PHYLOGENIES

Phylogeny is the origin and evolution of organisms. With an estimated four million organisms, though not even a quarter of them are currently known to science, it is necessary that they are properly classified and named. It will be of great advantage to understand the genetic and evolutionary relationships of organisms, in order to use them in a profitable manner, in biotechnology and elsewhere. Biologists have constructed very elegant systems of classifications for the known organisms, though problems persist. All this commendable work, with over three centuries of history, was done using externally visible, structural, chemical or functional attributes of organisms. This constitutes the field of taxonomy, which is called systematics when the theory of organic evolution is applied to it.

With the advancements in molecular biology, biologists have used data from the genetic material to characterise organisms and to verify their classification and relationships, inferred on the basis of other evidence. Since it is impractical to use entire genomes for this purpose, nucleotide sequences of genes in the genomes from the mitochondria and chloroplasts are used. These nucleotide sequences are compared using complex computer software. Extensive work was carried out this way, comparing a very large number of organisms of plants and animals. A number of systematists would be benefited if bioinformatists provide them with computer-based services to analyse their systematic data.

Amino acid sequences and charecteristics of proteins are also used in systematics. The metabolic protein enzyme cytochrome C, with 100 to 112 amino acids and a MW of about 12.59 kDa, was used to unravel phylogenetic relationships of a wide range of organisms. The protein is identified basing on its function, which is a certain guide of its nature and then the sequence comparisons are made.

Study of amino acid sequences of insulin, the peptide/protein hormone, which is involved in the mammalian carbohydrate metabolism, is another example. Such a study has also helped in choosing non-homologous insulin closest to human insulin, for use in the management of diabetes.

9. L- AND D-AMINO ACIDS

It is a much debated and yet unsolved perplexing feature that in nature all the carbohydrates are of the D-configuration and all the amino acids are of the L-configuration, although carbohydrates and amino acids of the alternative configuration do occur.

The 'dermorphin gene associated peptide', that mimics morphin activity, is composed of 11 D-amino acids. An all L-amino acid structure has no activity. It is hard to explain why. It is also not understood why peptides formed of D-amino acids are more susceptible to protein degradation

It will be very helpful if search is made for peptide drugs with partially or wholly D-amino acids. There are several bioactive but toxic L-amino acid peptides, which can be modified to contain some or all D-amino acids to reduce toxicity and to even improve bioactivity. There is a D-amino acid hexapeptide combinational library with structures of over 52,28,400 peptides, which is a very rich source of information for such research, in the very promising area of drug design.

10. DRUG MODIFICATION

Certain metabolic deficiencies such as diabetes require an exogenous supply of the active compound, insulin in this case, to maintain health. The insulin from bovine, sheep, horse, pig and human differs from that of the other, in the sequence of one or two amino acids at positions 8, 9 and 10, the rest of the sequence being identical. Clinical insulin used in the management of human diabetes is usually extracted from pig pancreas. If the differing amino acid in a non-human insulin is appropriately substituted, the product becomes human insulin. That, genetically modified bacteria now produce human insulin is a different matter.

Several synthetic products are quite useful but cannot be used by one and all for certain side effects in some people. For example, aspartame (marketed under different trade names) is a dipeptide of aspartic acid and phenylalanine, and is 300 times sweeter than cane sugar. Aspartame is widely used as an alternate sweetener by diabetics and others who cannot take sweeteners loaded with calories. Unfortunately, pregnant women and people suffering from phenylketonuria, a disorder due to an impaired metabolism of phenylalanine, should not use aspartame. It would be useful if phenylalanine were substituted by some other amino acid without affecting its sweetness, to remove the restriction on its use.

Cyclosporin A, an 11-amino acid cyclic peptide, is the most popular immunosuppressant widely used in tissue and organ transplantation to prevent tissue rejection. However, cyclosporin A has certain side affects and some antibiotic activity, which complicate post-transplant monitoring. It will be a great help if the side effects and antibiotic activity are removed through amino acid substitution, retaining immunosuppressant activity, to make the drug more reliable and safer.

11. ENLARGE THE SCOPE OF BIOINFORMATICS

From the foregoing it should be clear that bioinformatics has a far wider scope than now projected. Bioinfromatics is a versatile, vibrant, futuristic and important field, rich in applications. Enlarging the scope of bioinformatics will only be to the advantage of bioinformatics and the bioinformatists.

12. PARTNERSHIP IN BIOINFORMATICS

Bioinformatics operates under a three-partner system.

a) Data gatherers: Enormous amounts of basic data from biomolecular chemistry and related areas, very painstakingly gathered over long years by experimental and analytical scientists, are the body and substance of bioinformatics; these are the first party.

b) Data Processors: The second party use skills of complex software, to serve the needs of the 1st and the 3rd parties; should understand the area of the 1st party and the needs of the 3rd party.

c) Process product users: End users of products, the third party.
1st and the 3rd parties need not have the skills of the 2nd partner.
Teaching bioinformatics should be comprehensive covering all the three partnering areas.

13. CURRICULUM FOR BIOINFORMATICS

It is not easy to get everyone agree to any particular curriculum for any course of study. However, there is always a possibility for a generally acceptable curriculum, which can be suitably modified for special needs. The following curriculum for bioinformatics was drawn in consultation with specialists in different areas of bioinformatics and biotechnology and the curricula of some reputed international institutions. Whether conducted independently or as a part of a biotechnology course, instruction in bioinformatics should include a comprehensive background in biology and related areas, and the core and advanced areas of bioinformatics. The details of the syllabus can be worked out depending upon the level of the course and its needs.

Foundation courses:
Cell biology, Genetics
Biochemistry, Biophysics
Microbiology, Immunology
Molecular biology
Microbial biotechnology, Genetic engineering
Protein engineering, Immunotechnology
Computer courses

Level-one courses:
Information theory and biology
Internet use
Databases: Structure of databases, Sequence databases, Relational databases
Sequence analysis, Software resources
Sequence alignment and database searches
Phylogenetic analysis
Predictive methods
Informatics and automation in genome mapping
Genome mapping
Genome analysis

Level-two courses:
Genomics, Proteomics, Cheminformatics, Glycomics
Advanced bioinformatics
Neural network and Genetic algorithms
Molecular modelling in drug design

14. CONCLUSION

Bioinformatics should be an important component of biotechnology education and it should be taught from a broad based platform. Bioinformatics is an essential component of modern biology and not independent of it. Bioinformatics is not an area of information technology and cannot be restricted to biotechnology alone. The whole area of biology can immensely benefit from the bioinformatic approach.

We need large numbers of competent biotechnologists and bioinformatists, but not holders of mere degrees in these areas. Incentives are required to attract talent, but inducement, such as assured job placement, high salaries as in information technology, are not conducive to the long-term interests of any subject. Once the balloon of hype is pricked, in the face of un-kept promises, the resultant disillusionment will be detrimental to both biotechnology and bioinformatics. A rational assessment and projection of the scope and benefits of these two areas of biology are the need of the hour.

