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*As per decision of Council meeting held on May 03, 2014, Presidential Address will not be printed henceforth in Everyman’s Science as they are already printed in the above mentioned book.*
In Silico Toxicology Prediction: ADMET Property and QSAR

Quantitative structure–activity relationship (QSAR) methods and related approaches have been used to investigate the molecular features that influence the absorption, distribution, metabolism, excretion and toxicity of drugs. As the three-dimensional structures of several major ADMET proteins become available, structure-based (docking-scoriing) computations can be carried out to complement or to go beyond QSAR studies.

Traditionally, drugs were discovered by testing compounds synthesized in time-consuming multi-step processes against a battery of in vivo biological screens. Then the most promising compound is further studied for development by studying pharmacokinetic properties, metabolism and potential toxicity. Usually, poor pharmacokinetics, side effects and compound toxicity are frequent causes of late-stage failures in drug development.

Today, paradigm has been re-worked in several ways. The testing of drug metabolism, pharmacokinetics and toxicity is today done much earlier; that is, before a decision is taken to evaluate a compound in the clinic.

In response to these developments, a new approach to chemistry – combinatorial chemistry has been adopted to feed these highly efficient hit-finding machines. Combinatorial chemistry makes it possible to synthesize large series of closely related libraries of chemicals using the same chemical reaction, such libraries are then run through the HTS to find hits around which will be further studied.

As the capacity for biological screening and chemical synthesis have dramatically increased, so have the demands for large quantities of early information on absorption, distribution, metabolism, excretion and toxicity data which is together known as ADMET data. The ADMET profiling and prediction is mostly dependent on a number of molecular descriptors, for example, Lipinski's 'Rule of 5'. Recently a large number of articles have been reporting that it possible to do some prediction of the ADMET properties using the structural features of the molecules, utilizing several and multiple approaches. One of the most important approaches is the QSAR modelling of the data derived from their activity profiles and their different structural features.

The drug-like property optimization is another area of drug discovery process which enhances the chances of producing successful drugs. The term became commonly used following the pivotal work of Lipinski and colleagues. Their work examined the structural properties that affect the physicochemical properties of solubility and permeability and their effects on drug absorption. In this article a brief introduction of ADMET property and Descriptor is described.

The basic question before predicting drug-like property is which properties make drugs different from other chemicals?

Particularly influential example is to the analyse of the World Drug Index (WDI), which lead to Lipinski's 'rule-of-five' identifies several critical properties that should be considered for compounds with oral delivery in mind. These properties, which are usually viewed more as guidelines rather than absolute cut-offs, are as follows:

1. Molecular mass <500 Daltons (Da).
2. Calculated octanol/water partition coefficient (CLOGP) <5.
3. Number of hydrogen-bond donors <5 and

Since all numbers are the multiple of five that's why it is known as rule-of-five. These properties are then
typically used to construct predictive ADME models and form the basis for what has been called property-based design. To a certain extent, similar molecules can be expected to have similar ADME properties.

- First, a variety of \textit{in vitro} assays have been further automated through the use of robotics and miniaturization.
- Second, \textit{in silico} models are being used to assist in the selection of both appropriate assays, as well as in the selection of subsets of compounds to go through these screens.
- Third, predictive models have been developed that might ultimately become sophisticated enough to replace \textit{in vitro} assays and/or \textit{in vivo} experiments.

The need for ADMET information starts with the design of new compounds. This information can influence the decision to proceed with synthesis either via traditional medicinal chemistry or combinatorial chemistry strategies.

A deeper understanding of the relationships between important ADME parameters and molecular structure and properties has been used to develop \textit{in silico} models that allow the early estimation of several ADME properties.

We want to predict properties that provide information about dose size and dose frequency, such as oral absorption, bioavailability, brain penetration, clearance (for exposure) and volume of distribution (for frequency).

There are two aspects to consider:

1. Data modelling includes quantitative structure–activity relationship (QSAR) approaches.
2. Molecular modelling includes approaches such as protein modelling.

To understand the role of structure to govern effects could results in certain biological activity that is known as structure-activity relationship or SAR and an attempt to form a quantitative relationship between the biological activity and structure of chemicals then it is known as quantitative structure-activity relation or QSAR.

The purpose of \textit{in silico} studies includes following:

1. To predict biological activity and physio-chemical properties by rational means.
2. To comprehend and rationalize the mechanism of actions within a series of chemicals.
- Saving in the cost of product development.
- Reduction of animal tests will reduce the use of animals for experimental use.
- Other areas of promoting green and greener chemistry to increase efficiency and eliminate waste by not following leads unlikely to be successful.

The potential use of QSAR analysis and different areas where it can play major role is listed below:

- Integrating and harnessing new computational technologies and increasing speed and power of processing.
- Ability to react to new disease states (e.g. HIV).
- Ability to react to new toxicological problems for example: cardio-toxicity.
- Modelling the new problems with regards to the impact of chemicals on the environment.
- New and emerging issues, problems and opportunities for example: nanotechnology, properties of crystals, extension into other areas of chemistry (design of formulations).
- Integration with the-omics technologies to improve all areas of molecular design.

QSAR tool is broadly used for developing relationship between the effects of series of molecules with their structural properties. It is a dynamic area that integrates new technologies at a staggering rate. Some widely used tools for QSAR analysis are listed in the table.

A wide variety of descriptors for use in QSAR studies have been developed over the last 40 years. A subset of these descriptors is potentially useful for predicting ADME properties. Indeed, with the increased interest in the prediction of ADME properties, specifically tailored descriptors have already been reported.
<table>
<thead>
<tr>
<th>Software</th>
<th>Description</th>
<th>Company</th>
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<tbody>
<tr>
<td>clogP</td>
<td>Program for calculating log P_{oct-water} from structure</td>
<td>Tripos</td>
</tr>
<tr>
<td>Topomer CoMFA</td>
<td>3D Qsar tool that automates the creation of models for predicting the biological activity of compounds</td>
<td>Tripos</td>
</tr>
<tr>
<td>QSAR Pro</td>
<td>Used for evaluations of several molecular descriptors along with facility to the QSAR equation</td>
<td>VLife</td>
</tr>
<tr>
<td>Molegro Data Modeller</td>
<td>Principal component analysis, high-dimensional visualization, clustering and outlier detection</td>
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</table>

Good predictive models for ADMET parameters depend crucially on selecting the right mathematical approach, the right molecular descriptors for the particular ADMET endpoint, and a sufficiently large set of experimental data relating to this endpoint for the validation of the model. Some of widely used for ADMET property are listed below in a table:

<table>
<thead>
<tr>
<th>Software</th>
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<tbody>
<tr>
<td>VolSurf</td>
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<td>LeadScope, ToxScope</td>
<td>LeadScope</td>
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<td>ToxSys, QSARIS</td>
<td>SciVision</td>
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Computational chemists are now using ADMET filters in the very early stages of drug discovery, for example, in library design and virtual screening. Computational tools should allow chemists and drug-metabolism scientists to concentrate on compounds with the highest chances of meeting the required pharmacokinetic and safety criteria, and should contribute to a reduction in late stage compound attrition. Recent report on in silico technology estimates that ~10% of pharmaceutical R&D expenditure in 2006 will be on computer simulation and modelling, a figure set to rise to 20% by 2016. It seems clear that as a whole, the pharmaceutical R&D landscape will further change, and that computational ADMET will be part of this change.

Dr. (Mrs.) Vijay Laxmi Saxena

*Science is a beautiful gift to humanity; we should not distort it.*

---

A. P. J. Abdul Kalam
**BIO-REMEDIATION AND SUSTAINABILITY**

Anjali Bajpai*, Maya Sharma and Vidhya Raj

The heavy metal pollution is increasing due to anthropogenic activities. The treatment of polluted soil and water by sustainable technology is earnestly required. Some of the heavy metals are present in limited quantity on earth; hence the waste could be treated as their resource. Bioremediation is emerging as an effective technology for sustainable pollution control and recycling of heavy metals.

**INTRODUCTION**

The development of various sectors during the last century is a consequence of industrialization, urbanization and growing human population, which unfortunately has posed an adverse impact on the environment. The reason is the mentality of “greed over need”. The unsustainable and selfish human behaviour cannot be changed overnight, especially as emerging nations bring ever-larger populations to the table of consumption. This century must bring about a fundamental change in our attitude towards natural resources, which is essentially a socio-economic challenge. Further, the techno-scientific challenge must be accepted for improved waste management and rationalized utilization of resources.

Organic and inorganic wastes include metals and metalloids, some xenobiotic contaminants and salt leachates, sewage, sludge and other conventional wastes. Some redundant or back-up treatment may be necessary depending on the acuteness of toxicity to offset the variability of biological systems.

River pollution is becoming a serious problem worldwide. River water is easily polluted by domestic wastewater effluent, rainwater, agricultural run-off and industrial wastewater, which result in severe degradation of water quality. The river water becomes black with foul odour accompanied by decrease in fish population.

Sustainable technologies are available to revolutionise resource utilisation, which should be brought in use in time, so as to maintain our highly populated planet before the situation floods the alarming limit. Advances in technology such as nanotechnology, biotechnology and engineering, are finding possible solutions for the dismal situation involving water and soil contamination.

**HEAVY METAL POLLUTION**

The term heavy metal is strictly ascribed to transition metals with an atomic number of over 20 and specific gravity above 5 from a chemical point of view. In biological terms, “heavy” refers to a series of metals and also metalloids that can be toxic to both plants and animals, even at very low concentrations.

Soil contamination by heavy metals was originally restricted to metalliferous soils but in modern times it has become a general problem due to anthropogenic activities. Anthropogenic activities can be described as human activities such as burning of fossil fuel, increasing dependence on chemicals in agricultural fields, heavy industrialization and the utilization of industrial and urban effluents for irrigation in agricultural fields which is responsible for introducing and mobilization of heavy metals into the biosphere, ensuing in a serious threat to the environment and public health. Metal ions such as Zn\(^{2+}\), Mn\(^{2+}\), Fe\(^{2+}\), Cu\(^{2+}\), Pb\(^{2+}\), Cd\(^{2+}\), Ni\(^{2+}\) and Cr\(^{6+}\) present in agricultural inputs and sewage sludge gradually build up their concentrations in soil above their threshold.

**HAZARDS ASSOCIATED WITH LOW CARBON TECHNOLOGY**

Climate change due increasing carbon dioxide
concentration in environment and depleting fossil fuel led the drive for "low carbon technology" to enjoy a sustainable high standard of living in the future. Unfortunately, as new technologies are developed to tackle one challenge, we are creating another through resource deficit. Even many of the new low carbon technologies, such as wind turbines, electric cars, energy saving light bulbs, fuel cells and catalytic converters, etc. require rare and precious metals for their manufacture. However, traditional supplies of these elements are running out. For instance, reserves of Indium, which is vital for LCD screens, solar cells and semiconductors, may be used up in the next 13 years. Palladium and platinum are unique and finite elements, which are being quickly dispersed throughout our environment, making it more costly and difficult to recover them. Further there are no bio-derived alternatives for them. Additionally, it is not just the exotic elements that are getting more difficult to access, many other elements that play a crucial role in our lives, including phosphorous, aluminium and copper, are being depleted at an alarming rate. Some low carbon technologies are actually broadening concerns over future elemental sustainability for a wide range of elements. In order to address the rapid dispersion of metals, such as indium and silver, more innovative technologies are required to recover them and to turn wastes into resources. A multi-disciplinary blend of chemistry, extractive metallurgy, engineering and biotechnology is required to realise this ambition.

SUSTAINABLE PROCESSES FOR WASTE MANAGEMENT

Conventional processes for water treatment become inadequate and obsolete with the identification of progressively increasing contaminants in the water. Therefore, there is rapid development of novel water treatment technologies, to overcome the challenges associated with traditional processes, which hold great promises in providing green alternatives for better protection of public health and the environment. 'Green chemistry' takes account of energy, materials, resource economics, generation of dangerous secondary materials and finally, the life cycle of the products and their practical recycling into new materials. This provides the most effective way to reduce the harmful effects of water treatment processes.

Traditional approaches to municipal water monitoring barely include procedures for toxic heavy metals testing. Land fill is most common waste disposal practice, hence soil has become a major source of metal(loids) reaching food chain, mainly through plant uptake and animal transfer. The implications of contaminated soils on human and animal health catalapulated interest in developing technologies to remediate contaminated sites. Different chemical treatments are conventionally applied for contaminated sediments before reuse in other environmental settings. Environment-friendly techniques developed for soils and other environmental matrices have been investigated for applications over sediments. Biotechnological approaches are gaining prominence in this field and they are often considered as a promising strategy for the eventual treatment of contaminated sediments.

SUSTAINABLE MANAGEMENT OF SOLID MATRIX AS A RESOURCE

The demand for metals remains ever increasing with the advancement of the industrialized world. However, worldwide reserves of high grade ores are close to depletion. An evolution is in progress towards environmentaly sustainable solutions for management of solid wastes, from high impact (thermal treatment and cementification) to low impact (chemical and biological) processes. A new initiation is necessary for a really sustainable management of dredged sediments, in order to consider solid matrix as a resource rather than an environmental problem, taking into account its richness in oligoelements, microelements and organic nutrients.

Municipal solid waste (MSW) offers one of the largest potential resources for recycling and recovery of a multitude of elements. Conventional
remediation strategies include in-place sediment remediation strategies (e.g. in situ-capping) and relocation actions, in particular, landfill disposal and dumping at sea are still widely applied. Both these options are becoming unsustainable, due to problems associated with contaminant transport pathways, the uncertainties about long-term stability under various environmental conditions, the limited space capacity, costs and environmental compatibility. Japan has shown interest in the potential of its MSW as an 'urban mine' with national concerns over securing future natural resources, particularly due to its current utilisation of 30% of the world's annual consumption of rare metals.

BIOREMEDIATION

Bioresmediation is a natural process which relies on soil microorganisms and higher plants to alter metal(loid) bioavailability and can be enhanced by addition of organic amendments to soils. These organic amendments that are low in metal(loid)s can be used as a sink for reducing the bioavailability of metal(loid)s in contaminated soils and sediments through their effect on the adsorption, complexation, reduction and volatilization of metal(loid)s. In situ bioremediation is a highly promising and cost-effective technology for remediation of contaminated soil, groundwater and sediments. The characteristics of cost-effectiveness, environmental friendliness and fewer side-effects have centred-staged the development of plant based remediation technologies for contaminated soils.

Heavy metal-polluted soil environments can be reclaimed by chemical treatment, soil amendments or phytoaccumulators. Out of these, phytoremediation is a cost-effective and sustainable method of reclamation of metal-polluted environments. Plants may survive under high metal concentrations by sequestering metal ions into their tissues, exposing secondary consumers (human or animals) to the risk of metal toxicity.

Several phytoremediation technologies such as phytoextraction, phytostabilization, phyto-degradation, rhizofiltration, etc., are plant-based remediation technologies that have recently come to light to clean up contaminated soil and water. Phytoextraction is the most important phytoremediation process which helps to recover metal from the soil using non-food crops. The genetic and physiological potential of some plant species makes them effective in accumulating, translocating and tolerating high concentrations of metals and are used for phytoremediation. Phytoremediation has opened up the prospect for a cost efficient, practical and environmentally sound approach to clean-up low-level radiation waste sites.

The use of microalgae-based bioremediation is a viable method for treating waste streams and lipid-production. A heavily polluted river can be remediated using a combined engineering approach of aeration, microorganisms, biological aerated filtration, artificial biofilms and ecological floating beds. Microorganism assisted phytoextraction, using plants and bacteria to actively extract metal ions, has great potential, especially for moderately polluted sites.

Phytosorption, that is, the use of plants and their associated rhizospheric microorganisms to remove contaminants such as heavy metals from water, is a field offering a competitive and sustainable solution. The selection of the plant species that offer unique and intrinsic characteristics for achieving a maximum removal of metals is to be considered a critical initial step. A second critical decision is related to the type of phytosorption system to be selected for obtaining a maximum pollutant removal percentage.

ECO-REMEDICATION

In context of climate and anthropological changes, it is necessary to provide an integrated approach for the prevention and control of metal pollution, in order to limit its impact on water resources, biodiversity, trophic network and human health. For this purpose, introduction of constructed wetlands (CWs) between natural aquatic ecosystems and industrialised zones or catchments is a hopeful strategy for eco-remediation.
The key to apply sustainable ecosystems is the knowledge of the genetic and proteomic diversity, which is necessary for selection of plants and other organisms with optimal activities to transform or accumulate pollutants.  