Golden Lecture 20

PLANT FUNCTIONAL GENOMICS

Profile:

Name : Dr. Santanu Dasgupta

Address : Monsanto Research Centre
Bangalore

Academic Qualification : B.Sc., Chemistry.
M.Sc., Biochemistry from the Kalyani University
Ph.D. Molecular Biology from Bose Institute, Calcutta,
Post-doc, University of Kentucky, USA

Work Experience : He worked extensively on gene regulation and characterization of promoters to express genes in a directed manner
He involved in isolating inducible promoters for expression of genes in plant.
He was leading another project to produce clinically important monoclonal antibody in plants.
He taught and supervised number of undergraduate students and Graduate (Ph.D.) student in University of Kentucky

Awards : He is a recipient of First class first award from Kalyani University for his Master's degree.
He is also recipient of several fellowships, which include
felowship from Food and agricultural Organization of the United Nation, University Grant Commission, THRI post doctoral fellowship etc.

Present Position : Program Lead crop transformation and Functional Genomics

Publications : He has several research publications in International journals in the area of plant molecular biology.
He contributed papers in more than 20 National and international meetings and symposium.

PLANT FUNCTIONAL GENOMICS

Write up:

The fascinating world of biology has progressed rapidly in the last several years. Gregor Johan Mendel, the Father of Genetics, laid the foundation for modem plant biology over a hundred years ago. Today, we are in the era of genomics, a rapidly emerging area , which is revolutionizing the existing knowledge of biology. In the seventies, advancement gained momentum due to refinement in recombinant DNA technology. Now the technology of genome sequencing is helping scientists to break open the secret of genome. The complete genome sequences of several organisms have been deciphered and several more are underway.

High throughput sequencing is accumulating an enormous amount of data whose retrieval, management and analysis needed new computational tools with a deep insight into biology. These requirements lead to the development of a new discipline known as bioinformatics. The term bioinformatics, coined in late 1980, became popular in the 1990s as an integral tool of genome research. Bloinformatics can be defined as the science of developing computer databases and algorithms for the purpose of speeding up and enhancing biological research. It generally deals with the use of computation and large, cross-species databases of biological information to leverage the laboratory based biology. The genome sequence is the first step towards understanding the function of an organism. Bioinformatics is an important component of structural genomics as it can annotate and classify the genes comparing the sequences of known genes. The sequence similarity may suggest putative function of the gene(s). Genome sequence and subsequent analysis using bioinformatics are part of structural genomics.

Structural genomics gives a clue to move forward for elucidating the various functions of genes, which is the basis of functional genomics. Functional genomics is the application of global experimental approaches to assess the gene function by using the information provided by structural genomics. These functions can be biochemical, cellular like a role in signal transduction pathway, developmental like a role in pattern formation, adaptive like the contribution of the gene product to the fitness of the organism.

In the era of genomics, with the entire genome sequence of an organism in hand, it has become possible to analyze the organism in its entirety at the molecular level. To date about 60% of the higher plant genes can be assigned to some degree of function by comparing them with the genes of known functions. Unfortunately, this knowledge alone does not give an insight into its specific role in the organism. For example, about 13% of the Arabidopsis genes have been inferred to be involved in transcription and signal transduction. However, merely knowing that a gene codes for a transcription factor or a kinase, does not provide the information as to how they control or get controlled by other genes to give a phenotype. So the ultimate assignment of gene function must be on the basis of experimental evidences.

One of the important elements of functional genomics is DNA microarray technology. Within an organism, thousands of genes and their products express, interact with each other in a complicated and orchestrated way, and finally get translated into the action of life. So it is very important to study the genes and their expression together rather than gene-to-gene basis. This technology allows visualizing the activity of hundreds and thousands of genes simultaneously. This helps to monitor the whole genome on a single chip to give a clearer picture of the interactions of thousands of genes simultaneously. DNA microarray technology can address several biological issues like environmental stress responses, plant defense, seed development, fruit ripening, nutrient assimilation etc.

In a recent study, Seki et al.(2002) have demonstrated monitoring the expression profiles of 7000 Arabidopsis genes under different environmental stress conditions. They have not only identified 277 drought inducible, 53 cold-inducible and 194 high-salinity stress inducible genes but also have shown the cross talk of signaling cascades among these stresses. Such studies will bring revolution in understanding the stress biology in plant.

One of the high throughput approaches used to determine the function of genes is forward genetics. In forward genetics, the approach is to identify an unusual phenotype resulting from random mutagenesis of the whole genome and subsequent identification of the gene sequence, which might have disrupted. Mutation can be done either by chemical mutagenesis (using ethyl methanesulfonate, EMS, fast neutron radiation, FNR), insertional mutagenesis using T-DNA tagging (Tax and Vernon, 2001) or transposon tagging (example Ac-Ds transposable elements). In plant, arabidopsis has been used quite extensively for forward genetics screening due to its

amenability to chemical mutagenes s and availability of high throughput transforination method for insertiona mutagenesis.

The forward genetics approach may help scientists to go for a direct analysis of a particular process of their interest. This approach also has the advantage of producing thousands of mutants to cover the whole genome. However, forward genetics has some disadvantages like a laborious and lengthy process of positional cloning for a gene , biased phenotypic screening towards dominant mutations and occasional recovery of lethal mutants. Most mutations are loss of function mutations and may not always get reflected by a recognizable phenotype of the mutated plant. For example if the gene is a member of a multigene family, a loss of function of one of its members is not likely to give a mutant phenotype.

The other approach of deducing gene function is reverse genetics. Reverse genetics is a method that starts from the genes or mutant and then finding out the phenotype. A gene sequence identified by sequencing, with putative function based on sequence homology with well characterized gene from other species, and based on expression pattern from RT-PCR (reverse transcriptase polymerase chain reaction), northern analysis, microarray etc. can be over expressed or expressed in antisense and subsequently the phenotype can be monitored.

The gene can also be also isolated from any T-DNA mutant population (as described in forward genetics) by PCR to study the phenotype. Due to the availability of genome sequences, it has become possible to assign biological role to a gene with reverse genetics approach involving testing the gene in question using transgenic technology. The process thus initiates with the knowledge of gene sequence, followed by different steps like cloning the gene, bioinformatic analysis for putative function, expression study , generating transgeincs and study of the transgenics involving morphological, biochemical and physiological assays. These assays could constitute a standard panel for high throughput testing of several genes or could be biased assay based on the putative role of the gene. This process has some disadvantages like non-availability of high throughput transformation system for most of the crop plants. Another disadvantage could be over expression using constitutive promoters for high throughput study, which may result into lethal phenotype.

The entire genome can now be analyzed with respect to its expression at the transcript level, at the protein level and at the level of metabolites. Determination of gene function is a concerted effort among different technological platforms of functional genomics. A coordinated approach of analyzing different constituents of the cells like transcripts (transcriptomics), proteins (proteomics) and metabolites (metabolomics) can help to deduce the function of the gene. There are several methods of analyzing the transcripts (mRNA), the first step of gene expression. These includes DNA microarray as described above, massively parallel signature sequencing (Brenner e. al., 2000,), cDNA amplified fragment length polymorphism (Bachem *et* al., 1996,) and AP-PCR (arbitrarily primed polymerase chain reaction, Welsh *et* al., 1992). Out of all these methods, availability of genome sequences of model plants like arabidopsis and rice makes the high throughput microarray approach more popular. As described above, Seki *et* al.(2002) have demonstrated monitoring the expression profiles of 7000 arabidopsis genes together under different environmental stress conditions.