REFERENCES


INNATE IMMUNE RECEPTORS — AN EVOLVING THERAPEUTIC PARADIGM

Yashpal S. Malik, K. Karthik, K. Dhama and R.K. Singh

Advancement in our understanding of the host defence mechanisms probed innate and adaptive immune system, has led us to improve therapeutic protocols through the modulation of immune factors. Among the plethora of pattern recognition receptors (PRRs), the Toll-like receptors (TLRs), are receiving considerable attention as potential regulators and controllers of the immune response through their ability to recognize pathogen-associated molecular patterns (PAMPs). The discovery that endogenous ligands as well as microbial components are recognized by TLRs, raised interest in these receptors as potential targets for the development of new therapies for multiple diseases. This chapter focuses on the current uses of these innate immune TLRs in various disease conditions.

INTRODUCTION

Innate immune system, essential for the host survival, offers the first line of defense by recognizing and responding to pathogenic threats. During infection, the innate immune system recruits immune cells to the site, activates a number of processes to identify the cause of infection and subsequently ensures these are removed. Finally, the innate immune system activates the adaptive immune system to accomplish immunity for future infections. The innate immune system consists of phagocytic cells, complement proteins, natural killer cells, and anatomic barriers such as the skin and mucosal surfaces. Innate immune system recognizes microorganisms via a limited number of germ line-encoded Pattern-Recognition Receptors (PRRs) which bind conserved molecular structures found in large groups of pathogens, termed Pathogen-Associated Molecular Patterns (PAMPs). There are various groups of PRRs, which can be secreted, expressed on the cell surface, or are resident of intracellular compartments. These are expressed constitutively in the host and detect the pathogens regardless of their life-cycle stage and are independent of immunologic memory. Recent knowledge on germ line-encoded innate receptors, as a first line of host defence, has revolutionized our understanding of innate immunity. The trigger for the breakthrough in this field is viewed from the discovery of “Toll Like Receptors” (TLRs).

TOLL and TOLL LIKE RECEPTORS

Since first being described in the friut fly Drosophila melanogaster, TLRs have been proven to be of great interest to immunologists and researchers interested in related fields. The word 'TOLL' was originally coined by Christiane Nusslein-Volhard, while working on a weird-looking fly larva in which the ventral portion of the body was underdeveloped. Her spontaneous comment was “Dus war ja toll!” meaning “That was weird!” and proposed the name TOLL for the mutated gene. For the discovery she was honored with Nobel Prize in 1995. A decade later, TOLL was found to play a crucial role in antifungal defense. Later, mammalian proteins structurally related to Drosophila 'TOLL' were identified and named “TOLL-like receptors”. As shown in Fig.1, the TLR is made up of two domains, the extracellular domain consists of multiple LRRs (leucine rich repeats), which provides a platform for pathogen binding and intracellularly TLRs hold a signaling domain known as the TIR (Toll/IL-1 receptor). These TLRs are expressed on various immune cells, including macrophages, dendritic cells (DCs), B cells, specific types of T
Fig. 1. Structural organization of mammalian TLRs with signal peptide sequence, LRRCT (cysteine clusters on C-terminal side of LRRs), LRR domain in the ECD domain, transmembrane region and last TIR domain in cytoplasmic region.

Cells, and even on non-immune cells such as fibroblasts and epithelial cells. Expression of TLRs is not static but rather is modulated rapidly in response to pathogens, a variety of cytokines, and environmental stresses. TLRs are type 1 integral membrane glycoproteins recognizing a variety of conserved microbial PAMPs derived from bacteria, viruses, protozoa and fungi1. Through studying the structural homologies of these pathogen sensors across the species, a picture of an evolutionarily conserved sensing system is beginning to emerge. In the past, 13 TLRs have been identified, of which 1-9 are common in mice and human, TLR10 in humans, whereas 11-13 are unique to mice.

TLR LIGANDS AND SIGNALING

The lipid-based structures form the ligands for TLR2 and TLR4. These ligands are bacterial or mycobacterial lipopeptides or glycolipid-phosphatidylinositol anchors from parasites, both of which are recognized by TLR 2 (in combination with TLR1 or TLR6), and bacterial LPS (lipopolysaccharide), which is recognized by TLR4.

The microbial ligands for various TLRs are described in Table 1. The TLR3, TLR7, TLR8 and TLR9 all recognize viral and/or bacterial nucleic acids. The dsRNA is recognized by TLR3 and the unmethylated CpG motifs in DNA by TLR9 while TLR5 recognizes bacterial flagellin.²

Microbial ligands engagement with TLRs triggers the activation of signaling cascades, leading to the induction of genes involved in antimicrobial host defence. After ligand binding, TLRs dimerize and undergo conformational changes required for the recruitment of TIR-domain-containing adaptor molecules to the TIR domain of the TLR. Two adaptors play major role in antiviral TLR signaling, namely MyD88 and TRIF³. The MyD88 adaptor is critical for the signaling from all TLRs except TLR3. Stimulation with TLR3, TLR7, TLR8 and TLR9 ligands induces type I IFN production in addition to pro-inflammatory signals (Fig. 2).

Fig. 2. TLR signaling pathway for viral ligands

<table>
<thead>
<tr>
<th>TLR</th>
<th>Microbial ligands</th>
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<tbody>
<tr>
<td>TLR1/TLR2</td>
<td>Triacyl lipopeptide (Pam3 CSK4)</td>
</tr>
<tr>
<td>TLR2/TLR6</td>
<td>Diacyl lipopeptides (Pam2 CSK4) lipoteichoic acid, zymosan, porins, MALP2, bacterial peptidoglycan, lipourabinomannan</td>
</tr>
<tr>
<td>TLR</td>
<td>Microbial ligands</td>
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<tr>
<td>------</td>
<td>---------------------------------------</td>
</tr>
<tr>
<td>TLR3</td>
<td>dsRNA</td>
</tr>
<tr>
<td>TLR4</td>
<td>LPS; mannann; phospholipids, envelope proteins (MMTV, RSV)</td>
</tr>
<tr>
<td>TLR5</td>
<td>Flagellin</td>
</tr>
<tr>
<td>TLR7</td>
<td>ssRNA (viral)</td>
</tr>
<tr>
<td>TLR8</td>
<td>ssRNA (viral)</td>
</tr>
<tr>
<td>TLR9</td>
<td>DNA (bacterial/viral)</td>
</tr>
<tr>
<td>TLR10</td>
<td>unknown</td>
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</table>

**ROLE OF TOLL-LIKE RECEPTORS**

TLRs are found on surface and endosomal membrane of different types of cells like macrophages, dendritic cells (DC), neutrophils, mast cells, mucosal epithelial cells and endothelial cells, and fibroblasts. DCs are specialized cells of the immune system that are produced continuously in the bone marrow and traffic through different tissues in an immature state. DCs are critical for the initiation of all adaptive immune responses because these cells alone are capable of stimulating antigen naive T cells. Each cell type is associated with specific TLRs and the response of the immune system differs depending on which cell type is activated and the specific TLRs that are activated. The expression of TLRs and their influence on the major antigen presenting cells in the adaptive immune response has led to a better understanding of the interaction between the innate and adaptive immune responses.

TLR signaling in DCs, macrophages, and B cells affect their activation and differentiation. TLR signaling leads immature DCs to express co-stimulatory molecules and increase their antigen presenting function. For example, lipopolysaccharide (LPS) can increase this co-stimulatory (CD80, CD86) major histocompatibility (MHC)-Class II cell molecules as well as induce the production of cytokines such as IL-12, IL-15, and IL-18. DC production of IL-12 shifts T<sub>h</sub>0 development towards T<sub>h</sub>1 differentiation. In addition, LPS increases antigen loading onto MHC-Class II molecules. Therefore, signaling that occurs during the innate immune response also modulates the adaptive immune response.

**TLRS AS NOVEL THERAPEUTIC TARGETS**

TLRs are the extensively studied PRRs and their mechanism of prompting innate and humoral immunity is studied better. The role played by TLR during entry of pathogens has attracted researchers to target these PRRs for management and control of diseases. These innate receptors identify pathogen-derived factors and activate subsequent signaling pathways which triggers stimulation of transcription factors like nuclear factor-κB and interferon regulatory factors ultimately induces the immune and inflammatory genes (cytokines as tumor necrosis factor-α and type I interferon). A number of drugs are at different stages of clinical trials. On pharmacokinetic perspective, the wide tissue distribution of TLRs indicates complexity in deciding whether an agonist or an antagonist will be most effective therapeutically for specified indications and disease types. Recently, novel compounds have been designed, which act as potent TLR agonists and can be used as therapeutics. Some of the recent developments of novel therapeutics that target TLRs or their pathways in various diseases are highlighted here.

**(i) Antiviral therapy**

The viral components such as viral DNA, double stranded RNA, single-stranded RNA, and surface glycoproteins are recognized as PAMPs by TLRs to induce innate immune response as well as adaptive immunity through production of co-stimulatory molecules. Among the TLR family members, TLR3, TLR7, TLR8, and TLR9 are involved in the recognition of viral nucleic acid and agonists for these TLRs have shown promise to treat infectious
viral diseases. The induction of type I IFNs and IFN-dependent antiviral mechanisms accounts for this activity; however, additional mechanisms may contribute, such as enhancement of NK cell cytotoxicity and virus-specific T-cell responses. TLR7 agonists are the most advanced, with imiquimod being approved for treatment of genital warts caused by human papilloma virus. Orally available compounds from Anadys and Sumitomo are being evaluated for treatment of chronic hepatitis C virus (HCV). The ssRNA viruses and some synthetic antiviral imidazoquinoline components such as R848, Imiquimod, etc. and some guanine nucleotide analogs (loxoribine) are recognized by TLR7 and TLR8. Activation of TLR7 has also been found beneficial in patients infected with hepatitis C virus (HCV). Recently, the HIV-1 gag protein has been conjugated to the TLR7/8 agonist and shown to improve the magnitude and quality of T cell responses in non-human primates. The TLR7 agonist R-848 can enhance HBsAg-specific humoral and cellular immune responses and together with CpG oligonucleotide may be used as adjuvants for therapeutic and prophylactic HBV vaccine formulations. The TLRs use in infectious diseases is given in Table 2.

Table 2. TLRs directed for various viral infectious diseases.

<table>
<thead>
<tr>
<th>Drug/compound</th>
<th>Target viral infection</th>
<th>TLR targeted</th>
<th>Firm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aldara (imiquimod cream)</td>
<td>Papilloma virus induced genital warts</td>
<td>TLR7</td>
<td>3M Pharma</td>
</tr>
<tr>
<td>SM36032</td>
<td>Hepatitis C</td>
<td>TLR7</td>
<td>Sumitomo</td>
</tr>
<tr>
<td>CpG B and CpG-ODN</td>
<td>Hepatitis C</td>
<td>TLR9</td>
<td>Dynavax</td>
</tr>
<tr>
<td>IMO-2125, CpG-ODN</td>
<td>Hepatitis C</td>
<td>TLR9</td>
<td>Idera Pharmaeuticals</td>
</tr>
<tr>
<td>HIV</td>
<td>TLR3</td>
<td></td>
<td>Hemispheres</td>
</tr>
<tr>
<td>R851 (topical treatment)</td>
<td>Human Papilloma virus</td>
<td>TLR7</td>
<td>3M Pharma / Takeda</td>
</tr>
</tbody>
</table>

The TLR3 recognizes dsRNA viruses and induces interferon and co-stimulatory molecules. In addition to dsRNA ligand, synthetic analog polyinosine-poly(C) is also potent inducer of interferons. TLR3 is also expressed in a variety of epithelial cells, including airway, uterine, corneal, vaginal, cervical, biliary, and intestinal epithelial cells, which function as efficient barriers to infection. Expression of TLR3 is rapidly and dramatically upregulated by treatment with poly I: C or IFN-a/b. Furthermore, TLR3 is strongly expressed in the brain, specifically in astrocytes and glioblastoma cell lines, indicating a specific role in the brain and/or in the response to encephalitogenic viruses. Expression of TLR-4 in the nasal associated lymphoid tissue (NALT) of cattle infected with Foot and Mouth disease virus was greater than uninfected animals. TLR3 agonists may also have useful antiviral activity, with clinical development currently focused on H1IV.

A dose-dependent decrease in HCV RNA levels has also been observed in phase I studies with a CpG-ODN of the high IFN-inducing C-class. Prophylactic treatment with CpG-ODN can provide protection from challenge with a number of viral and bacterial pathogens for a period of days to a few weeks, and may confer protection against biological warfare agents. DNA viruses as herpes simplex virus 1 (HSV-1), HSV-2, and murine cytomegalovirus, contain genomes that are rich in CpG-DNA motifs, are recognized by TLR9 and activate inflammatory cytokines and type I IFN secretion. Poxviruses also stimulate various TLRs and induce innate as well as adaptive immunity because of their genomic DNA rich in unmethylated CpG motifs, ligand for TLR9 and viral glycoproteins, ligands for TLR2 and 4.

Some viral-envelope proteins also recognized by TLR4 or TLR2 and results in the production of pro-inflammatory cytokines, but not type I IFNs, implying that the response leads to the inflammation rather than specific antiviral responses. The fusion (F) protein from RSV has been identified as a viral component that activates TLR4. The envelope protein of mouse mammary tumor virus also
activates TLR4. Env directly activates B cells via TLR4 to allow an initial round of infection, and MMTV enhances the expression of its entry receptor, CD71, on DCs to facilitate virus entry. Thus, the MMTV-TLR interaction may favor the virus and represent a strategy to subvert the antiviral response. TLR2 is also activated by viruses or viral components such as measles virus (MV) haemagglutinin protein, human CMV, and HSV-1. It has been suggested that TLR2-mediated cytokine responses to HSV-1 are responsible for a significant portion of the morbidity and mortality associated with HSV-1 infection. Thus, various viral components are detected by TLRs, and this leads to the vigorous production of type I IFNs as well as pro-inflammatory cytokines.

(ii) Vaccine Adjuvantation

Availability of safe and efficacious vaccines remains the major global challenge. The effective therapeutic vaccines contain two primary constituents, antigen and adjuvant and successfully induce antibodies, interferons, cytokines/chemokines, cytotoxic T lymphocytes and/or NK cells. For these reasons, TLR ligands have become a focus for their potential use as adjuvants in vaccine formulations. By physically linking the TLR ligand and antigen, each antigen would achieve optimal antigen processing and presentation. Synthetic chimera molecules consisting of TLR agonists and target antigens have been found effective in induction of CTL to eliminate target cells in vivo. Extensive knowledge of TLR-dependent viral recognition may lead to the generation of new adjuvants and antiviral agents. CpG-oligomeric nucleotide (CpG-ODN), the common TLR9 agonist has also shown substantial potential as vaccine adjuvant, and as monotherapy or combination therapies for the treatment of cancer, infectious and allergic diseases. The clinical trials data has indicated that CpG-ODNs have antitumor activity as single agents and enhance the development of antitumor T-cell responses when used as therapeutic vaccine adjuvants.

A clear understanding of the molecular pathways involved in stimulation of the immune system by TLR agonist and other molecular adjuvants have opened a new dimension in the vaccination era. There is an enormous research activities going on to find suitable molecular adjuvant, but many of them are yet to reach clinical phase trials. These studies further substantiates the importance of administering vaccines with adjuvants in the form of TLR ligands as they will be capable of eliciting their positive effects across the entire spectrum of innate and adaptive immunity. As new and new synthetic and natural compounds are being tested as vaccine adjuvants, there is a need for proper clinical trials of these candidate adjuvants before it is released for human or veterinary use. Table 3 highlights on some of the TLRs targeted for improving the vaccine or adjuvantation.

<table>
<thead>
<tr>
<th>Indication</th>
<th>Drug/compound</th>
<th>Targeted TLRs</th>
<th>Firm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatitis B</td>
<td>Fendrix (HBV antigen and MPL adjuvant)</td>
<td>TLR4</td>
<td>Glaxo Smith Kline</td>
</tr>
<tr>
<td>Hepatitis B</td>
<td>Supervax (HBV antigen and synthetic MPL RC-529)</td>
<td>TLR4</td>
<td>Dynavax Technologies</td>
</tr>
<tr>
<td>Hepatitis B</td>
<td>Heplisav-HBV surface antigen and CpG-ODN 1018 ISS</td>
<td>TLR9</td>
<td>Dynavax Technologies</td>
</tr>
<tr>
<td>Human papilloma virus</td>
<td>Cervarix (HPV-16 and HPV-18 L1 virus-like particles with aluminium hydroxide MPL adjuvant)</td>
<td>TLR4</td>
<td>Glaxo Smith Kline</td>
</tr>
<tr>
<td>Influenza</td>
<td>Fusion proteins of flagellin to haemagglutinin or M2e</td>
<td>TLR5</td>
<td>Vaccinate</td>
</tr>
<tr>
<td>Influenza</td>
<td>Influenza antigens and CpG-ODN</td>
<td>TLR9</td>
<td>Dynavax Technologies</td>
</tr>
<tr>
<td>HIV</td>
<td>HIV Gag protein coupled to imidazoquinoline compound 3M-012</td>
<td>TLR7/8</td>
<td>NIH vaccine research center</td>
</tr>
<tr>
<td>General vaccine adjuvants</td>
<td>E60209(synthetic agonists)</td>
<td>TLR4</td>
<td>Eisa/SanoFil Pasteur</td>
</tr>
</tbody>
</table>
(iii) Allergic intervention

In the allergic condition an inappropriate TH2 T-cell response occurs to otherwise harmless environmental antigens. TH2 cell development and function can be counter balanced by cytokines from TH1 T cells. There is substantial evidence from animal models that TH1 activity can modulate allergy and asthma. Because of their ability to induce strong TH1 responses, TLR4 and TLR9 agonists are being developed for treatment of allergic rhinitis (Table 4 enlists TLR based anti-allergic compounds).

<table>
<thead>
<tr>
<th>Condition</th>
<th>Compound</th>
<th>Target</th>
<th>Firm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allergic rhinitis (multiple allergens)</td>
<td>Pollinex</td>
<td>TLR4</td>
<td>Allergy therapeutics</td>
</tr>
<tr>
<td></td>
<td>Quatro (modified allergens combined with MPT; ragweed, grass, tree, pollen, house dust mite allergy)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allergic rhinitis (ragweed)</td>
<td>Tolamba (Amb a l ragweed allergen with covalently linked CpG B class ODN 1018ISS)</td>
<td>TLR9</td>
<td>Dynavax Technologies</td>
</tr>
<tr>
<td>Allergic rhinitis (ragweed)</td>
<td>CRX-675 (given intranasal before intranasal challenges with ragweed allergen)</td>
<td>TLR4</td>
<td>Glaxo Smith Kline</td>
</tr>
<tr>
<td>Asthma</td>
<td>1018 ISS inhaled (CpG B ODN)</td>
<td>TLR9</td>
<td>Dynavax Technologies</td>
</tr>
<tr>
<td>Asthma</td>
<td>Second generation CpG-ODN</td>
<td>TLR9</td>
<td>Dynavax Technologies/AstraZeneca</td>
</tr>
<tr>
<td>Asthma</td>
<td>AEI0675(CpG-ODN)</td>
<td>TLR9</td>
<td>Coley Pharmaceuticals/SanoBi-Advance</td>
</tr>
</tbody>
</table>

Both types of agonists have been administered along with allergen to facilitate specific allergen immunotherapy. There has been significant clinical development with CpG-ODN covalently linked to a specific allergen. Linkage ensures that both the allergen and the TLR9 agonist are efficiently captured by the same DC, and the large ODN groups block cross-linking of allergen-specific IgE, resulting in reduced local and systemic reactions. The effectiveness of this approach has been reported in patients allergic to ragweed, and the expected shift in TH2/TH1 ratio has been observed. Intervventional success in this area is going to generate huge benefits to the allergic population which is on rise in the developing world.

(iv) Cancer therapy

Several TLR ligands show significant promise for the treatment of cancer, and the TLR7 agonist imiquimod is currently approved for treatment of superficial basal cell carcinoma. BCG, an agonist of TLR2, TLR3, TLR4, and to some extent TLR9 and MPL, a TLR4 agonist are the others approved by FDA for treatment of cancer. BCG has been used successfully against bladder cancer. BCG combined with whole lysate of tumor cells as vaccine has been used for treatment against colorectal cancer, renal cell cancer and melanoma. Imiquimod is used against skin tumors like basal cell carcinomas. 852A, another TLR7 agonist has been used against metastatic cancers of breast, ovaries, endometrium and cervix. Although monotherapy with TLR agonists may provide a benefit in certain types of cancer, interest is centered on the potential to complement existing modes of therapy with radiation, monoclonal antibodies or cytotoxic drugs. Evidence is provided by extensive studies of CpG-ODN in rodent models of cancer, and the susceptibility of human tumors to enhanced innate immunity has been demonstrated with human tumors transplanted into immunodeficient mice. The potential of TLR to act as adjuvants for therapeutic vaccination against tumor-associated antigens represents yet another TLR-based approach to cancer.

(v) Sepsis and autoimmunity

TLR antagonists currently under development are structural analogs of agonists and probably bind to the receptor so that it fails to signal. TLR
antagonists appear quite promising for a number of inflammatory and autoimmune diseases\textsuperscript{16}. The most obvious use for a TLR antagonist is to inhibit TLR4 recognition of LPS, the key triggering event in Gram-negative bacterial sepsis. Two lipid A analogs that act as potent antagonists of TLR4 have advanced into clinical trials for sepsis. The innate immune system faces the same fundamental challenge as the adaptive immune system—distinguishing self from non-self-antigens. There is now considerable evidence that self-recognition through TLR can occur and can contribute significantly to autoimmunity\textsuperscript{16}. Endogenous ligands have been identified for all human TLR except TLR5 and TLR10. Nearly all of these endogenous ligands are molecules released from damaged tissues or apoptotic cells. Thus, many different types of tissue damage may produce elevated concentrations of endogenous TLR ligands, leading to a continuing cycle of chronic activation and tissue damage by TLR-activated effector mechanisms. These endogenous ligands may further act as adjuvants to stimulate auto-reactive T and B cell responses.

**TLR RESEARCH WORK AT IVRI**

The research work on these innate immune receptors was undertaken under an Indian Council of Agricultural Research (ICAR) funded NAIP project on “Toll-like receptors in farm animals—Evolutionary lineages and application in disease resistance”. The focus of the project was on farm animals. We have successfully cloned and sequenced TLR genes of farm animals with their genetic and functional characterization. Additionally, the evolutionary relationship of Yak and Mithun TLRs with other animal species has also been clarified. The modelling of TLR with their ligands revealed differences in their ligand binding affinities along with differences in expression profiles and levels of TLR mRNAs among different tissues and organs. Significant changes were identified in LRR and TIR regions of several TLRs which could be reason for higher susceptibility of farm animal species to pathogens.

The role of TLR genes in conferring species-specific disease resistance was further confirmed using disease resistant models wherein buffalo and goats are either susceptible or resistant to bacterial, viral and parasitic diseases with convincing results as the innate disease resistance of animals was found related to higher levels of TLR mRNA expression.

**FUTURE DIRECTIONS**

Based on the rapid progress in TLR research, there is no doubt that an increased understanding of how TLRs and viruses interact at the molecular, cellular and whole animal levels, will give rise to many therapeutic opportunities. As we begin to understand more about innate immunity, including the role of TLRs, both for defending the body against invading pathogens, and for repairing damage caused by disease or injury, perhaps we will learn to harness this knowledge in the development of effective therapeutics. There have been some successes, but still this field of immunology is relatively new. As such, it would be exciting to see these innate molecules and future they hold.

**REFERENCES**

NANO IN OUR DAILY LIFE

Zeba Khanam\textsuperscript{1}, M.G.H. Zaidi\textsuperscript{2} and Vir Singh\textsuperscript{1}

Nanotechnology is now well-established and has progressively entered in the everyday life, conquering an increased importance in many fields. The close link of nanotechnology with nature like biomimetics, mimicking biology or natural concepts has been explained by various researchers. As an emerging strategy for development, nano-based ingredients have found a place as consumer products in the market such as paints, building materials, cosmetics and in medical treatment, the food industry and so much more. In fact, it’s becoming increasingly harder to keep track of where nanotech isn’t. We are using it in our daily lives and not even realizing it. Thus, efforts have done to compile the small wonders of nanotechnology that may already be in our world and some that may be on the horizon.

INTRODUCTION

Nanotechnology is the science and technology of very small things – in particular, things that are less than 100 nanometres in size. One nanometre is one billionth of a metre. A human hair is about 50,000 nanometres wide. Nanometre is a special point in overall length scale because it is the junction where the property of material changes as their size approaches the nanoscale. The interesting and sometimes unexpected properties of nanoparticles are due to the large surface area of the material. At this size scale, everything, regardless of what it is, has new exotic properties and these make “Nano” so fascinating!

The area of nanoscience and nanotechnology has become increasingly important in recent years. Many research groups all over the world are now involved in the preparation, characterization and evaluation of a wide range of nanostructures materials occurring at the size scale between 1nm and 100nm. This is the range that encompasses both the smallest artificial structures and ubiquitous molecules of the natural world and a wide variety of applications of these materials are expected in various branches of Science & Technology comprising Physics, Chemistry, Biology, Materials Science, Medicine, Computational Science, Environmental Science, Management Science etc. and thus providing a profound impact in our daily lives\textsuperscript{1-4}.

Nanotechnology, often inspired by the natural world, plays a big part in the manufacture of many familiar products. It is changing every part of our lives from the medicines we take, to the cosmetics we wear, the phones and laptops we use, the bikes we cycle and the clothes we wear. We can even see nanoscience in nature – peacock feathers and butterfly wings contain nano-scale features which provide iridescent colours. We are using it in our daily lives and not even realizing it. Nanotechnology has found a place in consumer products, medical treatment, the food industry and so much more. In fact, it’s becoming increasingly harder to keep track of where nanotech isn’t.

As we wake up in the morning, nanotechnology is probably the farthest thing from our mind. Yet throughout the day at every step we have unknowingly encountered it. From the wrinkle-free shirt and sunglasses we wear to computer hard drives and even cosmetic products, to the way to office, nanotechnology is there. The nanoparticle in the bumper of car reduces weight. Other side, nanoparticles boosted our sunscreen’s ability to reflect harmful ultraviolet radiation, rendered shirt with that just-ironed look and armoured our designer...
shades against unwanted scratches. The gadgets also used nanotechnology to store our snaps and songs on their respective hard-drives and flash memory. Nanotechnology is an inescapable part of modern everyday life, both on holiday and at office. “There are things we’ve been using for a long time which contain nanosize components, like the lasers in DVD and CD players,” says Milo Shaffer, head of the London Centre for Nanotechnology. Yet most of the time it goes unnoticed. “On the whole people aren’t very aware of the nanotechnology all around them,” Shaffer said.

RESEARCHERS FOUND NANOTECHNOLOGY IN NATURE

"Nature is all about nanoscale structures. It starts with the cell,” explains Julian Vincent, a former biologist and now professor of mechanical engineering at the University of Bath. "Biology plays around with the molecular scale all the time, it’s the level at which all biological reactions occur," he adds. Silk is a prime example of naturally occurring nanotechnology. "Silk is strong because of the way its molecules are aligned into a set of cross-links," says Vincent. Kevlar, used in everything from flak-jackets to frying pans, was constructed by engineering its constituent molecules in a similar fashion. Mimicking nature’s nanotech is becoming big business. Teams of researchers have turned to geckos and mussels in order to develop adhesives that bind to dry and wet surfaces alike. They’ve drawn inspiration from nanofibres in the geckos’ foot hairs, which allow the lizards to cling upside down on inclined surfaces, and the nanoscale structures used by mussels to “glue” themselves to rocks despite being underwater. Similarly, non-reflective materials have been improved by imitating the nanostructures found in the wings of cicada insects. Their wings contain small projections, spaced about 200 nanometres (a nanometre is equivalent to one billionth of a metre) apart, which allow 98% of light to pass through them. Nanostructures are also responsible for the brilliant white colouring of the cyphochilus beetle. The arrangement of molecules within the beetle’s scales scatters almost all incoming light. Mimicking this molecular arrangement in made-made materials would eliminate the need for potentially toxic pigments, which are often currently used to create white paint and paper. Plants too are big exploiters of natural nanotech. Nanostructures on the surface of lotus leaves repel water which carries away dirt as it rolls off the leaf, allowing the lotus to remain spotless despite growing in muddy water. This “lotus effect” is the basis behind self-cleaning windows. But rather than shedding water, beetles in the Namib desert are using a series of alternating waxy and non-waxy nanostructures to capture precious moisture from the early morning fog. Applying the idea to buildings could allow them to trap moisture for use inside.

Whether in your office, home or while sunning yourself on holiday, it is impossible not to encounter technology based on the manipulation of the very small. Many technologies in the modern world rely on nanostructures, often inspired by evolution in the natural world. But there is much untapped potential left to explore. "The overlap between the way nature solves these problems and the way we do, using technical solutions, is only 10-20%," Vincent explains. "I’d like to see a world where we can truly utilise the tried and tested methods nature has employed," he says.

APPLICATIONS IN VARIOUS FIELDS

Though nanotechnology is a relatively new science, it already has numerous applications in everyday life, ranging from consumer goods to medicine to improving the environment. Nowadays nanomaterials are progressively entering in the everyday life, conquering an increased importance in many fields of technology; nano-based ingredients can be found in many products on the market such as paints, building materials, cosmetics and also food. Some are covered below.

**Medicine:** One application of nanotechnology in medicine currently being developed involves
employing nanoparticles to deliver drugs, heat, light or other substances to specific types of cells, such as cancer cells. Particles are engineered so that they are attracted to diseased cells, which allow direct treatment of those cells. This technique reduces damage to healthy cells in the body and allows for earlier detection of disease. For example, nanoparticles that deliver chemotherapy drugs directly to cancer cells are under development. Drugs containing dendrimers are targeted delivery.

**Electronics:** Nanoelectronics holds some answers on expanding the capabilities of electronics devices can be expanded while reducing their weight and power consumption. These include improving display screens on electronics devices and increasing the density of memory chips. Nanotechnology can also reduce the size of transistors used in integrated circuits.

**Environment:** Nanotechnology is being used in several applications to improve the environment. This includes cleaning up existing pollution, improving manufacturing methods to reduce the generation of new pollution, and making alternative energy sources more cost effective. Potential applications include:

- Researchers have shown that iron nanoparticles can be effective in cleaning up organic solvents that are polluting groundwater. The iron nanoparticles disperse throughout the body of water and decompose the organic solvent in place. This method can be more effective and cost significantly less than treatment methods that require the water to be pumped out of the ground.
- Researchers have demonstrated that the use of silver nanoclusters as catalysts can significantly reduce the polluting byproducts generated in the process used to manufacture propylene oxide. Propylene oxide is used to produce common materials such as plastics, paint, detergents and brake fluid.
- Diesel fuel containing cerium oxide to reduce emissions.
- Increasing the electricity generated by windmills. Epoxy containing carbon nanotubes is being used to make windmill blades. The resulting blades are stronger and lower weight and therefore the amount of electricity generated by each windmill is greater.
- Producing solar cells that generate electricity at a competitive cost. Researchers have demonstrated that an array silicon nanowire embedded in a polymer results in low-cost but high-efficiency solar cells. This may result in solar cells that generate electricity as cost effectively as coal or oil.

**Consumer Products:** Nanotechnology has already found its way into numerous consumer products you use every day, from clothing to skin lotion. They include:

- Silver nanoparticles in fabric that kill bacteria making clothing odor-resistant.
- Skin care products that use nanoparticles to deliver vitamins deeper into the skin.
- Lithium ion batteries that use nanoparticle-based electrodes powering plug in electric cars.
- Flame retardant formed by coating the foam used in furniture with carbon nanofibers.
- Certain sunscreens containing titanium dioxide and a face cream containing fullerenes.
- Certain food products, for example vegetable oils, containing nanodrops of components such as vitamins, minerals, and phytochemicals.

**Sporting Goods:** Current nanotechnology applications in the sports arena include:

- Increasing the strength of tennis racquets by adding nanotubes to the frames which increases control and power when you hit the ball.
- Filling any imperfections in golf club shaft materials with nanoparticles; this improves the uniformity of the material that makes up the shaft and thereby improving your swing.
- Reducing the rate at which air leaks from tennis balls so they keep their bounce longer.
SOME EXAMPLES OF NANO MATERIALS

Nanocomposites: A plastic nanocomposite is being used for "step assists" in the GM Safari and Astro Vans. It is scratch-resistant, light-weight, and rust-proof, and generates improvements in strength and reductions in weight, which lead to fuel savings and increased longevity. And in 2001, Toyota started using nanocomposites in a bumper that makes it 60% lighter and twice as resistant to denting and scratching.

Nanocrystals: Smith & Nephew markets an antimicrobial dressing covered with nanocrystalline silver (A patented Technology of NUCRYST Pharmaceuticals). The nanocrystalline coating of silver rapidly kills a broad spectrum of bacteria in as little as 30 minutes.