The next level of analysis towards function of the gene is profiling the proteins, the proteomics. Proteornics helps to study many different aspects of gene function. Wilkins et al (1996) conceptualized the term "proteome" to define the expressed complement of a genome. All the proteins expressed by a genome could define a proteorne or there could be specific proteomes. A specific proteome could be for a particular tissue, subcelluar level, defense-associated response, an organelle etc. (Komatsu *et* al., 1999; Panter *et* al., 2000; Rakwal and Komatsu, 2000; Peltier *et* al., 2000). Development of new methods of protein extraction helps to construct proteomes for specific classes of proteins. For example, a specific proteome was developed on envelope membrane proteins that displayed several transmembrane segments based on a selective extraction of hydrophobic proteins and the subsequent analysis (Seigneurin *et al.,* 1999). Proteomics refers to the study of the proteome (Roberts, 2002). There are different methodologies to analyze proteome including two dimensional gel electrophoresis, isoelectric focusing coupled to ion cyclotron resonance mass spectrometers or to non-porous liquid chromatographic separations and mass spectrometric identification (Wall *et. al.,* 2000), isotope tagging to identify low copy number proteins (Goodlett *et al.,* 2000). The proteomic tools are very useful to analyze various plant-specific processes and the response to environmental factors. A combination of genome sequence data and analysis of proteome will greatly enhance the understanding of gene function.

Another important platform for functional assignment to genes is metabolomics which may help to determine the biochemical functions and the underlying biological roles of the gene. Similarly, as transcriptome and proteome, a

set of metabolites synthesized by an organism constitutes its metabolome (Oliver *et al.*, 1998). There can also be specific metabolome for tissues, cells or cell compartments. An estimated size of a plant metabolome could be in the range of **90,000 to 200,000** (Feihn *et al.*, 2001). This enormous and complex range of metabolites can be monitored only with highly automated and sensitive detection and quantitation method and then subsequently can be analyzed by sophisticated software like automated mass spectral deconvolution and identification software (AMDIS) (Stein, 1999). In the commonly used method for plant transgenic research, over expression of a particular gene may not always result in a visible phenotype. Metabolic profiling of the transgenic can give a better insight. An increased level of any important metabolites or reduced level of any undesirable metabolite, like a potential allergen, could be of tremendous value which otherwise would not have been noticed.

Determination of the complete sequence of plant genome made it possible to know the enormous amount of information contained within the genome. This is making a revolution in the area of plant biology and completely changing the perspective of plant research what was there a few years back. Efficient assignment of gene function is now the critical challenge for contemporary life science. Knowing the function of almost all the gene in the organism would help in modifying specific genes more precisely and predictably in transgenic crop plants. The options of plant biotechnology are exponentially growing in the era of genomics. The enormous amount of information coming out from the genome analysis has to be converted into useful traits. The genomics information can be used to solve the real-world problems, like better crop yield and nutrition, metabolic engineering to produce specific biochemical resistance to biotic and abiotic stresses.

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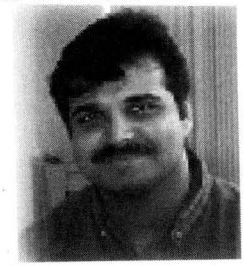
Golden Lecture 21

AVENUES FOR PRODUCTION OF EDIBLE VACCINES IN PLANTS



Profile:

Name : Dr. Rajesh Ullanat

Address : Avestha Gengrain Technologies
ITPL, Bangalore

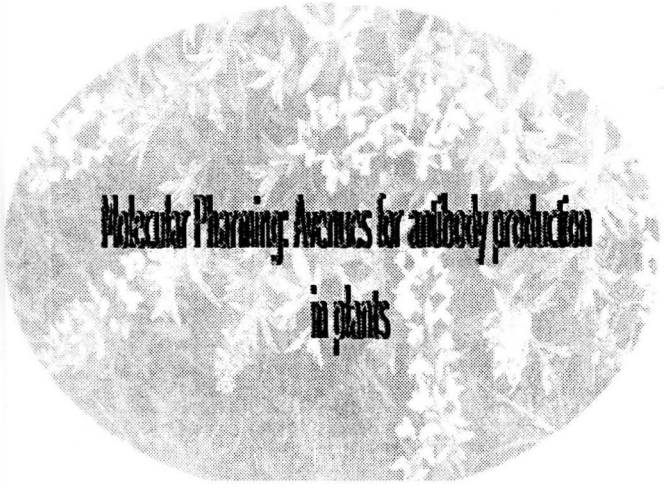
Academic Qualification : Ph.D in 2000 "Light and phytohormone regulated gene expression in higher plant systems" at the Department of Biochemistry, Indian Institute of Science, Bangalore, India
He pursued his post-doctoral studies at Plant Research International, Wageningen, The Netherlands

Work Experience : His research at Avesthagen is focused on the isolation of novel lead compounds of therapeutical or nutraceutical relevance from the medicinal plant flora prevalent in India and their subsequent target identification.
He believes that thinking at a molecular level may be one of the most promising ways to address some of the most important biochemical puzzles that we face today

Present Position : Sr.Scientist, Avestha Gengrain Technologies

AVENUES FOR PRODUCTION OF EDIBLE VACCINES IN PLANTS

Write up:

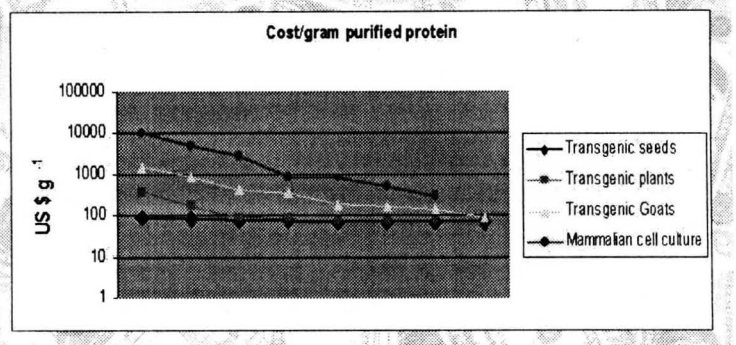


Molecular pharming:

- production of biomolecules of interest in suitable systems such as microorganisms, eukaryotic cell culture systems and plants
- represents an area of convergence of the agricultural and pharmaceutical sectors

Why Plants ?