Nanoparticles: Stain-repellent Eddie Bauer NanoCare™ khakis, with surface fibers of 10 to 100 nanometers, uses a process that coats each fiber of fabric with "nano-whiskers." Developed by NanoTex, a Burlington Industries subsidiary. Dockers also make khakis, a dress shirt and even a tie treated with what they call "Stain Defender", another example of the same nanoscale cloth treatment. In the cosmetics sector, BASF has for several years been among the leading suppliers of UV absorbers based on nanoparticulate zinc oxide. Incorporated in sun creams, the small particles filter the high-energy radiation out of sunlight. Because of their tiny size, they remain invisible to the naked eye and so the cream is transparent on the skin. Using aluminum nanoparticles, Argonide has created rocket propellants that burn at double the rate. They also produce copper nanoparticles that are incorporated into automotive lubricant to reduce engine wear. AngstroMedica has produced a nanoparticulate-based synthetic bone. "Human bone is made of a calcium and phosphate composite called Hydroxyapatite. By manipulation calcium and phosphate at the molecular level, they have created a patented material that is identical in structure and composition to natural bone. This novel synthetic bone can be used in areas where natural bone is damaged or removed, such as in the in the treatment of fractures and soft tissue injuries."

Nanostructured Materials: Nanodyne makes a tungsten-carbide-cobalt composite powder (grain size less than 15nm) that is used to make a sintered alloy as hard as diamond, which is in turn used to make cutting tools, drill bits, armor plate, and jet engine parts. Kodak is producing OLED color screens (made of nanostructured polymer films) for use in car stereos and cell phones. OLEDs (organic light emitting diodes) may enable thinner, lighter, more flexible, less power consuming displays, and other consumer products such as cameras, PDAs, laptops, televisions, and other as yet undreamt of applications.

Nanoclays and Nanocomposites: Used in packaging, like beer bottles, as a barrier, allowing for thinner material, with a subsequently lighter weight, and greater shelf-life. Nanoclays help to hold the pressure and carbonation inside the bottle, increasing shelf life. It is estimated that beer in these containers will gain an extra 60 days (from 120 to 180) of shelf life, reducing spoilage, and decreasing overall costs to the end user. Nanocor is one company producing nanoclays and nanocomposites, for a variety of uses, including flame retardants, barrier film (as in juice containers), and bottle barrier (as shown above). "They are not only used to improve existing products, but also are extending their reach into areas formerly dominated by metal, glass and wood."

Nanocomposite Coatings: Wilson Double Core tennis balls have a nanocomposite coating that keeps it bouncing twice as long as an old style ball. Made by InMat LLC, this nanocomposite is a mix of butyl rubber, intermingled with nanoclay particles, giving the ball substantially longer shelf life.

Nanotubes: Nanolidge makes carbon nanotubes for commercial uses, of which one mundane (marketing tactic) use is in a tennis racket, made by Babolat. The yoke of the racket bends less during ball impact, improving the player's performance. Applied Nanotech recently demonstrated a 14" monochrome display based on electron emission from carbon nanotubes.
**Nanofilters:** Argonide Nanomaterials, an Orlando based manufacturer of nanoparticles and nanofiltration products, makes a filter that is capable of filtering the smallest of particles. This disposable filter retains 99.9999% of viruses at water flow rates several hundred times greater than virus-rated ultra porous membranes. It is useful for sterilization of biological, pharmaceutical and medical serums, protein separation, collector/concentrator for biological warfare detectors, and several other applications.

**Various Products in the market**

- **Cosmetics and personal care products:** RevitaLiftR Intense Lift Treatment Mask (L'OrealR) — uses nanosomes, tiny capsule-like structures, to transport active ingredients into the skin’s outer layer and then release them.

- **Serge Lutens Blusher** (Barneys New YorkR) — “Nano Dispersion technology” creates a fine powder.

- **Chemical-Free** [sic] Sunscreen SPF 15 (Burts BeesR, Inc.) — contains nano-sized particles of titanium dioxide as the active ingredient.

- **Food supplements and food storage:** MesoZinc™ (Purest Colloids, Inc.) — nutritional supplement containing 30 parts per million (ppm) zinc nanoparticles.

- **FresherLonger™** Miracle Food Storage (Sharper ImageR) — food storage containers are infused with silver nanoparticles as an antibacterial agent.

- **Silver Nano Baby Milk Bottle** (Baby DreamR Co., Ltd.) — “silver nano poly system” acts as an antibacterial and deodorizer.

- **Appliances:** SamsungR Washing Machine (SamsungR) — Silver Nano technology “sterilizes your clothes. DaewooR Vacuum Cleaner (DaewooR) — nano-silver coated cyclone canister removes bacteria. Samsung R Air Conditioner — contains silver nano filter and silver nano evaporator.

- **Clothing:** Sport Anklet Sock (AgActive) — treated with nanoparticles of silver (typically 25 nm) as bactericide and fungicide. NANO-PEL™ clothing (NordstromR Inc.) — fabric used in clothing such as pants is treated with Nano-Tex process to bind water-repellent molecules to cotton fibers, in order to impart stain resistance.

- **Coatings:** Pilkington Activ™ Self Cleaning Glass (Pilkington plc) — glass coating that works with ultraviolet (UV) light and rain to keep glass free from organic dirt.

- **Turtle WaxR F21™ Car Wash** (Turtle WaxR, Inc.) — nanotechnology formula comprising synthetic polymers provides protection against UV light.

- **BehrR PREMIUM PLUSR Exterior Paint** (BehrR Process Corporation) — proprietary nanoparticles improve adhesion and anti-mildew properties.

- **UltimaR Photo Paper** (Eastman KodakR Company) — nine-layer composition incorporates ceramic nanoparticles to resist the effects of heat, humidity, light, and ozone.

- **Electronics and computers:** Invisicon™ (FikosR) — Invisicon™ ink used to create transparent conductive coatings and manufacture printed circuits on transparent plastic films; Invisicon™ incorporates carbon nanotubes with a 1000:1 aspect ratio. Applications include flat panel displays, XBOX 360R (MicrosoftR) — microprocessor chip manufactured by IBM using IBM’s 90 nanometer Silicon on Insulator (SOI) technology to reduce heat and improve performance.

- **Sporting goods:** WilsonR Tour Davis Cup Official Tennis Ball (WilsonR) — incorporates “NanoPlay” technology to increase durability.

- **HeadR Nano Titanium Tennis Racquet** (HeadR) — integrates nanoscale materials. WilsonR [K]FactorR Tennis Racket — contains Karophite Black, created by bonding carbon black, graphite, and silicon dioxide together at the nano level.
REFERENCES


MOLECULAR MARKER TECHNIQUES IN PLANT BIOTECHNOLOGY

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During the last few decades, the development and use of molecular markers for the detection and revealing polymorphism at the DNA level, has been playing an increasing part in plant biotechnology and their genetic studies. There are two types of DNA based markers viz. non PCR based (RFLP) and PCR based markers (RAPD, AFLP, SSR, SNP etc.), DNA based marker techniques such as RFLP (restriction fragment length polymorphism), RAPD (random amplified polymorphic DNA), SSR (simple sequence repeats) and AFLP (amplified fragment length polymorphism) are routinely being used in ecological, evolutionary, taxonomical, phylogenic and genetic studies of plant sciences. Day by day development of such new and specific types of markers makes their importance in understanding the genomic variability and the diversity between the same as well as different species of the plants.

INTRODUCTION

In genetics, a molecular marker (identified as genetic marker) is a fragment of DNA that is associated with a certain location within the genome. Molecular markers are used in molecular biology and biotechnology to identify a particular sequence of DNA in a pool of unknown DNA.

A molecular marker is defined as a particular segment of DNA that is representative of the differences at the genome level. Molecular markers, sometimes called DNA markers, should be thought of as signs along the DNA trail that pinpoint the location of desirable genetic traits or indicate specific genetic differences. DNA based molecular markers have been extensively utilized in the fields like taxonomy, physiology, embryology, genetics, etc. for authentication of different plant species. Molecular markers offer numerous advantages over conventional phenotype based alternatives as they are stable and detectable in all tissues regardless of growth, differentiation, development, or defense status of the cell are not confounded by the environment, pleiotropic and epistatic effects. An ideal molecular marker technique should have the following criteria:

1. Be polymorphic and evenly distributed throughout the genome,
2. Provide adequate resolution of genetic differences
3. Generate multiple, independent and reliable markers
4. Simple, quick and inexpensive
5. Need small amounts of tissue and DNA samples
6. Have linkage to distinct phenotypes and
7. Require no prior information about the genome of an organism.

Unfortunately no molecular marker technique is ideal for every situation. Techniques differ from each other with respect to important features such as

Unluckily, no molecular marker technique is ideal for every situation. Techniques differ from each other with respect to important features such as

![Fig. 1. Types of Molecular markers](image)

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requirements and cost. The plant genome consists of DNA, the sequence of which is unique for each genotype. Based on the sequence variation that exists between different species of the same genus, powerful molecular markers have been developed.

MOLECULAR MARKERS IN PLANTS

Molecular markers have wide application in different branches of life-sciences. In genetics, molecular marker is defined as a fragment of DNA sequence that is associated to a part of the genome carrying genes responsible for a trait. It is usually defined as an allele or DNA sequence or chromosome fragment indicating the existence of a metabolism or chemical or physical process. Sometimes this category of markers is referred to as biomarkers or bio-signatures or molecular signatures. In medicines, it could be a substance that is introduced into an organism as a means to detect something (Ex: rubidium chloride is used as radioactive isotopes to evaluate perfusion of heart muscle). In biology, it could be a substance native to the organism whose detection indicates a particular disease state (Ex: presence of an antibody might indicate an infection). Molecular markers are also sub-divided into Biochemical markers (Eg: Isozymes, Allozymes), DNA based markers- (Eg: RFLP, RAPD, SSRs), Physiological markers, Biomarkers and Protein markers (also called biochemical markers) depending on the biochemistry, physiology and origin of markers within the plant. Molecular markers are phenotypically neutral. This is a significant advantage compared to traditional phenotypic markers.

APPLICATIONS OF DNA MARKERS

The molecular markers produced a greater impact on genome mapping, gene tagging and evolutionary studies of crop plants. As far as mapping genomes and genes is concerned, the success depends on the availability of suitable base populations of F, backcross progenies, Doubled Haploids 2 (DH), Recombinant Inbred Lines (RIL), and Near Isogenic Lines (NIL). Exploiting the available populations in conjunction with molecular marker techniques, molecular linkage maps have been constructed for several crop species and very many major and minor genes have been mapped with molecular markers. These "molecular and gene tags" are to be used to exercise marker aided selection, map based cloning and physical mapping of genes of agronomic importance. There are success stories on cloning genes based on their map positions. Apart from genome/gene mapping, molecular markers are employed in assessing the extent of genetic diversity in plant populations. The following section deals with various applications of molecular marker technology individually.

GENOME MAPPING

The genome map of an organism summarizes much of the genetic information available for that species and can serve as a reference for the development and testing of additional genetic hypotheses. However, generation of a complete linkage map remains a daunting task, for many of suitable population. For the construction of linkage maps with molecular markers; parents are chosen that show the maximum of polymorphic loci in order to ensure the mapping of as many markers as possible. Several strategies are followed to have a mapping population. In most of the map construction, F segregating populations are used. These populations are the result of selfing F of two homozygous inbred lines. Most of the molecular maps to date are based on segregation data from F2 progenies. In some cases, the segregating progenies of F backcrossed to recurrent 1 parent were also used to construct linkage maps. Developing a population of RIL is an alternative strategy in mapping projects. Recombinant inbred lines are developed by continuous selfing of F individuals until the 2 homozygosity is achieved. Doubled haploids from anther or microspore culture are also used for linkage map construction in various crop species. Though the strategy sounds good, construction of DH population is a genotype-
dependent process to the *in vitro* culture conditions.

**MAPPING GENES**

Molecular markers offer a tool for locating genes governing agronomically important characters via linkage to mapped DNA sequences. Phenotypic evaluation at the whole plant level or at the cellular level provides information, which can be used to determine the chromosomal location of the genes that confer the phenotype of interest. This is accomplished by analyzing linkage between mapped molecular markers and expression of the target phenotype in a range of related individuals. Markers linked to the genes of interest function as "gene tags" facilitating selection of favorable alleles in a breeding programme. Like for linkage map construction, gene tagging component also needs a suitable population in which the trait to be tagged with molecular markers shows clear-cut segregation with a higher level of polymorphism for the molecular markers.

**GENETIC DIVERSITY ANALYSIS**

Study of genetic diversity is the process by which variation among individuals or groups of individuals or populations is analyzed by a specific method or a combination of methods. The data often involve numerical measurements and in many cases, combinations of different types of variables. Diverse data sets have been used by researchers to analyze genetic diversity in crop plants; most important among such data sets are pedigree data, passport data, morphological data, biochemical data obtained by analysis of isozyme and storage proteins, and, recently, DNA-based marker data that allow more reliable differentiation of genotypes. Since each of these data sets provide different types of information, the choice of analytical method(s) depends on the objective(s) of the experiment, the level of resolution required, the resources and technological infrastructure available, and the operational and time constraints, if any. The advances in DNA marker technology really revolutionized the process of portraying diversity within plant population, crop germplasm and establishing DNA fingerprints for each genotype.

A wide range of molecular marker technologies is now available for genetic studies. Amongst these, RAPD, AFLP, ISSR and SSR marker systems are emerging as the lead technologies. Using RAPD marker system is not felt convenient because of its inconsistency. However, RAPD assay is still used for DNA fingerprinting along with other dominant markers *viz*. AFLP and ISSR markers. SSR markers remain the markers of choice for genome mapping and genetic diversity analysis.

**DNA Based Markers**:

The advent of molecular DNA markers has opened up new vistas for an easy and precise detection and better understanding of soma-clonal variations. The molecular markers can be used at various development stages in tissue culture, and thus help in identifying the specific culture conditions that induce variations.

Several DNA based techniques are available now and new ones continue to be developed. Different techniques are helpful in ascertaining genetic stability/instability related problems in the micropropagated plants. DNA based molecular markers have acted as versatile tools and have found their own 8 position in various fields like taxonomy, physiology, embryology and genetic engineering. Ever since their development, they are constantly being modified to enhance their utility and to bring about automation in the process of genome analysis. The discovery of PCR was a landmark in these efforts and proved to be a unique process that brought about a new class of DNA profiling markers. Un-like protein markers, DNA markers segregate as a single gene and they are not affected by environment. DNA is easily extracted from plant material and its analysis can be cost and labour effective. Now a day's DNA based markers are being preferred over others to test the genetic stability in tissue culture derived plants. This is mainly due to the inertness of DNA to developmental, physiological or environmental changes for screening the variation induced under *in*
vitro conditions. A vast array of DNA-based genetic markers has been discovered since 1980 and new marker types are developed every year. DNA-based markers can be classified into two categories:

(i) Non-PCR based techniques or hybridization based techniques: Genetic marker systems based on DNA-DNA hybridization were developed in the 1970s. Eukaryotic genomes are very large and there was no simple way to observe genetic polymorphisms of individual genes or sequences. The property of complementary base pairing allowed for methods to be developed whereby small pieces of DNA could be used as probes to reveal polymorphisms in just the sequences homologous to the probe. The genetic system derived using this approach is called restriction fragment length polymorphism (RFLP).

Restriction Fragment length Polymorphism (RFLPs): These markers were used for the first time in the construction of genetic maps. Restriction Fragment Length Polymorphism (RFLP) is a technique in which organisms may be differentiated by analysis of patterns derived from cleavage of their DNA. If two organisms differ in the distance between sites of cleavage of particular Restriction Endonucleases, the length of the fragments produced will differ when the DNA is digested with a restriction enzyme. The similarity of the patterns generated can be used to differentiate species (and even strains) from one another. This technique is mainly based on the special class of enzyme i.e. Restriction Endonucleases. In RFLP, DNA polymorphism is detected by hybridizing a chemically labeled DNA probe to a Southern blot of DNA digested by restriction endonucleases, resulting in differential DNA fragment profile. This differential profile is generated due to nucleotide substitutions or DNA rearrangements like insertion or deletion or single nucleotide polymorphisms. Size fractionation is achieved by gel electrophoresis and, after transfer to a membrane by Southern blotting; fragments of interest are identified by hybridization with radioactive labeled probe. Different sizes or lengths of restriction fragments are typically produced when different individuals are tested. Such a polymorphism can be used to distinguish plant species, genotypes and, in some cases, individual plants.