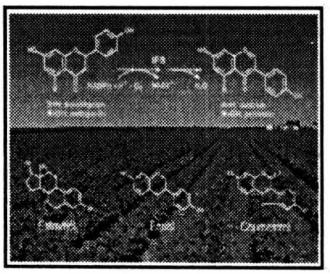
- Plant systems are more economical than industrial facilities using fermentation or bioreactor systems
- Amenability to large scale-ups at low costs
- Availability of technology for harvesting and processing plants/ plant products on a large-scale
- Reduced risk of occurrence of mammalian pathogens
- Elimination of purification requirements when the plant tissue containing the recombinant protein is used as food (edible vaccines)



Expression mg/L Cell Culture; mg/L Milk; mg/kg Plant/Seed

Avesthagen

Molecular pharming in plants



- Metabolic engineering of secondary metabolite (eg.isoflavone) biosynthesis using cytochrome P450s
- Modification of seed quality by altering the amino acid content
- Expression of recombinant proteins of therapeutic interest

Avesthagen

Recombinant protein expression in transgenic plants

- Plants, unlike microorganisms can produce multimeric proteins such as antibodies in the right conformation.
- Depending on the promoter used, transgenic proteins can deposited throughout the plant or in specific tissues like seeds or even specific organelles such as chloroplasts.
- In addition to very high recombinant protein expression levels, Chloroplast transformation also has the advantage of increased biological containment due to the apparent elimination of transmission of transgenes through pollen.
- Proteins expressed in seeds have shown remarkable stability.

Avesthagen

Divergent glycosylation patterns.

Problems:

- Plants have a higher number of glycoforms in comaprison to mammals
- Plants specific glycosylation (xylose and fucose) have an immunogenic potential
- Recent research efforts have focussed on the "humanization" of the plant glycans

Solutions:

- A cross between a tobacco plant expressing a human version of galactosyl transferase and one that was engineered to make mouse antibody was found to result in the production of plantibodies with very similar glycosylation patterns to that produced in mammalian cell culture systems
- Studies are underway to engineer sialic acid synthesis in plants

Avesthagen

Post-transcriptional gene silencing (PTGS)

- PTGS is a sequence specific RNA-degradation mechanism that is widespread in eukaryotes. It is often associated with the methylation of the transcribed region of the silenced gene and / or with the accumulation of small RNA molecules (21-25 nt) that are homologous to the silenced gene.
- In plants, PTGS can be triggered locally and then spread throughout the organism via a mobile signal that can cross a graft-junction.
- Silencing of introduced transgenes has been frequently observed in plants and hence poses a serious commercial problem

Avesthagen

Timelines and Regulatory issues.

- In terms of timelines for protein production, plants are comparable with a. imal systems (eg: goat and chicken)
- In corn, the first commercial lot (~1kg of protein) is estimated to be available in 36 months after the initiation of gene transfer.Subsequently, each generation provides a 100 X scale-up
- Species such as tobacco with an extremely high number of seeds per plant may allow an even faster scale-up
- The regulatory framework for plant-derived recombinant pharmaceuticals remains to be fully established
- Although GMP-regulations will apply, these may need refinement to make them relevant and applicable to plant-based expression systems

Avesthagen

Conclusions

- Plants are a safe, cost-effective system for the commercial production of therapeutic biomolecules of interest
- Considerable progress has been achieved in the "humanization" of recombinant proteins (esp. plantibodies) expressed in plants
- Edible vaccines have the potential to revolutionize the process of vaccination especially with reference ce to developing countries

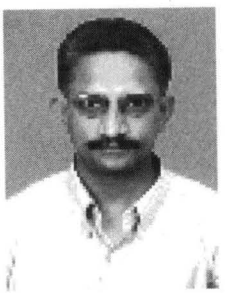
Avesthagen

Plants have been exploited as a source of useful molecules for many years. Molecular pharming refers to the production of biomolecules of interest in suitable systems such as micro-organisms, eukaryotic cell culture systems and plants. This also represents an area of convergence of the agricultural and pharmaceutical sectors. Presently, transgenic technology allows the usage of plants as bioreactors for the production of foreign or customized molecules of interest. Transgenic plants have become attractive systems for the production of human therapeutic products due to several reasons such as (a) the availability of technology for harvesting and processing plants / plant products on a large-scale thus making plant systems more economical than industrial facilities that use fermentation or bio-reactor systems (b) the amenability to large scale-ups at low costs and (c) the reduced risk of the occurrence of mammalian pathogen contaminants. Plants, unlike micro-organisms can produce multimeric proteins such as antibodies in the right conformation. Depending on the promoter used, transgenic proteins can be deposited throughout the plant or in specific tissues like seeds or even specific organelles such as chloroplasts. In addition to very high recombinant protein expression levels, chloroplast transformation also has the advantage of increased biological containment due to the apparent elimination of transmission of trans-genes through pollen. Therefore, plants now offer a number of expression systems for the temporally and spatially controlled production of recombinant proteins for many different purposes. The two major bottlenecks associated with transgenic expression in plant systems are (a) differences in glycosylation patterns of plants and mammals and (b) post-transcriptional gene silencing (PTGS). Although plant specific oligosaccharide linkages (α-1,3-fucose and β-1,2-xylose residues linked to the glycosylation core) have been reported to have an immunogenic potential, recent clinical trials of pharmaceutical products produced in transgenic plants are encouraging, with plant glycans showing reassuringly poor immunogenicity. Strategies for the elimination or minimization of PTGS include (a) careful design of transgene constructs by eliminating repeat sequences (b) positioning of matrix attachment regions on either side of the transgene and (c) the introduction of the helper-component proteinase of plant potyviruses into plants. Nevertheless, our increasing understanding of protein targeting and accumulation should help in the further improvement of the potential of molecular pharming in plant systems. It must also be noted that the regulatory framework for plant-derived recombinant pharmaceuticals remains to be fully established. Although the GMP-regulations will apply, these may need refinement to make them more relevant and applicable to plant-based expression systems.

Golden Lecture 22

BIOTECHNOLOGY AND BIOINFORMATICS IN MODERN ERA

Profile:



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Academic Qualification :M.Sc., Microbiology from Gobind Ballabh Pant University of Agriculture and Technology, Pantnagar, Uttaranchal.
Ph.D., Plant Sciences, Osmania University, Hyderabad.

Work Experience : Teacher for the last decade teaching both undergraduate and postgraduate students in life sciences at Bharatiya Vidya Bhavan's Vivekananda College of Science, Humanities and Commerce, Sainikpuri, Secunderabad.
Worked on enhancement of Colchicine yield in *Gloriosa superba*
Worked on enhancement of xylanase yield in *Melanocarpus albomyces*
Working on bioremediation of effluents using a unique cross-flow sieve reactor system and a filamentous fungal organism, *Aspergillus fumigatus.* Using this system, effluent quality is sought to be improved and the excess fungal organisms could be made available as a single-cell protein source

Awards :Awarded Research Fellowship by Council for Scientific and Industrial Research, New Delhi.

Present Position :Programme Director, Centre for Life Technologies, facilitators Wageningen University, the Netherlands

BIOTECHNOLOGY AND BIOINFORMATICS IN MODERN ERA

Write up:

The aim of today's biotechnology is to discover new products or develop new process using modern strategies. The advances that have been made in the past decade in areas such as organic chemistry, combinatorial biosynthesis, metabolic pathway elucidation, gene shuffling, and directed evolution of proteins have caused shift in the strategy from traditional biology to bioinformatics that is revolutionizing biology. Means of detecting novel organisms, novel chemical structures, and novel biocatalytic activities in this era will ensure that natural products will continue to be a primary resource for biotechnology

The course of biotechnology search starts with the assembly of appropriate biological materials, moves through screening for a desired attribute and selecting the best option from among a short list of positive screening hits, and culminates with the development of a commercial product or process. The concept of exploitable biology outlined above remains valid and continues to be the core strategy for industrial practice overall. However, advances of the past decade are revolutionizing the approaches to exploitable biology such that the process is undergoing a major reevaluation. The intention of this article is to look at strategies adopted as a consequence of the bioinformatics revolution and to consider some of the opportunities and challenges that it presents for biotechnology.