Fig. 2: Outline of the different steps of restriction fragment length polymorphism (RFLP) markers. Double stranded DNA fragments generated by restriction enzymes are separated according to length by gel electrophoresis. A sheet of either nitrocellulose or nylon paper (membrane) is laid over the gel, and the separated DNA fragments are transferred to the sheet by blotting (Southern transfer). The gel is supported on a layer of sponge in a bath of alkali solution, and the buffer is sucked through the gel and the nitrocellulose paper by paper towels stacked on top of the nitrocellulose. As the buffer is sucked through, it denatures the DNA and transfers the single-stranded fragments from the gel to the surface of the nitrocellulose sheet, where they adhere firmly. This transfer is necessary to keep the DNA firmly in place while the hybridization procedure is carried out. The nitrocellulose sheet containing the bound single-stranded DNA fragments is carefully peeled off the gel and placed in a sealed plastic bag that contained a radioactively labeled DNA probe for hybridization. The sheet is removed from the bag and washed thoroughly, so that only probe molecules that have hybridized to the DNA on the paper remain attached. After autoradiography, the DNA that has hybridized to the labeled probe will show up as bands on the autoradiograph.

In RFLP analysis, restriction enzyme-digested genomic DNA is resolved by gel electrophoresis and then blotted onto a nitrocellulose membrane. Specific banding patterns are then visualized by hybridization with labeled probe. Labeling of the probe may be performed with a radioactive isotope or with alternative non-radioactive stains, such as
digoxigenin or fluorescein. These probes are mostly species-specific single locus probes of about 0.5–3.0 kb in size, obtained from a cDNA library or a genomic library. Though genomic library probes may exhibit greater variability than gene probes from cDNA libraries, a few studies reveal the converse. The RFLP markers are relatively highly polymorphic, co-dominantly inherited and highly reproducible. Because of their presence throughout the plant genome, high heritability and locus specificity the RFLP markers are considered superior. The method also provides opportunity to simultaneously screen numerous samples.

Restriction fragment length polymorphisms (RFLPs) are very reliable markers in linkage analysis and crop breeding however, time consuming, expensive and require large amount of DNA for restriction and hybridization analysis.

Advantages of RFLP technique: RFLPs are generally found to be moderately polymorphic. In addition to their high genomic abundance and their random distribution, RFLPs have the advantages of showing co-dominant alleles and having high reproducibility. RFLPs, being co-dominant markers, can detect coupling phase of DNA molecules, as DNA fragments from all homologous chromosomes are detected. RFLP are very reliable markers in linkage analysis and breeding, since they make it possible to determine if a linked trait is present in a homozygous or heterozygous state in an individual. This information is highly desirable, especially for recessive traits.

Disadvantages of RFLP technique: The of utility RFLPs has been hampered due to the large quantities (1–10 μg) of purified, high molecular weight DNA are required for each DNA digestion and Southern blotting. Larger quantities are needed for species with larger genomes, and for the greater number of times needed to probe each blot. The requirement of radioactive isotope makes the analysis relatively expensive and hazardous. The assay is time-consuming and laboratory intensive and only one out of several markers may be polymorphic, which is highly in convenient especially for crosses between closely related species. Their inability to detect single base changes restricts their use in detecting point mutations occurring within the regions at which they are detecting polymorphism.

ii) PCR based techniques: The development of PCR for amplifying DNA sequences led to the revolution in the applicability of molecular methods, and a range of new technologies were developed which could overcome the technical limitations of hybridization-based methods. In a PCR, arbitrary or known sequence primers are used to amplify one or discrete DNA segments that can be resolved in agarose or polyacrylamide gels. Each product is derived from a region of the genome containing two DNA sites with sequences complementary to the primer(s) on the opposite strand and sufficiently closes for the amplification to work.

Random amplified polymorphic DNA (RAPD): Due to advances in molecular biology techniques, large numbers of highly informative DNA markers have been developed for the identification of genetic polymorphism. In the last decade, the random amplified polymorphic DNA (RAPD) technique based on the polymerase chain reaction (PCR) has been one of the most commonly used molecular techniques to develop DNA markers. Williams et al. (1990) invented Random Amplified Polymorphic DNA or RAPD. RAPD markers are amplification products of anonymous DNA sequences using single, short and arbitrary oligonucleotide primers, and thus do not require prior knowledge of a DNA sequence. The primer is usually 10-nucleotide long and the GC content is almost 50%. To obtain good amplification with a single arbitrary primer there must be two identical target sequences close to each other. The distance between the two sites should be within amplifiable units of 4-5 Kb. The two sites should be present on the two opposite strands in inverse orientation. The polymorphism obtained by the RAPD primers can result due to the following reasons:

1. Insertion of DNA fragment between the two annealing sites, because of which the original fragment becomes too large to be amplified
2. Deletion of DNA fragments carrying the primer annealing sites
3. Nucleotide substitution, which may affect the annealing of the primers at a given site due to change in homology, which in turn results in presence or absence of the fragment or a change in size of the amplified product
4. Insertion or deletion of small piece of DNA, which may result in change in size of the amplified product

Low expense, efficiency in developing a large number of DNA markers in a short time and requirement for less sophisticated equipment has made the RAPD technique valuable although reproducibility of the RAPD profile is still the centre of debate.

**Principle of the RAPD Technique:** The standard RAPD technology utilizes short synthetic oligonucleotides (10 bases long) of random sequences as primers to amplify nanogram amounts of total genomic DNA under low annealing temperatures by PCR. Amplification products are generally separated on agarose gels and stained with ethidium bromide. Decamer primers are commercially available from various sources (e.g., Eurofins Genomics, India Pvt. Ltd. Bangalore, India). PCR amplification with primers shorter than 10 nucleotides (DNA amplification fingerprinting (DAF)) has also been used producing more complex DNA fingerprinting profiles. Although these approaches are different with respect to the length of the random primers, amplification conditions and visualization methods, they all differ from the standard PCR condition in that only a single oligonucleotide of random sequence is employed and no prior knowledge of the genome subjected to analysis is required.

At an appropriate annealing temperature during the thermal cycle, oligonucleotide primers of random sequence bind several priming sites on the complementary sequences in the template genomic DNA and produce discrete DNA products if these priming sites are within an amplifiable distance of each other. The amplified DNA primarily depends on

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*Fig. 3: The random amplified polymorphic DNA (RAPD) marker system involves a small number of steps and all are generally easy to apply in forest trees: Step 1, DNA is isolated; Step 2, DNA is amplified by PCR and Step 3, RAPD products are electrophoresed and bands are visualized by staining gels with ethidium bromide.*

Nucleotide sequence homology between the template DNA and oligonucleotide primer at the end of each amplified product. Nucleotide variation between different sets of template DNAs will result in the presence or absence of bands because of changes in the priming sites. Recently, sequence characterised amplified regions (SCARs) analysis of RAPD polymorphisms showed that one cause of RAPD polymorphisms is chromosomal rearrangements such as insertions/deletions. Therefore, amplification products from the same alleles in a heterozygote differ in length and will be detected as presence and absence of bands in the RAPD profile. The RAPD bands is similar to that of low stringency minisatellite DNA fingerprinting patterns and is therefore also termed RAPD fingerprinting. On average, each primer directs amplification of several
discrete loci in the genome so that allelism is not distinguishable in RAPD patterns. In other words, it is not possible to distinguish whether a DNA segment is amplified from a locus that is heterozygous or homozygous.

RAPD markers have found a wide range of applications in gene mapping, population genetics, molecular evolutionary genetics and plant and animal breeding. This is mainly due to the speed, cost and efficiency of the RAPD technique to generate large numbers of markers in a short period compared with previous methods. Therefore, RAPD technique can be performed in a moderate laboratory for most of its applications. Despite the reproducibility problem, the RAPD method will probably be important as long as other DNA-based techniques remain unavailable in terms of cost, time and labour.

Reproducibility of RAPD Markers: Although the RAPD method is relatively fast, cheap and easy to perform in comparison with other methods that have been used as DNA markers, the issue of reproducibility has been of much concern since the publication of the technique. In fact, ordinary PCR is also sensitive to changes in reaction conditions, but the RAPD reaction is far more sensitive than conventional PCR because of the length of a single and arbitrary primer used to amplify anonymous regions of a given genome.

Several factors have been reported to influence the reproducibility of RAPD reaction: quantity of template DNA, PCR buffer, concentration of magnesium chloride, primer to template ratio, annealing temperature, Taq DNA polymerase brand or source, and thermal cycler brand. The first and most commonly used of these enzymes is Taq DNA polymerase (from Thermus aquaticus), whereas Pfu DNA polymerase (from Pyrococcus furiosus) is used widely because of its higher fidelity when copying DNA. The concern about reproducibility of RAPD markers, however, could be overcome through choice of an appropriate DNA extraction protocol to remove any, contaminants, by optimizing the parameters used in testing several oligonucleotide primers and scoring only the reproducible DNA fragments, and by using appropriate DNA polymerase brand. The presence of artifactual bands (false positives) corresponding to rearranged fragments produced by nested primer binding and intrasstrand annealing and interactions during PCR have also been reported to influence the reliability of RAPD data. The presence of both false negatives and false positives may, if frequent, seriously restrict the reliability of RAPDs for various purposes, including genetic diversity and mapping studies. All pair wise comparison of RAPD fragments among samples begins with the assumption that co- migrating bands (i.e., bands that migrate equal distance) represent homologous loci. However, as in any study based on electrophoretic resolution, the assumption that equal length equals homology may not be necessarily true, especially in polyploid species. For example, some RAPD bands scored as identical (equal length) have been found not to be homologous; more accurate resolution of fragment size using polyacrylamide gels and AgNO₃ staining have been reported to reduce such errors. The other limitation of RAPD markers is that the majority of the alleles segregate as dominant markers, and hence the technique does not allow identifying dominant homozygotes from heterozygotes. The RAPD assays produce fragments from homozygous dominant or heterozygous alleles. No fragment is produced from homozygous recessive alleles because amplification is disrupted in both alleles.

Advantages of RAPD Markers: The main advantage of RAPDs is that they are quick and easy to assay. Because PCR is involved, only low quantities of template DNA are required, usually 5–50 ng per reaction. Since random primers are commercially available, no sequence data for primer construction are needed. Moreover, RAPDs have a very high genomic abundance and are randomly distributed throughout the genome. They are dominant markers and hence have limitations in their use as markers for mapping, which can be overcome to some extent by selecting those markers that are linked in coupling. RAPD assay has been used by several groups as efficient tools for identification of markers linked to agronomically important traits,
which are introgressed during the development of near isogenic lines.

Disadvantages of RAPD Markers: The main drawback of RAPDs is their low reproducibility, and hence highly standardized experimental procedures are needed because of their sensitivity to the reaction conditions. RAPD analyses generally require purified, high molecular weight DNA, and precautions are needed to avoid contamination of DNA samples because short random primers are used that are able to amplify DNA fragments in a variety of organisms. Altogether, the inherent problems of reproducibility make RAPDs unsuitable markers for transference or comparison of results among research teams working in a similar species and subject. As for most other multi-locus techniques, RAPD markers are not locus-specific, band profiles cannot be interpreted in terms of loci and alleles (dominance of markers), and similar sized fragments may not be homologous. RAPD markers were found to be easy to perform by different laboratories, but reproducibility was not achieved to a satisfactory level and, therefore, the method was utilized less for routine identifications. RAPD marker diversity was used also applied for diversity studies within and among some other Asteraceae species.

Inter-Simple Sequence Repeat (ISSR): ISSR marker system is another newly developed method, which relies on one primer for PCR that anneals to an SSR region and amplifies region between inversely oriented adjacent SSRs. This microsatellite primed-PCR employs 3' and 5' anchored 15 to 20-mer primer and is referred as Inter SSR amplification, ISA or ISSR.3 ISSR assay can be undertaken for any species that contains a sufficient number and distribution of SSR motifs and has the advantage that genomic sequence data is not required. This technique amplifies large numbers of DNA fragments per reaction, representing multiple loci from across the genome; it is an ideal method for fingerprinting varieties.

The technique has many inherent advantages because of which it has been regularly employed for a variety of purposes. The ISSR technique has the

![Fig. 4. A schematic representation of ISSR-PCR with a single primer (AG)₈, unanchored (a), 3'-anchored (b) and 5'-anchored (c) targeting a (TC)n repeat used to amplify inter simple sequence repeat region flanked by two inversely oriented (TC)n sequences. (a) Unanchored (AG)n primer can anneal anywhere in the (TC)n repeat region on the template DNA leading to slippage and ultimately smear formation; (b) (AG)n primer anchored with 2 nucleotides (NN) at the 3' end anneals at specific regions on the template DNA and produces clear bands; (c) (AG)n primer anchored with 2 nucleotides (NN) at the 5' end anneals at specific regions and amplifies part of the repeat region also leading to larger bands](image_url)
of high annealing temperature (45–60°C) leading to higher stringency. This technique overcomes most limitations such as low reproducibility and high cost.\textsuperscript{12,13} It is widely used by the research community in various fields of plant science such as breeding, germplasm conservation and genetic mapping. Because of the multi-locus fingerprinting profiles obtained, ISSR analysis can be applied in studies involving genetic identity, parentage, clone and strain identification, and taxonomic studies of closely related species.\textsuperscript{4}

**Advantages of ISSR Markers:** The technique has many inherent advantages because of which it has been regularly employed for a variety of purposes. The ISSR technique has the reliability and advantages associated with any SSR marker system along with the broad taxonomic applications of RAPDs. Abundance of SSRs is exploited to generate a complex banding pattern. The ISSR technique is simple and reproducible. The main advantage of ISSR is that no sequence data for primer construction are needed, low quantity of template DNA are required, randomly distributed in the genome and are dominant markers. These are advantages over AFLPs in term of high multiplex ratio and over RAPDs in term of 3–5 folds greater variability.\textsuperscript{14}

**Disadvantages of ISSR Markers:** ISSR is a multi-locus technique; disadvantages include the possible non-homology of similar sized fragments. Moreover, ISSRs, like RAPDs, can have reproducibility problems.

**Amplified Fragment Length Polymorphism (AFLPs):** Amplified fragment length polymorphism (AFLP), which is essentially intermediate between RFLPs and PCR. AFLP is based on a selectively amplifying a subset of restriction fragments from a complex mixture of DNA fragments obtained after digestion of genomic DNA with restriction endonucleases. Polymorphisms are detected from differences in the length of the amplified fragments by polyacrylamide gel electrophoresis (PAGE) or by capillary electrophoresis. The technique involves four steps:

1. Restriction of DNA and ligation of oligonucleotide adapters
2. Preselective amplification
3. Selective amplification
4. Gel analysis of amplified fragments.

AFLP is a DNA fingerprinting technique, which detects DNA restriction fragments by means of PCR amplification. AFLP involves the restriction of genomic DNA, followed by ligation of adapters complementary to the restriction sites and selective PCR amplification of a subset of the adapted restriction fragments. These fragments are viewed on denaturing polyacrylamide gels either through autoradiography or fluorescence. AFLPs are DNA fragments (80–500 bp) obtained from digestion with restriction enzymes, followed by ligation of oligonucleotide adapters to the digestion products and selective amplification by the PCR. AFLPs therefore involve both RFLP and PCR. The PCR primers consist of a core sequence (part of the adapter) and a restriction enzyme specific sequence and 1–5 selective nucleotides (the higher the number of selective nucleotides, the lower the number of bands obtained per profile). The AFLP banding profiles are the result of variations in the restriction sites or in the intervening region. The AFLP technique simultaneously generates fragments from many genomic sites (usually 50–100 fragments per reaction) that are separated by polyacrylamide gel electrophoresis and that are generally scored as dominant markers.

Selective Fragment Length Amplification (SFLA) and Selective Restriction Fragment Amplification (SRFA) are synonyms sometimes used to refer to AFLPs. A variation of the AFLP technique is known as Selectively Amplified Microsatellite Polymorphic Locus (SAMPL). The potential of SAMPL (Selectively Amplified Microsatellite Polymorphic Locus) analysis was studied in lettuce to detect PCR-based co-dominant microsatellite markers.\textsuperscript{15} SAMPL is a method of amplifying microsatellite loci using general PCR primers. SAMPL analysis uses one AFLP primer in combination with a primer
AFLP markers present various advantages over the different methodologies available for genetic polymorphism screening at the genomic level, including the identification of a large number of polymorphism distributed across the genome and the reproducibility of the amplicon. In addition, the AFLP technique is relatively easy to perform, uses small amount of DNA and does not require prior knowledge of sequence. AFLP markers are being widely applied for the analysis of soma-clonal variants. Although AFLPs are highly reproducible, homoplaspy (co-migration of non-homologous fragments) is a major issue in the analysis and interpretation of AFLP data that results in an under-estimation of genetic diversity among samples and a loss of resolution in the analysis.