Biotechnology Biotechnology can be broadly defined as "using living organisms or their products for commercial purposes. Biotechnology currently can be defined as a reliable, and relatively low risk technology capable of being implemented on a large scale and across the full range of industrial sectors. The impact of biotechnology to date has been most pronounced in the pharmaceuticals sector, but it is clear that enormous potential exists in all of the other sectors for biotechnology penetration.

The principal drivers of biotechnology are economic demand, led by industry and advances in science and technology. Biotechnology is one of the key enabling technologies for the 21st century, and confidence in this view stems from the impact that it has had and will have on major global problems -disease, malnutrition, and environmental pollution, the promise it holds for achieving industrial sustainability optimal use of renewable resources, reduction of global warming, and introduction of clean or cleaner products and processes and achieving economic competitiveness, generating new markets, and having wide industrial applicability.

The search for natural products has been the mainstay of the biotechnology industries. Natural-product search however, is not synonymous with drug discovery. All the available evidence points to natural-product discovery continuing strongly and accelerating as a consequence of new search strategies and innovative biology. In drug discovery, for example, novel natural-product chemotypes with interesting structures and biological activities continue to be reported. Without such discoveries "there would be a significant therapeutic deficit in several important clinical areas, such as neurodegenerative disease, cardiovascular disease, most solid tumors, and immune-inflammatory diseases".

One prerequisite to natural-product discovery that remains paramount is the range and novelty of molecular diversity. This diversity surpasses that of combinatorial chemical libraries and consequently provides unique lead compounds for drug and other developments. Newly discovered bioactive products do not usually become drugs but may enter a chemical transformation program in which the bioactivity and pharmacodynamic properties are modified to suit particular therapeutic needs.

Development of screening assays, particularly as a response to the need to evaluate large numbers of samples in high-throughput screens and the expectation that many new targets will be identified in the wake of genome sequencing projects have been developed. High-throughput screening involves the robotic handling of very large numbers of candidate samples, the registering of appropriate signals from the assay system, and data management and interpretation. For example, the ability of *Saccharomyces cerevisiae* to express heterologous proteins makes it an attractive option; its use in screens based on substitution assays and differential expression assays is proving to be an effective route to drug discovery. Biotechnology goes well beyond drugs: novel crop protection agents, food and feed ingredients, biocatalysts, and biomaterials are among the many important industrial targets

Currently we are witnessing a major change in the way which we do search-and-discovery research in biotechnology. This shift demands a major reorientation of methodology so that old questions may be approached a new. In the modern era, biology has shifted from what we refer to as traditional biology to bioinformatics. In traditional biology the search strategy is based upon specimen collection, system observation, and laboratory experimentation in order to organize knowledge in a systematic way and to formulate concepts.

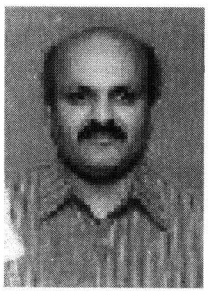
In bioinformatics the search strategy is based upon data collection and storage and the mining of the databases in order to generate knowledge, i.e., generation of knowledge from information or data. *Bioinformatics* is currently defined as the study of information content and information flow in biological systems and processes. It has evolved to serve as the bridge between observations (data) in diverse biologically related disciplines and the derivations of understanding (information) about how the systems or processes function, and subsequently the application (knowledge).

The above shift is being actuated by a number of key factors: (i) the phenomenal pace of technological advances, e.g., bioinformatics, combinatorial syntheses, high-throughput screening, and laboratories on a chip; (ii) the need for significant breakthrough discoveries; (iii) pressure to reduce costs; (iv) the requirement to reduce cycle times; and (v) biotechnology acquisitions and mergers, i.e., survival in global markets. Bioinformatics databases include DNA (genomes), RNA, and protein sequences, proteomes, macromolecular structures, chemical diversity, biotransformation, metabolic pathways (metabolomes), biodiversity and systematics. Thus, experiments can be made in silico rather than *in vivo* or *in vitro*, so that only essential experiments need be undertaken.

In conclusion the novel means of detecting novel organisms, novel chemical structures, and novel biocatalytic activities will ensure that natural products will continue to be a primary resource for biotechnology. The shift has been driven by a convergence of complementary technologies, exemplified by DNA sequencing and amplification, genome sequencing and annotation, proteome analysis, and phenotypic inventorying, resulting in the establishment of huge databases that can be mined in order to generate useful knowledge such as the identity and characterization of organisms and the identity of biotechnology targets. The integration of information from complementary databases should facilitate answers to complex questions involving sequence, biochemical, physiological, taxonomic, and ecological information of the sort posed in biology. The shift is not absolute in the sense that it will replace established biology; rather, it reinforces our view that innovative biology is essential for releasing the potential of biotechnology penetration throughout industry.

Golden Lecture 23

SUPER CRITICAL FLUID TECHNOLOGY FOR EXTRACTION OF HIGH VALUE BIO-ACTIVE COMPOUNDS



Profile:

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Insurance Surveyor – 3 yrs

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Publications : Published about 3 papers

SUPER CRITICAL FLUID TECHNOLOGY FOR EXTRACTION OF HIGH VALUE BIO-ACTIVE COMPOUNDS

Write up:

Importance of Nature.

Good Health is everybody's concern and "Health – the Natural Way" is everybody's favorite. Nature has immense curative power, but it needs to be tapped selectively. Nature's cure is like mother's gentle care and works wonders by restoring equilibrium and harmony in the entire body system. Nutrition plays a vital role in strengthening the body's immune system. It is believed that there are at least 43 chemical components, called essential nutrients, which must be present in our food. Nutritional deficiencies may occur during preservation, processing, transportation, contamination or degradation. Mother Nature provides us with all the essential nutritients, these are present in seeds, nuts, grains, spices, fruits and vegetables. However, as some of the nutrients are lost or depleted by thermal, natural or bacteriological degradation between the time of harvesting and the time of ingestion, it becomes absolutely essential to supplement these vitamins and antioxidants. Hence, it is desirable that these nutrients be derived or concentrated from natural sources, in the form of natural extracts (Nutraceuticals). Besides it is also necessary to remove some of the toxins or harmful components present. Health food is food grown organically, and consumed in its natural form after it is transformed by using natural substances into a high-nutrition value product, such as "NUTRACEUTICALS". Scientific research into biological activity of these natural products and their healing potential has confirmed their therapeutic uses for cosmetic benefits. Nutraceuticals (often referred to as dietary supplements or functional foods) are bioactive compounds that have health promoting, disease preventing properties. Nutraceuticals and functional foods are food components that provide physiological benefits or reduce the risk of chronic diseases, apart from their basic nutritional functions. A functional food is similar to a conventional food, while a nutraceutical is isolated from a food and made available in dosage form, in both cases the active components occur naturally in the food.