AFLP markers are useful in genetic studies, such as biodiversity evaluation, analysis of germplasm collections, genotyping of individuals and genetic distance analyses. The availability of many different restriction enzymes and corresponding primer combinations provides a great deal of flexibility, enabling the direct manipulation of AFLP fragment generation for defined applications (e.g. polymorphism screening, QTL analysis, genetic mapping). Applications for AFLP in plant mapping include establishing linkage groups in crosses, saturating regions with markers or gene mapping efforts and assessing the degree of relatedness or variability among cultivars.

**Advantages of AFLP Markers:** The strengths of AFLPs lie in their high genomic abundance, considerable reproducibility, the generation of many informative bands per reaction, their wide range of applications, and the fact that no sequence data for primer construction are required. AFLPs may not be totally randomly distributed around the genome as clustering in certain genomic regions, such as centromers, has been reported for some crops. AFLPs can be analyzed on automatic sequencers, but software problems concerning the scoring of AFLPs are encountered on some systems. The use of AFLP in genetic marker technologies has become the main tool due to its capability to disclose a high number of...
polymorphic markers by single reaction.

**Disadvantages of AFLP Markers:** Disadvantages include the need for purified, high molecular weight DNA, the dominance of alleles, and the possible non-homology of co-migrating fragments belonging to different loci. In addition, due to the high number and different intensity of bands per primer combination, there is a need to adopt certain strict but subjectively determined criteria for acceptance of bands in the analysis. Special attention should be paid to the fact that AFLP bands are not always independent. For example, in case of an insertion between two restrictions sites the amplified DNA fragment results in increased band size. This will be interpreted as the loss of a small band and at the same time as the gain of a larger band. This is important for the analysis of genetic relatedness, because it would enhance the weight of non-independent bands compared to the other bands. However, the major disadvantage of AFLP markers is that these are dominant markers.

**Microsatellite or SSR Markers:** The term microsatellites was coined by Litt and Luty and it also known as Simple Sequence Repeats (SSRs), are sections of DNA, consisting of tandemly repeating mono, di, tri, tetra or penta-nucleotide units that are arranged throughout the genomes of most eukaryotic species. Microsatellite markers, developed from genomic libraries, can belong to either the transcribed region or the non-transcribed region of the genome, and rarely is there information available regarding their functions. Microsatellite sequences are especially suited to distinguish closely related genotypes; because of their high degree of variability, they are, therefore, favoured in population studies and for the identification of closely related cultivars. Microsatellite polymorphism can be detected by Southern hybridisation or PCR. Microsatellites, like minisatellites, represent tandem repeats, but their repeat motifs are shorter (1–6 base pairs). If nucleotide sequences in the flanking regions of the microsatellite are known, specific primers (generally 20–25 bp) can be designed to amplify the microsatellite by PCR. Microsatellites and their flanking sequences can be identified by constructing a small-insert genomic library, screening the library with a synthetically labeled oligonucleotide repeat and sequencing the positive clones. Alternatively, microsatellite may be identified by screening sequence databases for microsatellite sequence motifs from which adjacent primers may then be designed. In addition, primers may be used that have already been designed for closely related species. Polymerase slippage during DNA replication, or slipped strand mispairing, is considered to be the main cause of variation in the number of repeat units of a microsatellite, resulting in length polymorphisms that can be detected by gel electrophoresis. Other causes have also been reported. In plant genome analysis, microsatellites are frequently used to fingerprint genotypes.

**Advantages of SSR Markers:** The strengths of microsatellites include the co-dominance of alleles, their high genomic abundance in eukaryotes and their random distribution throughout the genome, with preferential association in low-copy regions. Because the technique is PCR-based, only low quantities of template DNA (10–100 ng per reaction) are required. Due to the use of long PCR primers, the reproducibility of microsatellites is high and analyses do not require high quality DNA. Although microsatellite analysis is, in principle, a single-locus technique, multiple microsatellites may be multiplexed during PCR or gel electrophoresis if the size ranges of the alleles of different loci do not overlap. This decreases significantly the analytical costs. Furthermore, the screening of microsatellite variation can be automated, if the use of automatic sequencers is an option EST-SSR markers are one class of marker that can contribute to 'direct allele selection', if they are shown to be completely associated or even responsible for a targeted trait. A large number of genic SSRs have been placed on the genetic maps of wheat.

**Disadvantages of SSR Markers:** One of the main drawbacks of microsatellites is that high development costs are involved if adequate primer sequences for the species of interest are unavailable, making them difficult to apply to unstudied groups.
Fig. 6: SSR technique
Microsatellites can also be implemented as mono-locus, co-dominant markers by converting individual microsatellite loci into PCR-based markers by designing primers from unique sequences flanking the microsatellite. Microsatellite containing genomic fragment have to be cloned and sequenced in order to design primers for specific PCR amplification. This approach was called sequence-tagged microsatellite site (STMS). In the longer term, development of allelespecific markers for the genes controlling agronomic traits will be important for advancing the science of plant breeding. In this context, generic microsatellites are but one class of marker that can be deployed, along with single nucleotide polymorphisms and other types of markers that target functional polymorphisms within genes. The choice of the most appropriate marker system needs to be decided upon on a case by case basis and will depend on many issues, including the availability of technology platforms, costs for marker development, species transferability, information content and ease of documentation.

Although microsatellites are in principle co-dominant markers, mutations in the primer annealing sites may result in the occurrence of null alleles (no amplification of the intended PCR product), which may lead to errors in genotype scoring. The potential presence of null alleles increases with the use of microsatellite primers generated from germplasm unrelated to the species used to generate the microsatellite primers (poor “cross-species amplification”). Null alleles may result in a biased estimate of the allelic and genotypic frequencies and an underestimation of heterozygosity.

Furthermore, the underlying mutation model of microsatellites (infinite allele model or stepwise mutation model) is still under debate. Homoplasy may occur at microsatellite loci due to different forward and backward mutations, which may cause underestimation of genetic divergence. A very common observation in microsatellite analysis is the appearance of stutter bands that are artifacts in the technique that occur by DNA slippage during PCR amplification. These can complicate the interpretation of the band profiles because size determination of the fragments is more difficult and heterozygotes may be confused with homozygotes. However, the interpretation may be clarified by including appropriate reference genotypes of known band sizes in the experiment.

The decision about the most appropriate marker system to use will vary greatly depending on the species, the objective of the marker work and resources available.

REFERENCES
THREATS TO GLOBAL BIODIVERSITY

Supriya Tiwari

Over the last century, degrading environmental biodiversity has become an issue of concern for the environmentalists worldwide. Biodiversity losses can be attributed to the resource demands of our rapidly growing human population. With increasing population, the natural habitat of plants and animals are decreasing as more and more land is being utilized for human habitation and development. This review deals with the important causes which result in threats to global biodiversity.

Biological diversity refers to the variety and variability among living organisms along with the ecological complexes in which they occur. Diversity can be defined as the number of different items and their relative frequencies which are organized at many levels, ranging from complete ecosystems to the chemical structures that are the molecular basis of heredity. Biodiversity refers to the number and variety of species, of ecosystems, and of the genetic variation contained within species. According to a study by United Nations Environmental Programme (UNEP), 8.7 million is the latest number of species on Earth, of which 6.5 millions species are found on land and 2.2 millions inhabit the oceans. However, in spite of 250 years of taxonomic classification, the results suggest that 86% of all species on land and 91% of those in the seas are yet to be discovered. The entire flora and fauna of the World is divided into several groups and number of described species in each group is given in Table 1.

Biodiversity is not evenly distributed; rather it varies greatly across the globe as well as within regions. Among other factors, the diversity of all living things (biota) depends on temperature, precipitation, altitude, soils, geography and the presence of other species. Generally, there is an increase in biodiversity from the poles to the tropics. Thus localities at lower latitudes have more species than localities at higher latitudes. This is often referred to as the latitudinal gradient in species diversity. The

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of Described Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteria and blue-green algae</td>
<td>4,760</td>
</tr>
<tr>
<td>Fungi</td>
<td>46,583</td>
</tr>
<tr>
<td>Algae</td>
<td>26,500</td>
</tr>
<tr>
<td>Bryophytes (mosses and liverworts)</td>
<td>17,000</td>
</tr>
<tr>
<td>Gymnosperms (conifers)</td>
<td>750</td>
</tr>
<tr>
<td>Angiosperms (flowering plants)</td>
<td>250,000</td>
</tr>
<tr>
<td>Protozoans</td>
<td>30,800</td>
</tr>
<tr>
<td>Sponges</td>
<td>5,000</td>
</tr>
<tr>
<td>Corals and Jellyfish</td>
<td>9,000</td>
</tr>
<tr>
<td>Roundworms and earthworms</td>
<td>24,000</td>
</tr>
<tr>
<td>Crustaceans</td>
<td>38,000</td>
</tr>
<tr>
<td>Insects</td>
<td>751,000</td>
</tr>
<tr>
<td>Other Arthropods and minor invertebrates</td>
<td>132,461</td>
</tr>
<tr>
<td>Mollusks</td>
<td>50,000</td>
</tr>
<tr>
<td>Starfish</td>
<td>8,100</td>
</tr>
<tr>
<td>Fishes (teleosts)</td>
<td>19,056</td>
</tr>
<tr>
<td>Amphibians</td>
<td>4,184</td>
</tr>
<tr>
<td>Reptiles</td>
<td>6,300</td>
</tr>
<tr>
<td>Birds</td>
<td>9,198</td>
</tr>
<tr>
<td>Mammals</td>
<td>4,170</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>1,435,662</strong></td>
</tr>
</tbody>
</table>

Table 1. Table showing the groups into which the organisms are divided along with their numbers.

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species are now extinct and many are at the verge of extinction. Many reports suggest a substantial and largely irreversible loss in the diversity of life on Earth, with 11,877 species of animals and 10,896 of plant species threatened with extinction, due to human actions. The current extinction rate is now approaching 1,000 times the background rate and may climb to 10,000 times the background rate during the next century. Background rate refers to the normal extinction rate which can be defined as the standard rate of extinction in Earth's geological and biological history before humans became primary contributors to extinction. Several factors can be attributed to the loss of global biodiversity. Fig 1 shows the contribution of some of the existing factors which result in biodiversity loss. A few of such important factors are discussed in this article.

![Known Causes of Animal Extinctions Since 1600](image)

**Fig 1.** Figure showing the percentage of different factors responsible to cause animal extinction. **Source:** World Conservation Monitoring Centre, "Global Biodiversity" Chapman & Hall, London, 1992.

1. **Overexploitation of natural resources:** The unsustainable use of natural resources and overexploitation, which occurs when harvesting exceeds reproduction of wild plant and animal species, continues to be a major threat to biodiversity. As the human population passes the six billion mark, we have destroyed roughly half of the world's forests and roughly half of the world's net primary productivity for human use. Besides human population utilizes most available fresh water and virtually all of the available productivity of the oceans. This overexploitation of the natural resources by human beings is an important factor which poses threat to the global biodiversity. The ecological footprint analysis suggest that the overall biological resources use and waste emission is well above the biological capacity indicating that the present day society is not able to meet its consumption demands within its own borders.

In today's scenario overexploitation and misuse of natural resources is an ever present threat for species richness. This is more prominent in case of islands, whose endemic populations are more prone to extinction from overexploitation, as they often exist at low densities with reduced reproductive rates. A good example of this is island snails, such as the Hawaiian *Achatinella* and the French Polynesian *Partulidae*. *Achatinella* snails have 15 species listed as extinct and 24 critically endangered while 60 species of *Partulidae* are considered extinct with 14 listed as critically endangered. Overexploitation, coupled with very low lifetime fecundity is responsible for the extreme vulnerability exhibited among these species.

2. **Over-hunting:** has been a significant cause of the extinction of hundreds of species and the endangerment of many more, such as whales and many African large mammals. Most extinctions over past several hundred years are mainly due to over-harvesting for food and profit. Important species that have become extinct in the last century, owing to the anthropogenic activities include Atlas Bear (native to Africa), Zanzibar leopard (native to Tanzania), Quagg (native to Southern Africa), Great Auk (flightless water birds), Tasmanian Tiger (native to Australia and Tasmania), Dodo (native Mauritius) etc. Beside these species that have become extinct, the hunting activities have also forced a few species in the list of endangered ones. Polar bear, Musk Ox, Mediterranean Monk seals, Flying Fox and Great White Sharks are a few to be included in the IUCN list of endangered species.

3. **Habitat fragmentation:** is an important cause of known extinctions. One of the major ways that habitat fragmentation affects biodiversity is by reduction in the amount of available habitat (such as rainforests, boreal forests, oceans, marshlands, etc.)
for all organisms in an ecological niche. Habitat fragmentation invariably involves some amount of habitat destruction. Humans in their zest to exploit natural resources cause destruction of natural habitats of organisms. Agriculture, mining, logging etc are important human activities that lead to habitat destruction. This generally causes formation of patches in the natural habitats of organisms resulting in habitat fragmentation. Tropical rain forests are most severely affected. Out of 16 million square kilometers of tropical rain forest habitats that normally existed in the World, less than 9 million square kilometers exist today. Plants and other sessile organisms in these areas are usually directly destroyed. Mobile animals (especially birds and mammals) retreat into remnant patches of habitat. This can lead to crowding effects and increased competition. The size of the fragment will influence the number of species which are present when the fragment was initially created, and will influence the ability of these species to persist in the fragment. Small fragments of habitat can only support small populations of plants and animals and small populations are more vulnerable to extinction. Minor fluctuations in climate, resources, or other factors that would be unremarkable and quickly corrected in large populations can be catastrophic in small, isolated populations. As deforestation proceeds in tropical forests, this promises to become an important threat to global biodiversity. Tropical forests are so important because they harbor at least 50%, and perhaps more, of world's biodiversity. Habitat fragmentation is a further aspect of habitat loss that often goes unrecognized. Any species that requires a large home range, such as a grizzly bear, will not survive if the area is too small.

Additionally, habitat fragmentation leads to edge effects. Microclimatic changes in light, temperature and wind can alter the ecology around the fragment, and in the interior and exterior portions of the fragment. Fires become more likely in the area as humidity drops and temperature and wind levels rise. Exotic and pest species may establish themselves easily in such disturbed environments, and the proximity of domestic animals often upsets the natural ecology.

4. **Invasion of non-native species:** is an important and often-overlooked cause of extinctions. The African Great Lakes - Victoria, Malawi and Tanganyika - are famous for their great diversity of endemic species, of cichlid fishes. In Lake Victoria, a single, exotic species, the Nile Perch, has become established and may cause the extinction of most of the native species, by simply eating them all. The major plant Forest Invasive Species (FIS) include *Lantana camara*, *Eupatorium glandulosum*, *Parthenium hystrix*, *Mimosa species*, *Eichhornia crassipes*, *Mikania micrantha*, *Ulex europaeus*, *Prosoptis juliflora*, *Cytisus scoparius*, *Euphorbia royleana* etc. Alien aquatic weeds like water hyacinth and water lettuce are increasingly choking waterways and degrading freshwater ecosystems.

5. **Domino effects:** are likely when two or more species are highly inter-dependent, or when the affected species is a "keystone" species, meaning that it has strong connections to many other species. The seeds of the tree *Calvaria major*, now found exclusively on the island of Mauritius, must pass through the abrasive gut of a large animal in order to germinate. Their tough seed coats are protection against digestion, but also a kind of living coffin, for the seed can not germinate unless abraded. None of the animals currently on Mauritius have that ability. The dodo (a 25 kg pigeon), hunted to extinction in the late 17th century, probably was the key to recruitment in this species. Some seeds, abraded, roughened, and excerced by dodos, germinated and grew. Today, no seeds germinate, and only a few very old trees now survive. Similarly, the blackfooted ferret was once very abundant in the western prairies. It preyed upon prairie dogs and used their burrows to nest in. Poisoning of prairie dogs has greatly reduced their abundance, and the blackfooted ferret is now the rarest mammal in North America.

6. **Climate change:** The relationship between climate change and biodiversity is well established. A changing global climate threatens species and ecosystems. From a human perspective, the rapid
climate change and accelerating biodiversity loss risks human security. Medicines and other resources on which we rely may be harder to obtain as the plants they are derived from may reduce or disappear due to rapidly changing climatic conditions. The distribution of species (biogeography) is largely determined by climate, as is the distribution of ecosystems and plant vegetation zones (biomes). Climate change may simply shift these distributions but, for a number of reasons, plants and animals may not be able to adjust. The pace of climate change almost certainly will be more rapid than most plants are able to migrate. Parks and nature reserves are fixed locations. The climate that characterizes present-day Yellowstone Park will shift several hundred miles northward. Forests, which are the home to several species are very much threatened due to the global warming induced as a result of climate change (Fig 2). For these reasons, some plant and animal species are likely to be eliminated by climate change. Agricultural production likely will show regional variation in gains and losses, depending upon crop and climate.