Natural Extracts.

Over the past decade, concern for the quality and safety of foods and medicines, regulations for nutritive and toxicity levels, has increased the preference for "Natural" as opposed to synthetic substances. Furthermore, the present popular belief that everything " natural " is good, provides a positive incentive towards growth of the natural products industry, particularly in the food, flavoring, perfumery, and pharmaceutical sectors. "Mother Nature" is considered a highly efficient synthesizer of desirable blends of constituents ideally suitable for human consumption. For example, the subtle nuances and characteristic notes possessed by natural extracts or concentrates have not yet been matched by mixtures of their major ingredients produced synthetically, although considerable efforts are made in mimicking the natural molecules.

No double, safety of both producers and consumers is now a major requirement of any product or process. Accordingly, compelling regulations on the usage of hazardous, carcinogenic, or toxic solvents, as well as high energy costs for solvent regeneration have encouraged the growth of the natural extract industries. To suppress the competitive edge of synthetic materials, alternative extraction methodologies that comply with both consumer preference and regulatory control and that are cost effective, are becoming more popular. One of such major technologies that has emerged over the last two decades as the alternative to the traditional solvent extraction of natural products is the supercritical fluids extraction technique. It uses a clean, safe, inexpensive, nonflammable, nontoxic, environment-friendly, nonpolluting solvent, such as carbon dioxide. Besides, the energy costs associated with this novel extraction technique are lower than the costs for traditional solvent extraction methods.

Super Critical Fluids.

A SCF is defined as a substance above its critical temperature (T_C) and critical pressure (P_C). The critical point represents the highest temperature and pressure at which the substance can exist as a vapour and liquid in equilibrium. The unique solvent properties of supercritical fluids (substances above their critical temperature and critical pressure) were first observed more than a century ago. When a gas is compressed to a sufficiently high pressure, it becomes liquid. If, on the other hand, the gas is heated beyond a specific temperature, no amount of compression of the hot gas will cause it to become a liquid. This temperature is called the critical temperature and the corresponding vapor pressure is called the critical pressure. These values of temperature and pressure define a

critical point, which is unique to a given substance. The state of the substance is called supercritical fluid (SCF) when both the temperature and pressure exceed the critical point values. This "fluid" now takes on many of the properties of both gas and liquid. It is the region where the maximum solvent capacity and the largest variations in solvent properties can be achieved with small changes in temperature and pressure. It offers very attractive extraction characteristics, owing to its favorable diffusivity, viscosity, surface tension and other physical properties. Its diffusivity is one or two orders of magnitude higher than those of other liquids, which facilitates rapid mass transfer and faster completion of extraction then conventional liquid solvents. It low viscosity and surface tension enable it to easily penetrate the botanical material from which the active component is to be extracted. The gas-like characteristics of SCF provide ideal conditions for extraction of solutes gas-like characteristics of SCF provide ideal conditions for extraction of solutes giving a high degree of recovery in a short period of time. However, it also has the superior dissolving properties of a liquid solvent. It can also selectively extract target compounds from a complex mixture. Sometimes the target compound is the active ingredient of interest. At other times, it may be an undesirable component, which needs to be removed from the final product. The strong pressure and temperature (or density) dependence of solubility of certain solutes in an SCF solvent is the most crucial phenomenon that is exploited in supercritical fluid extraction (SCFE).

CO2 as Solvent.

The most desirable SCF solvent for extraction of natural products for foods and medicines today is carbon dioxide (CO2). It is inert, inexpensive, easily available, odorless, tasteless, environment-friendly, and GRAS (generally regarded as safe) solvent. Further, in SCF processing with CO2, there is no solvent residue in the extract, since it is a gas in the ambient condition. Also, its near-ambient critical temperature (31.1 deg C) makes it ideally suitable for thermolabile natural products. Due to its low latent heat of vaporization, low energy input is required for the extract separation system, which renders the most natural smelling and natural-tasting extracts. Further, the energy required for attaining supercritical (SC) state of CO2 is often less than the energy associated with distillation of conventional organic solvent. In general, the extractability of the compounds with supercritical CO2 depends on the occurrence of the individual functional groups in these compounds, their molecular weights, and polarity.

Commercial CO2 required for supercritical fluid extraction process is already present in the environmental system, obtained as a by-product from the fermentation process or the fertilizer industry. So its use as an extractant does not cause any further increase in the amount of CO2 present in the earth's atmosphere. Therefore, there is no additional "green house effect" from using CO2 as the SCF solvent.

Introduction to reactions in supercritical fluids.

In general, reactions fall into two main categories as to why they have been chosen for investigation in supercritical carbon dioxide. The first class exploits the high solubility of the light gases (e.g. Hydrogen and CO) in supercritical carbon dioxide to enhance reaction rates. Hydrogenation and hydroformylation are particularly good examples of this. The second category of reactions investigated in supercritical carbon dioxide are those where enhanced selectivity is observed in the reaction, which usually originates from the unusual solvent properties of supercritical carbon dioxide, and the ability to vary its solvent properties by adjusting pressure. This is potentially the most important and intriguing aspect of synthetic chemistry in supercritical carbon dioxide, and is the area where we have made our most significant contribution. At present, it is also one of the least well investigated and least understood areas, although a number of groups around the world are active in this area.

Solvent properties of supercritical fluids.

A crucial aspect of carrying out reactions in supercritical carbon dioxide is solubility. Pure supercritical carbon dioxide is a relatively non-polar solvent, but has some limited affinity with polar molecules due to its large molecular quadrupole. Modifiers can often be added (e.g. MeOH) to improve the solubility of polar molecules. Alternatively, when reactions involve more than one reagent, less polar reagents can in effect act as modifiers enhancing the solubility of more polar reagents avoiding the need to resort to additional co-solvents.

Another approach widely used to enhance solubility in supercritical carbon dioxide is to introduce fluorinated substitutents, often onto a ligand or counterion for orgamometallic catalysis. However, expense of reagents can be a limiting factor, necessitating recycling.

There are also a number of practical advantages associated with the use of supercritical carbon dioxide as a solvent. Product isolation to total dryness is achieved by simple evaporation. This could prove to be particularly useful in the final steps of pharmaceutical syntheses where even trace amounts of solvent residues are considered problematic. There are also two very useful complementary routes to particle formation with SCFs and supercritical carbon dioxide in particular, rapid expansion of supercritical solutions (RESS) and supercritical anti-solvent precipitation (SASP).

One of the main differences between supercritical fluids and conventional solvents is their compressibility. Conventional solvents in the liquid phase require very large pressures to change the density, whereas for supercritical fluids, very significant changes in density and hence solvating properties can be achieved by comparatively small pressure and/or temperature changes, particularly around the critical point. This provides an infinite range of solvent properties, which can in some cases, be tuned to significantly affect the outcome of a reaction. Note that in general, supercritical fluids are considerably less dense than conventional solvents. This can lead to problems of solubility in some cases, but also means they are considerably less viscous than conventional solvents which leads to a significantly greater diffusivity. This can result in significantly faster reaction rates if diffusion is rate limiting.

Carbon dioxide Extracts.

Two types of materials are obtained by SCFE method, viz., CO2 selects and CO2 totals.

CO2 Selects.

Essential oils or CO2 selects are obtained at relatively low pressure and contain only volatile CO2 soluble components. These tend to resemble the classic steam distillate but with the advantage of no temperature degradation and the potential for additional volatile substances that may not be distilled out of the plant under normal steam distillation. The consistency of essential oils extracted with the SCFE method will vary from batch to batch, just as the plants themselves vary and just as steam distilled oils vary.

CO2 Totals.

Extracts called "Totals" are obtained at higher pressures and contain all CO2 soluble components including waxes, resins, colorants, resembling a classical hexane extract, with the advantage of no solvent residue. CO2 totals are usually thick and pasty due to the beneficial fats, resins, and waxes they contain that come from the plant material itself. These totals are soluble in essential oils and vegetable oils.

Golden Lecture 24

THE ROLE OF MEDIA IN BIOTECHNOLOGY

Profile:

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Academic Qualification : Masters Degree in English Literature
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Work Experience : She served in diverse work environments such as the country's foremost telecommunications Industry ,the premier Science Institute and Infrastructure Industry-both Indian and foreign.
Media Relations,Government Relations,Event Management
Helping in starting business operations in the State, preparing and making presentations to the Government ,potential clients and Regulatory Authorities,liaising with various departments and financial Institutions, Government Relations,as well as marketing the project.
Establishing the PR section and handling editorial,creating audio-visuals,a permanent exhibition centre towards interface with the industry, media coverages ,co-ordinating for seminars/conferences,special lectures and organising VVIP visits

Awards : Gold Medalist for the State in Hindustani Classical Music- Sitar

Present Position : CEO, IKON PR Consultancy Promoter

THE ROLE OF MEDIA IN BIOTECHNOLOGY

Write up:

At the outset, let me thank you for asking me to address this august gathering on the "role of media in Biotechnology. The topic is indeed most relevant to the present times. Biotechnology is a sunrise industry the world over, and we in India, can boast of the best research institutes and top notch professionals in the field of Biotechnology . Biotechnology is no more a futuristic technology, but is very much a "here and now" technology, with its seen and unseen presence in almost every sphere of life, whether the urban or the rural.

Once upon a time we would have dismissed selective and genetic re-engineering as Science " trying to play God". But with the current trends , case in point being "Dolly the sheep", the day is not very far when we could create a child of our choice, with features tailored to our special specifications. And believe me, this would be no Frankenstien's monster but surely a product of " the Brave New World" . Truth being stranger than fiction, Aldous Huxley would , in retrospect, seem to be a scientific and literary Nostradamus. In the world of plants, we have a tomato having strains of a fish and so on.

In this context of Biotechnology embracing every day life, with its applications having far reaching implications, the media has a very pivotal role to play. The role of the media would, in my opinion, be more proactive and responsible, as it would have to play, not only the role of the informant, but also the moral watch dog, as Biotechnology would involve a host of moral and ethical issues , with humankind being the beneficiary. Again Dolly had ,and is still raising a hue and cry globally, amongst the purists and the moralists. Who knows the day might not be far, when, we could, through the miracle of Biotechnology , engineer the right type of media man having all the qualities required for giving excellent publicity to the Scientist, integrated into his genes !!!

In the sociological division of the rulers and opinion leaders in society, the media has been called the "Fourth Estate", considering the power that it has in informing the public, moulding public opinion and leading to decision making, even provoking revolutions.

The 19th century British Sociologist and Historian, Edmund Burke , had divided Society into 3 estates.

The first three estates being Priesthood, Aristocracy and the Commons . With the discovery of the power of letters – namely the media, which kept in check the other 3 Estates – eminent Historian Carlyle, called the media the Fourth Estate. The media became the "guardians of public sphere".

Over the years , the media has evolved, having grown in stature, in its role and functions and being absolutely powerful . Power being one side, the other side of this coin is the immense social responsibility that comes with it. Media has to be more proactive, judicious, impartial and objective. It is only then that it can contribute to the progress of mankind . And this is imperative for translating pure science and technology to day-to-day life. And greater than pure science and technology, the emerging field of biotechnology needs the media along with it to take the giant strides that it is taking. There is a sense of urgency to all this given the fact that there is a mind-boggling population explosion leading to fast depleting natural reserves and increasing levels of pollution and global degradation.

Bio technology would prove to be the single most effective saviour of life force in whatever form. Strains of plants and animals need to be discovered , and are being discovered, that could withstand the onslaughts and ravages of modern day lifestyles. We have had talks by eminent bio-technologists at these very premises, who have showcased their various research – be they in the form of a new vegetable, a new crop, or a new flower, etc., Biotechnology has not only been a saviour at the grass-root levels for the farmer, but also at very sophisticated levels of pure science and technology such as genetics, pharmaceuticals, aviation medicine , amongst a host of others.

We have seen that aviation medicine has made great strides, making it possible for a single capsule/pill to have all the nutritional contents and gastronomic needs that keep the astronauts fit and going in space voyages. Our very own CFTRI from the State has researched , produced and supplied food to space flights of NASA.

In between the figurative and literal fields of grass-root agriculture and high – flying space technology, is the miracle of reproduction biology and life sciences. Geneticists, garnerning the skills of bio-engineering, have brought light into dark homes, through their successful research in the areas of fertility, genetic disorders and baffling diseases.

With increasing levels of education, particularly amongst modern women, bringing with them the problems of late marriages and late motherhoods, Biotechnology has been a boon as it has enabled increasingly greater number of women to have healthy, normal babies, as opposed to the lesser privileged women about 40-50 years ago, who had the high risk of giving birth to genetically and physically impaired children, during late motherhood.

Biotechnology has admirably helped women fight middle life crises with the boon of the discovery of HRT whose virtues are still debatable. However a time will soon come when bio-genetics can overcome even the associated hazards of such medical treatments.

Biotechnology, by its very nature, and the implications that it has, involves a host of policy making issues and governance. The fraternity of scientists and engineers needs the support of a discerning media. The media here plays the role of opinion leader, aiding in governmental awareness and support on the one hand and public understanding and participation on the other.

Without patronage, funds , legislations, incentives and encouragement, the benefits of this wonder discipline would not be available to the beneficiaries. And public consent and consensus, understanding and faith would be necessary for popularizing these discoveries, and giving them public acceptance and legitimacy. Only the media could bring about this transformation through sustained and clear flow of information. The media presents the vital link between the scientists and the general public.

There is a greater need for a higher level of interaction between the scientific community and the media. It is imperative to have timely and accurate dissemination of information to the public. This would lead to increased recognition of the place of Science and Technology in daily life . Scientists must step out of their ivory towers and become more accessible and media savvy. With the happy marriage of science , technology and engineering, scientists are increasingly recognizing this need for being less remote and more transparent. With the drastic reduction in time frames of table-top research reaching out to the public, in keeping with the urgent demands of time, scientists need to keep the expectations of the public in mind and pursue application–oriented research. When controversial, ethical and legal issues are concerned, there is a need for candid exchange of views, ideas and information, between the scientists and the media .