The Arctic, Antarctic and high latitudes have had the highest rates of warming, and this trend is projected to continue. In the Arctic, it is not just a reduction in the extent of sea ice, but its thickness and age. In terms of biodiversity, the prospect of ice-free summers in the Arctic Ocean implies the loss of an entire biome. The inhabitants of the Arctic are adapted to life on top of or under ice — from the algae that grow on the underside of multi-year ice, forming up to 25% of the Arctic Ocean's primary production, to the invertebrates, birds, fish and marine mammals further up the food chain. Thus the entire Arctic flora and fauna along with the iconic polar bear at the top of that food chain is at the risk of extinction.

One of the most important components of climate change is the increasing concentration of greenhouse gases in the atmosphere. Rapidly rising greenhouse gas concentrations are driving ocean systems toward conditions not seen for millions of years, with an associated risk of fundamental and irreversible ecological transformation. Changes in biological function in the ocean caused by anthropogenic climate change go far beyond death, extinctions and

Fig 2. Figure showing different forests regions of the World which are threatened due to climate change.
habitat loss leading to the alteration in the fundamental processes of the marine ecosystems. The National Oceanic and Atmospheric Administration (NOAA), USA has hinted towards the increasing ocean acidification. More CO₂ in the atmosphere means more CO₂ in the ocean which results in increased ocean acidification. The resulting changes in the chemistry of the oceans disrupt the ability of plants and animals in the sea to make shells and skeletons of calcium carbonate, while dissolving shells already formed.

As a consequence of these multiple forces, many scientists fear that by end of next century, perhaps 25% of existing species will be lost. Biodiversity loss is an expensive and irreversible loss of the society. The lost species cannot be regenerated at any cost. The magnitude of this loss is aptly expressed by E.O. Wilson in his following lines:

“The worst thing that can happen during the 1980s is not energy depletion, economic collapse, limited nuclear war, or conquest by a totalitarian government. As terrible as these catastrophes would be for us, they can be repaired within a few generations. The one process ongoing in the 1980s that will take millions of years to correct is the loss of genetic and species diversity by the destruction of natural habitats. This is the folly that our descendents are least likely to forgive us”.

REFERENCES


7) Biodiversity and Climate Change, Convention on Biological Diversity, December, 2009.

8) NOAA Ocean Acidification Demonstration, National Oceanic and Atmospheric Administration, February 26, 2010.
URBAN SOUND POLLUTION AND ITS IMPACT ON ANIMAL HEALTH: A GLANCE

Debojyoti Borkotoky\(^1\), S.K. Mukhopadhyay, Ruma Devi and R.K. Singh\(^1\)

Animals struggle to adapt to the noisy environment in the urban areas. They have developed a range of adaptive strategies available for mitigating the adverse effects of environmental noise on their use of acoustic information. However, these adoptions are in the cost of energetic expenditure, increased risk of predation, or lost opportunities for preening, feeding or mating. Obvious consequences may be reduced fecundity rates and ultimately jeopardizing their viability or survival in the urban areas. There is an opportunity of research to better understand how anthropogenic noise can affect patterns of animal movement, reproduction, social relations, and communication.

In this increasingly noisy world, an elaborate understanding on well being of the pets, domestic animal and wild life is the need of the hour. Buzzing of a mosquito, barking dogs or caw of crows in our locality may be exceedingly disturbing at times especially when we are up in the bed for a nap. Conversely, honking of the vehicles, passing supersonic jet, rock music, roaring street public addressing system and innumerable human noise is obviously nuisance and a stress to the city animals. Cities are dynamic grounds for research on the evolution of animal communication systems, with broader implications for conservation in human-altered environments.

Scientifically, sounds are mechanical waves of pressure that are transmitted through mediums of solid, liquid, gas, or plasma. Generally humans can perceive frequencies between about 20 Hz and 20,000 Hz (20 kHz), though this differs depending on humans. Other species (Table 1) such as dogs (45 kHz) and cats (64 kHz) hear higher frequencies compared to humans while Homing pigeons can detect sounds up to 200 Hz and extremely low-frequency sounds (infrasound) as low as 0.05 Hz. Natural infra-sounds come from many sources, including weather patterns, topographic features (volcanoes, avalanches, earthquakes etc), ocean wave activity and cosmic activities (meteorites etc).

A person hears sounds when the vibrations pass through the ear and resonate off the ear drum. Sounds include any noise, music, speech, etc. A noise is a type of sound, but is usually used to refer to loud and unwanted sounds.

**Table 1: Audible frequency range (Hz) of common urban and zoo animals**

<table>
<thead>
<tr>
<th>Species</th>
<th>Approximate Range (Hz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dog (Canis lupus familiaris)</td>
<td>67-45,000</td>
</tr>
<tr>
<td>Cat (Felis catus)</td>
<td>45-64,000</td>
</tr>
<tr>
<td>Cow (Bos taurus)</td>
<td>23-35,000</td>
</tr>
<tr>
<td>Horse (Equus caballus)</td>
<td>55-33,500</td>
</tr>
<tr>
<td>Sheep (Ovis aries)</td>
<td>100-30,000</td>
</tr>
<tr>
<td>Rabbit (Oryctolagus cuniculus)</td>
<td>350-42,000</td>
</tr>
<tr>
<td>Rat (Rattus rattus)</td>
<td>200-76,000</td>
</tr>
<tr>
<td>Mouse (Apodemus sylvaticus)</td>
<td>1,000-91,000</td>
</tr>
<tr>
<td>Bat (Myotis spp.)</td>
<td>2,000-110,000</td>
</tr>
<tr>
<td>Goldfish (Carassius auratus)</td>
<td>20-3,000</td>
</tr>
<tr>
<td>Canary (Serinus canaria)</td>
<td>250-8,000</td>
</tr>
<tr>
<td>Cockatoo (Cacatuidae)</td>
<td>250-8,000</td>
</tr>
<tr>
<td>European Robins (Erithacus rubecula)</td>
<td>21,000</td>
</tr>
<tr>
<td>House Sparrow (Passer domesticus)</td>
<td>675-18,000</td>
</tr>
<tr>
<td>Budgerigars (Melopsittacus undulatus)</td>
<td>14-14,000</td>
</tr>
<tr>
<td>Barn Owl (Tyto alba)</td>
<td>200-12,000</td>
</tr>
<tr>
<td>American Crows (Corvus brachyrhynchos)</td>
<td>300-8,000</td>
</tr>
<tr>
<td>Chicken (Gallus gallus domesticus)</td>
<td>125-2,000</td>
</tr>
</tbody>
</table>

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Animals and birds use different acoustic signals to maintain and establish contact with the members of the family or social group, as aids to navigation, message of distress, presence of food, mating, demarcation of territory and probably many others. Growth in transportation systems, resource extraction, motorized recreation and urban development is responsible for chronic noise exposure in most terrestrial areas, including remote wilderness locations. Animal acoustic communications must compete with the rapid and dramatic increases in the levels of ambient noise in the urban area. Increased noise levels reduce the distance and area over which acoustic signals can be perceived by animals. This is an area where knowledge of physiology, developmental neurobiology, animal behavior, and behavioral ecology all contribute to understanding how animals adjust (or fail to adjust) to anthropogenic change. Most researchers agree that noise can affect an animal's physiology and behavior, and if it becomes a chronic stress, noise can be injurious to an animal's energy resources, reproductive success and long-term survival.

**URBAN NOISE POLLUTION**

The source of most outdoor noise (environmental noise) worldwide is mainly caused by machines and transportation systems and social events. Poor urban planning may give rise to noise pollution, since side-by-side industrial and residential buildings can result in noise pollution in the residential areas. Indoor noise can be caused by machines, building activities, and music performances, television etc.

**BEHAVIOURAL CHANGES IN ANIMALS**

Animals frequently interrupt their activity to look up and to scan their surrounding environment for potential predators (vigilance). As vigilance and other activities are often mutually exclusive, as such behaviours are at the expense of feeding, sleeping or preening. A study on American Crows (*Corvus brachyrhynchos*) revealed that the birds were more vigilant in areas of high human disturbance than in areas of low human disturbance. Prey have evolved anti-predator responses to generalized threatening stimuli, such as loud noises and hastily approaching objects. Therefore, when encountering disturbance stimuli ranging from a low-flying aircraft to the quiet wildlife videographer, animal responses are likely to follow the same economic principles used by prey encountering predators. Some similar to predation risk, disturbance stimuli can indirectly affect fitness and population dynamics via the energetic and lost opportunity costs of risk avoidance. Studies showed strong evidence of reduced densities of many bird species of forest/woodland and open habitat birds in broad zones adjacent to busy roads. The density reduction is related to a reduced habitat quality, and traffic noise is probably the most critical factor. Intense noise induces an increase of scanning rate and eating speed in rats. In a report it was established that urban European Robins (*Erithacus rubecula*), highly territorial birds reliant on vocal communication, reduce acoustic interference by singing during the night in areas that are noisy during the day. In another study in West Bengal, India, it was concluded that in spite of heavy noise of trains, crowdy travelers, and lack of nest sites, House Sparrow (*Passer domesticus*), remain at the railway stations because of availability of food in the nearby roadside market.

**BREEDING BEHAVIOUR**

Bird song is a sexual trait important to attract mate and known to be shaped by environmental selection. Acoustic features, including minimum and maximum frequency, and delivery rate of song notes showed significant differences between habitats in a study conducted on little Greenbul in Central Africa. Noisy territories were home to Great-tit males (a common bird species throughout Europe, the Middle East, Central and Northern Asia, and parts of North Africa in any sort of woodland) whose songs had a high average minimum frequency. Birds in quiet territories sang more notes that reached the lowest frequencies measured for the population in an observation at the city. There are indications of birds having a higher-pitched song with frequencies, amplitude and call length well above those of noise makes a bird less susceptible to noise pollution.
studied in humming birds (*Lampornis clemenciae*), tree swallow (*Tachycineta bicolor*), nightingales (*Luscinia megarhynchos*), budgerigars (*Melopsittacus undulates*) and zebra finches (*Taeniopygia guttata*), canaries (*Serinus canaria*) paralleling the well-known Lombard effect in humans which is the reflexive increase in speech intensity during communication in noise. The adjustment of vocal amplitude may serve to maintain a specific signal-to-noise ratio that is favorable for signal production. Similar, adjustment of vocal amplitude to counteract masking effect was found in common marmoset (*Callithrix jacchus*), a new world monkey. The amplitude regulation of vocalizations contributes to signal transmission distance along with the established relationships between singing behavior, acoustic structure and habitat. Another study found a significant reduction in ovenbird pairing success at industrial sites (77%) compared with noiseless place (92%). These differences were apparent regardless of territory quality or individual male quality. Significantly more inexperienced birds breeding for the first time were found near noise-generating compressor stations (industrial sites) than noiseless wellpads (48% vs. 30%). It was hypothesized that noise interferes with a male’s song, such that females may not hear the male’s song at greater distances and/or females may perceive males to be of lower quality because of distortion of song characteristics. These works demonstrate that chronic background noise could be an important factor affecting bird populations.

**Physiological Changes**

There is scientific evidence of reduction or even cessation of milk yield in cow and goats during fright caused by sudden loud sounds. A tractor engine sound (97 dB) can significantly increase the blood glucose and total leucocyte count and decreases the level of haemoglobin in milk cows. At 105 dB there is a reduction of feed consumption, milk yield and release of milk. There is also influence in the hormonal system with increase in plasma 10-OH corticosterone and catecholamine and decrease corticosterone level in swine at general noise of 108-120 dB. There is also excess secretion of aldosterone (93 dB) and trichycardia (120-135 dB) as evident from the studies on pigs. Sheep expresses higher heart and respiratory rate, lower feeding efficiency and thyroid activity at 90-100 dB white noise. Similar finding were record in a study on black buck. Studies on domestic fowl indicate decrease in weight of chicken (156.3 dB), increase in plasma 11-hydrocorticosteroid (100 dB), interruption of brooding (115 dB) and reduced egg production by keeping hens from feed and water due to noise stress.

**Neurobiological Affects**

Ecological research in the past few decades has made known that most animals acquire and respond adaptively to information that affects survival and reproduction. At the same time, neurobiological studies have established that the rate of information processing by the brain is much lower than the rate at which information is encountered in the environment, and that attentional mechanisms enable the brain to focus only on the most essential information at any given time. Data indicates that limited attention affects diet choice and constrains animals’ ability simultaneously to feed and attend to predators. Acoustic cues play a role in detection of insect prey by bats and mouse lemurs.

**Conclusion**

The ill effect of noise pollution and its implication in human health is well documented. There is notable addition of noise with rapid urbanization. Existence of human in the earth in intrinsically associated with the environment with its mechanism. Anthropogenic noise is undoubtedly harming our livestock and city dwelling birds and animals, as evident from a number of research reports.

**References**


GLIMPSES OF CHEMISTRY AND CHEMISTS AROUND THE GREEN MOLECULE CHLOROPHYLL

R.P. Chamoli

Chlorophyll is a green pigment present in the green parts of the plants. It plays a significant role in the process of photosynthesis which provides oxygen. Chlorophyll facilitates the formation of carbohydrates, fats, amino acids and vitamins in plants. The contributions of some prominent organic chemists: Willstatter, Hans Fischer, and R.B. Woodward in the chemistry of chlorophyll have been described with their inspiring life sketches.

INTRODUCTION

We are well familiar with the fascinating green molecule chlorophyll which is an integral part of the vegetable kingdom. One of the major chemical constituents of the plants is chlorophyll. This green coloured pigment makes our earth green, provides oxygen to breath, and thus plays a pivotal role in the existence of life on earth. Due to its close association with life it is also known as 'green blood'. This fact is evident by a remark 'All flesh is grass' made by Prophet Isaiah, 8th century B.C.¹. The essential chemicals like oxygen, carbohydrates, fats, amino acids and vitamins are generated by the plants with the help of chlorophyll. During the beginning of 20th century, it was recognised that plants are capable of using chlorophyll to trap the light energy from the sun and under its influence converting carbon dioxide and water into carbohydrates (sugars and starches). This process was named as photosynthesis and represented in a simplified manner by the following equation:

\[ 6\text{CO}_2 + 6\text{H}_2\text{O} \xrightarrow{\text{Light}} \text{C}_6\text{H}_{12}\text{O}_6 + 6\text{O}_2 \]

The name 'chlorophyll' does not mean that this green compound present in leaves and green stems of the plants contains the element chlorine. The name is derived from Greek words 'chloros' meaning yellowish-green, and 'phyll' meaning leaf². The history of discovery, chemistry and biochemistry of chlorophyll constitute an interesting and inspiring story of hard work of a number of talented chemists³. Some of them have been honoured with prestigious Nobel Prize for their outstanding contributions in this area⁴. During the 18th century many chemists and botanists started to realise that in presence of sunlight green plants are capable of enriching the atmosphere with 'air' which is needed to sustain life on earth. In 1780, Joseph Priestley, an English chemist, discovered that plants generate oxygen. After 14 years, in 1794, the French chemist Antoine Lavoisier discovered the concept of oxidation. But unfortunately, soon after, this brilliant chemist was awarded death sentence during the French Revolution on the charge of being a Monarchist sympathizer. While pronouncing the judgement the judge remarked 'The Republic has no need for scientists'. Encouraged by Priestley's experiments, a Dutchman, Jan Ingenhousz who was a court physician to the Austrian empress, conducted 500 experiments and concluded that light plays a significant role in photosynthesis. Subsequently, three more important discoveries were made in this field. Jean Senebier, a Swiss pastor, discovered that fixed air (CO₂) from atmosphere was taken up during the photosynthesis, and Theodore de Saussure, while working in Geneva, found that the other reactant necessary for this process was water. Finally, a German surgeon, Julius Robert Mayer recognised that green plants convert solar energy.

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into chemical energy. He said: 'The plants take in one form of power, light; and produce another power, chemical difference.'

**EXTRACTION OF CHLOROPHYLL.**

In 1817, a green pigment was isolated from the green leaves by two French chemists, Pierre Joseph Pelletier and Joseph Bienaimé Caventou. They named it chlorophyll in 1818. After isolation of chlorophyll, some leading chemists made attempts to explore the other aspects of its chemistry. Jons Jakob Berzelius in Sweden and Englishman Edward Schunk started efforts to purify and analyse this pigment but they achieved very little success. However, meanwhile there was some progress in some other related areas. The German plant physiologist Julius von Sachs discovered chloroplasts. These are very small bodies inside the plant cells and secrete chlorophyll. In 1864, the Anglo-Irish physicist George Stokes studied the absorption spectrum of the chlorophyll and mapped its prominent absorption bands.