In this regard, institutions such as the Institution of Engineers is taking a step in the right direction. Creating a platform for showcasing topical issues and giving them wide publicity amongst peer groups , scientific community, the Industry, governmental and non-governmental organizations and the media, the Organisers are reaching out to a large cross section of society, communicating, influencing and recommending the right approach to be taken in bringing science and technology into the mainstream of everyday life. It would be realistic to co-opt members of the media to the Advisory Boards and Committees on Science and Technology of Research Institutes and Scientific Bodies such as the ICCR,UGC,ICMR,BARC,IISc,Department of Science andTechnology,amongst others.The Institution of Engineers could lead by example and induct a media representative into its Board.This would give a realistic perspective, right at the planning stages. The very fact that I am standing in front of you and giving this talk is a plan in action in the right direction, by the Institution of Engineers. I commend you on this .

A person like me who is in the field of communications is a jack of all trades – who knows a little of something and something of everything. Therefore the Communications expert would admirably bridge the gap between a subjective scientist and an expectant public.

Having rightly considered this topic as important enough for the golden lecture series, I would be delighted if you could have a session devoted to this at the forthcoming Conference.

I would like to once again thank the Chairman, Senior fraternity and Committee Members of the Institution of Engineers for giving me this opportunity to address you and share my thoughts with you. I wish the forthcoming Conference every success that it deserves and hope the Institution continues the good work that it is doing in revolutionizing Science and Technology and bringing it within reach of the ultimate beneficiary – the common man.

Golden Lecture 25

VIRUS INDUCED GENE SILENCING (VIGS) APPROACH TO STUDY GENE FUNCTION IN PLANTS

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University of Agricultural Sciences,
(UAS) Bangalore Merit Scholarship
Indian Council of Agricultural Research (ICAR) Junior Research Fellowship.
ICAR Senior Research Fellowship
Council of Scientific and Industrial Research (CSIR) Senior Research Fellowship.
National Eligibility Test (NET)
Dr. CS Venkata Ram Memorial Award during the year 1998 for the best original research scientific work.
BOYSCAST Fellowship (for training and research at Yale University, CT, USA).

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Publications : In indexed journal: 10
In workshops/national/international symposia: 25
Popular scientific articles: 04.

VIRUS INDUCED GENE SILENCING (VIGS) APPROACH TO STUDY GENE FUNCTION IN PLANTS

Write up:

Introduction

The primary goal of current genome projects is to identify the biological functions of every gene discovered. Several approaches are available to study gene function in plants. Two attractive functional genomic approaches are T-DNA and transposon-based insertion mutagenesis. These methods have limitations because there is gene target bias, and it is difficult to disrupt or tag all genes. In addition, insertion or deletion of a single gene may fail to induce a phenotypic change because of the high degree of gene duplication in plant genomes. Finally, using these approaches mutations that lead to lethality of organism cannot be uncovered. To suppress gene function, an approach involving a single-stranded self-complementary (hairpin) RNA has been successfully employed (Wesley, *et al.*, 2001). However, all of these approaches rely on the generation of transgenic lines, which, is a difficult task. To over come the limitations of the above approaches, a technique involving targeted post-transcriptional gene silencing (PTGS) has been developed (Liu et al., 2002).

Post-transcriptional gene silencing and its relevance

Post-transcriptional gene silencing is a process during which, the accumulation of an RNA species in the cytoplasm is suppressed based on its homology to an introduced gene. This is known as RNA silencing and was first discovered in plants. PTGS is an epigenetic form of mRNA degradation essential in the defense of plants against infection by viruses (Waterhouse *et al.*, 2001; Klahre, *et al.*, 2002). In addition to this, it is believed that such a phenomenon normally function to regulate gene expression in plants (Vance and Vaucheret, 2001). This phenomenon has been observed in a diverse array of organisms, including ciliates, fungi, insects, nematodes, fish and mice (Mlotshwa *et al.*, 2002). The sequence of RNA degradation is triggered by double stranded RNA (dsRNA) and known to occur in a two-step process. In the first step, double stranded RNA (dsRNA) is processed by endonucleolytic cleavage into shorter, 21-25 nucleotide long sense and antisense units. These small RNAs, called shortinterfering RNA or siRNA, in the second step act as guide sequences to identify homologous transcripts and target them for destruction. A protein complex called RNA induced silencing complex (RISC) are essential for selecting the target RNA and effecting their degradation (Mlotshwa *et al.*, 2002).

Virus-Induced Gene Silencing systems

Based on the PTGS information, Virus-Induced Gene Silencing (VIGS) systems have been developed to study gene function in plants (Baulcombe, 1999). VIGS occurs when plants are infected with a virus carrying target sequences with homology to a host nuclear gene. The virus infection triggers the cytoplasmic degradation of any RNA with sufficient homology to the target sequence (Vance and Vaucheret, 2001). This activity results in post-transcriptional silencing of homologous nuclear genes and the phenotype of the plant silenced by VIGS for a particular gene mimics the phenotype of loss of function mutant. Many studies have demonstrated that VIGS is a powerful tool to determine gene function (Liu, *et al.*, 2002a; 2002b, 2002c, Liu, *et al* 2002; Vance and Vaucheret, 2001). Several plant viruses have been used to develop VIGS vectors such as tobacco mosaic virus (TMV), potato virus X, tomato golden mosaic virus (TGMV) and tobacco rattle virus (TRV) (Angell and Baulcombe,1997; Ratcliff *et al.*, 2001; Liu *et al.*, 2002). For efficient gene silencing, the VIGS vector should be able to infect the plant rapidly and systemically spread uniformly in the system. There should not be production of any strong suppressor of silencing in the plant system. From this context, TRV based VIGS system has been very successful, which can silence genes in all plant tissues including meristems and flowers. TRV based VIGS infects plants without any chlorotic or necrotic symptoms, which facilitates identification of phenotype.

Recently, Dr. S.P. Dinesh_Kumar's laboratory at Yale University (CT, USA) has developed an efficient VIGS vector using TRV (Liu *et al.*, 2002). The cDNA clones of RNA1 and RNA2 of TRV in a T-DNA expression cassette have been constructed with multiple cloning sites (MCS). The MCS included in RNA2 allows the cloning

of target gene sequences for VIGS. For PTGS, the *Agrobacterium tumefaciens* cultures harboring TRV-RNA1 and TRV-RNA2 constructs having the gene of interest are mixed and infiltrated into the leaves. Upon infection, the viral RNA gets synthesized from the T-DNA and the RNA transcripts then serve as templates for the further replication of viral RNA by the RNA dependent RNA polymerase encoded by RNA1. Systemic infections with in the plants by the virus bring about PTGS of the targeted plant host sequences.

Conclusion

The Virus-Induced Gene Silencing system is being considered as a good and attractive system to study gene function in plants. VIGS enables silencing of any specific gene if the gene sequence is known. The silencing here is conditional, thus loss of mutations due to organismal lethality will not occur. It is an excellent system to study gene function in plants that are recalcitrant to *Agrobacterium*-mediated transformation. This approach is fast, reliable and specific. Within a short time, the construction of a VIGS vector with host gene sequence, the silencing of this gene's expression, and the analysis of the phenotype can be performed.

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