These spectral studies revealed that chlorophyll was a mixture. This work apparently did not receive much attention.

**STRUCTURE OF CHLOROPHYLL.**

Further significant progress in the chemistry of chlorophyll started only when Willstätter entered this field. Richard Martin Willstätter was a German organic chemist born in a Jewish family on 13 August 1872 in Karlsruhe in Baden. He was the son of Sophie Ulman and Maxwell Willstätter, a textile merchant. At the age of 18 he was admitted in the chemistry department of the University of Munich as a student of Adolf von Baeyer. He received his doctorate degree in 1894 for his work on the synthesis and structure of plant alkaloids such as strychnine and cocaine. Thereafter, in 1896 and 1902, he was appointed as a Lecturer and a Junior Professor of Chemistry, respectively. He left Munich in 1905 and joined ETH, Zurich as a Professor of Chemistry and started work on the plant pigment chlorophyll. He first determined its empirical formula. In 1912, Willstätter became Professor of Chemistry at the University of Berlin and Director of the Kaiser Wilhelm Institute of Chemistry and started the study of structure of pigments of flowers and fruits. It was here that Willstätter discovered that chlorophyll was a mixture of two compounds which were designated as chlorophyll-a and chlorophyll-b. In 1915, he was awarded Nobel Prize for his outstanding work in the field of chlorophyll and other plant pigments. In 1916, he came back to Munich as a successor to his mentor, Adolf von Baeyer. In 1924, brilliant career of this great chemist came to a sudden end when he took retirement as a protest against increasing antisemitism at the University. Even after retirement, he continued his research interests privately in collaboration with one of his former students. Ultimately in 1939, Willstätter emigrated to Switzerland where he died of heart attack in 1942.

During the period 1905-1912, Willstätter made significant investigations on chlorophyll. In 1908, he noticed that when dried leaves are powdered and digested with ethanol, a 'crystalline' chlorophyll is obtained after concentration of the solution. But if water or aqueous acetone is used in place of ethanol, then the product is 'amorphous' chlorophyll. During the isolation of 'crystalline' chlorophyll, a molecule of phytol alcohol is replaced by ethanol under the influence of an enzyme, chlorophyllase which is present in leaves. During the isolation of the chlorophyll from the leaves, two other pigments, carotene and xanthophyll were also isolated by him. Originally, in 1911, Willstätter et al. worked out the molecular formula of the chlorophyll as $C_{55}H_{72}MgN_{6}O_{4}$. In 1913, Willstätter assigned molecular formula $C_{35}H_{27}N_{4}O_{4}Mg$ and $C_{45}H_{30}N_{4}O_{4}Mg$, to chlorophyll-a and chlorophyll-b respectively. In the following years, Willstätter gathered some other valuable information regarding the structure of chlorophyll on the basis of some degradation reactions. He pointed out the structural similarities between chlorophyll and haemin. Haemin is red pigment of the blood. In human blood, haemin exists in combination with the protein globin and forms haemoglobin which resides in red blood cells or erythrocytes. Haemin is
composed of an aromatic porphyrin macrocycle comprising four pyrrole units and containing an iron atom at centre. Haemoglobin absorbs oxygen from the lungs and carries it to different parts of the body, and then picks up carbon dioxide and transports it back to lungs for exhalation. How is this carbon dioxide produced? The intake of oxygen leads to the breakdown of larger molecules like carbohydrates and fats in our metabolism. In this way oxygen provides us energy for all other metabolic processes. The carbon dioxide is generated in this process and is expelled out from the lungs by exhalation.

Willstatter showed that chlorophyll molecule holds an atom of magnesium with its central part in the same way as an atom of iron is held at the centre of haemin molecule. In 1930, a German organic chemist Hans Fischer was awarded Nobel Prize for his researches on haemin and chlorophyll, especially for his synthesis of haemin. In addition to his work on haemin, he was also involved in the structure elucidation of chlorophyll. While delivering the Nobel Lecture, he emphasized the need for further investigation in this area in order to give a correct structure to chlorophyll. He provided a more detailed view of the structural similarities between haemin and chlorophyll. He showed that chlorophyll is also constructed from the same pyrrole constituents that make up haemin. By the year 1940, Fischer et al. elucidated the final structure of chlorophyll (Fig. 1).

Fig. 1. Structure of Chlorophyll
Hans Fischer was born on 27 July, 1881 in Hoechst on Main. He was son of Dr. Eugen Fischer, and Anna Herdegen. After matriculation in 1899, he studied chemistry and medicine first at the University of Lausanne and then at University of Marburg. He completed graduation in 1904, and subsequently obtained his M.D. in 1908. He started his career first at a Medical Clinic in Munich and then at Berlin Chemical Institute under Emil Fischer. His independent academic career began in Munich where he became a Lecturer in Internal Medicine and then a Lecturer in Physiology in Physiological Institute. Later he joined the University of Innsbruck as Professor of Medical Chemistry, and from there he went to the University of Vienna in 1918. In 1921, Hans Fischer returned back to Munich to take up the position of Professor of Organic Chemistry at the Technical University. He made significant contribution in the field of pigments in blood, bile, chlorophyll and the chemistry of pyrrole, from which these compounds were derived. His synthesis of bilirubin and haemin are of special importance. At the age of 63, he committed suicide in Munich on 31 March, 1945. Deep despair over the destruction of his institute and his work during the last days of World War II compelled him to take this drastic step.

SYNTHESIS OF CHLOROPHYLL
The final step in the structural determination of a new compound is its synthesis. Before the invention of modern physical methods, synthesis was the only means for the final proof of the assigned structure. Although, structural elucidation work of chlorophyll was almost complete by 1940, its synthesis was accomplished after twenty years in 1960. Robert Burns Woodward and his research group at Harvard University synthesized chlorophyll. The synthetic procedure used by Woodward group involved 55 separate chemical reactions which were brilliantly and laboriously applied by himself and the other members of his team. This challenging venture took four years for successful completion. In the synthesis of chlorophyll, Woodward was supported by about a dozen of brilliant postdoctoral fellows of his laboratory.

In the field of science, a group of three great scientists, Galileo, Newton and Einstein, is popularly known as the trinity of physics. In a similar manner in the arena of chemistry of natural
products, three great chemists, Willstatter (Germany), Robinson (Great Britain) and Woodward (USA) constitute a triunity of world fame. Their epoch-making contributions to the chemistry of natural products are part of the folklore. Woodward is the greatest synthetic organic chemist that the world has ever seen. He was born on 10th April, 1917 in Boston, Massachusetts. His father, Arthur Chester Woodward died of influenza just after 18 months of his birth. His mother, Margaret Woodward remarried and settled in Quincy, Massachusetts. At the age of 16, Woodward was admitted to Massachusetts Institute of Technology in 1933. He was whole heartedly devoted to the study of chemistry but neglected the compulsory subjects which did not interest him. Consequently, he was expelled at the end of the term in 1934. But luckily he was readmitted by MIT in the 1935 term on the recommendation of some of his teachers. In 1936 he was awarded B.Sc. degree. In 1937, he completed his Ph.D. degree in one year only at the age of 21. For his doctoral degree, Woodward worked on 'Investigations related to the synthesis of female sex hormone estrone'. After a brief postdoctoral stint at the University of Illinois, he entered the Harvard University as a Junior Research Fellow and remained at Harvard in various capacities throughout his life.

During the 1930s, British organic chemists Christopher Ingold, Robert Robinson and many other organic chemists developed the concept of Organic Reaction Mechanism. They formulated some empirical rules to predict the reactivity of organic molecules. In fact, it was the commencement of a new era in the field of organic chemistry. Woodward was the first synthetic organic chemist who started the use of these theoretical rules as a predictive framework in the synthesis of medicinally important and structurally complex natural products. The synthetic strategies devised by him always had an element of art in them. He was very careful about elegance as well as utility in synthesis. Judicious use of newly developed techniques of infrared spectroscopy and later nuclear magnetic resonance spectroscopy was an important feature of his synthetic work. It is a general experience that most of the medicinally useful natural products are usually chiral molecules and depict their physiological activity in a particular stereochemical form only. Consequently, there is necessity of stereoselective synthesis. Woodward was a pioneer in showing how a compound of desired configuration could be synthesized by a stereoselective synthesis. This skill of Woodward is depicted interestingly in the synthesis of reserpine and strychnine.

About 400 research students and coworkers from all over the world had the proud privilege of working with Woodward. He synthesized many complex natural products which include quinine (1944), patulin (1950), cholesterol (1951), cortisone (1951), lanosterol (1954), lysergic acid (1954), strychnine (1954), reserpine (1956), chlorophyll (1960), tetracycline (1962), colchicine (1963), cephalosporin (1965), and vitamin B_{12} (1972). Woodward started a new era of synthetic organic chemistry which is known as 'Woodwardian era'. Woodward expressed the significance of synthetic work in these words, 'The structure known, but not accessible yet by synthesis, is to the chemists what the unclimbed mountain, the uncharted sea, the untiled field, the unreached planet are to other men'. For his outstanding achievements in the art of organic synthesis, Woodward was awarded Nobel prize in Chemistry in 1965.

Apart from the above mentioned synthetic work, Woodward in collaboration with Ronald Hoffmann, devised a set of rules in organic chemistry in 1965 to predict the ease and stereochemical outcome of pericyclic reactions. These rules are known as Woodward–Hoffmann Orbital Symmetry Rules. For this excellent work, Hoffmann was awarded the Nobel Prize in Chemistry in 1981 with Kenichi Fukui who developed similar model using frontier molecular orbital (FMO) theory. Woodward died before the 1981 Nobel Prize was awarded, and thus missed this prize which certainly would have been his second.
Nobel Prize. Nobel Prizes are not awarded posthumously.

Woodward was famous for his encyclopaedic knowledge of chemistry and extraordinary memory. His lectures usually lasted for three to four hours. During his lectures, he generally avoided the use of slides and wrote all the structures beautifully on the blackboard by multicoloured chalks. He was very hardworking chemists and extremely busy with his laboratory work and late night brain-storming seminars. During nights, he usually slept for less than four hours. His dedication to chemistry can be imagined by a number of anecdotes. Once a British student who was a new entrant, enquired from Woodward about the schedule of his holidays. He replied that he knew only one holiday and that is Christmas day off. The illustrious Indian chemist Professor Subramania Ranganathan who had the privilege of working with Woodward as a postdoctoral student (1962-1964), remembers his association in these words: “On an average he used to put in 14-15 hours a day (Saturday half day); when I joined he told me that he expected to me to work for 100 hours a week ! Towards the end it came to much more than that. His only regular round was between 10:30-11pm perhaps ensuring our presence”. To ward off the stress caused by tiresome schedule and heavy work load, Woodward resorted to chain smoking and heavy drinking which made an adverse effect on his health and family life.

R.B. Woodward married twice but both the marriages failed miserably and ended in divorce. In fact, chemistry was his only love and he was deeply obsessed with it. It is irony of fate that the man who had an extraordinary knack of synthesising giant organic molecules by arranging a friendly alliance between the various organic molecules, had to face pathetic failure at the front of his own matrimonial alliance. On 8thJuly, 1979, Woodward died in Cambridge, Massachusetts from a heart attack in sleep at the age of 62 years. During that time, he was working on the synthesis of an antibiotic, erythromycin.

REFERENCES


TUBERCULOSIS: AN OVERVIEW
Kanchan Srivastava, Surya Kant, Ajay Verma and Abhishek Dubey

Tuberculosis (TB) was recognized in ancient times and is still a major global cause of death. Because TB principally attacks young people during their economically productive years, affected communities get caught in a cycle of illness and poverty. This affects the growth of countries. One of the most remarkable features of Mycobacterium tuberculosis (MTB) is its capacity to cause both symptomatic and asymptomatic infections in human.

By 1650, TB became the leading cause of death and was considered incurable. A rate of more than 1,000 TB cases per 100,000 populations was seen in the late 1700s and early 1800s. The first widely practiced TB treatment was the introduction of sanatoria in 1859, where patients took bed-rest, ate healthy diets and performed exercises. In 1880, surgical procedures were introduced which probably improved cure rates. Since 1882, the fight against TB was changed dramatically with the discovery of tubercle bacillus; the disease causing bacteria. Then, following the development of Bacilli Calmette-Guerin (BCG) vaccine, the discovery of a series of new anti-tuberculosis drugs and the application of the combined antibiotic chemotherapy method, TB was considered completely curable in 20th century. Since the mid-1980s, TB rates began to increase again. Co-infection of TB with HIV and a surge in multidrug-resistant tuberculosis (MDR-TB), a form of disease that is resistant to at least two first-line TB drugs have threatened to disrupt the recent global successes in TB. The rise of MDR-TB and TB/HIV co-infection has given a new sense of urgency to develop new tools, drugs, diagnostics, and vaccines to vastly improve the way the world responds to the disease. The aim of this review was to study the current/historical incidences and clinical aspects of TB.

INTRODUCTION

Tuberculosis (TB) is one of the most ancient diseases known to mankind and has co-evolved with humans for many thousands of years or perhaps for several million years. TB is a devastating public health problem with grave socio-economic consequences and causes an enormous burden of morbidity and mortality around the world. Although as early as 1689, it was established by Dr. Richard Morton that the pulmonary form was associated with “tubercles.” The word “tuberculosis” is a derivative of the Latin word ‘tubercula’, meaning a small lump. TB also gets its name from the Latin word tuber, which is a botanical term for an underground structure consisting of a solid rounded outgrowth of a stem of a more or less rounded form which new plants may arise. The most familiar example is the potato. When conducting autopsies of TB patients, doctors described small, round, firm and white swellings on the surface or within an organ, most typically the lungs. Because of the color of these tubercles, the disease was commonly referred to as the “White Plague.”

TB, then, is a combination of both the word tubercle and the Greek suffix –osis, which signifies an abnormal or diseased condition, action, or process. The word made its first appearance in the English language in an 1860 textbook. TB was not identified as a single disease until the 1820s and was eventually named “tuberculosis” in 1839 by J. L. Schönlein. TB is caused by a group of closely related bacterial species termed Mycobacterium tuberculosis complex (MTBC). Today the principal cause of human TB is Mycobacterium tuberculosis (MTB). It is estimated that in the pre-antibiotic era, M. bovis
was responsible for about 6% of TB deaths in humans.\(^5\)\(^6\)

In spite of newer modalities for diagnosis and treatment of TB, unfortunately, millions of people are still suffering and dying from this disease. TB is among the top three infectious killing diseases in the world along with Malaria & AIDs. TB kills ~2 million people each year.\(^5\)\(^7\) Even though tubercle bacilli were identified nearly 130 years ago, a definitive understanding of pathogenesis of this disease is still deficient.\(^7\) Although most people carrying TB germs in their bodies do not have symptoms and are not contagious, “active” TB is debilitating and contagious. It can affect people of any age, individuals with weakened immune systems, e.g., HIV infection, are at increased risk. Since the immune system in healthy people wails off the causative bacteria, TB infection in healthy people is often asymptomatic.\(^7\)\(^1\)

**THE RECENT TB EPIDEMIC**

TB is the second leading cause of death among infectious diseases worldwide.\(^7\) TB infects about a third of the global population and is causing an estimated two million deaths every year. A new TB case develops every two minutes and one dies due to TB every ten minutes. It causes a great deal of ill health in the populations of mostly low income countries.\(^6\)\(^7\)\(^8\) India and China had the largest number of cases (24% and 11% of the global total, respectively).

**GLOBAL SCENARIO**

TB is infecting about a third of the global population and is a devastating public health problem with grave socio-economic consequences. TB causes an enormous burden of morbidity and mortality around the world. Yet today, despite the availability of affordable, effective treatment, the annual toll of 9 million new TB cases and 2 million TB deaths worldwide represents an intolerable burden of human suffering and unacceptable barrier to socioeconomic development.

According to WHO, TB is a pandemic. Among the 15 countries with the highest estimated TB incidence rates, 13 are in Africa, while half of all new cases are in six Asian countries, viz., Bangladesh, China, India, Indonesia, Pakistan and Philippines.\(^4\) The global community woke up to this disease when, in 1993, WHO declared TB as a global emergency. It was estimated that by 2004, the world as a whole would have achieved the Millennium Development Goal (MDG) of halting and reversing the incidence to half of its 1990’s prevalence and mortality rate. Now the revised time limit to achieve that MDG is by 2015.\(^4\)

According to “WHO Global Tuberculosis Report 2013”, it was believed that world is on track to meet the targets of the 2015 MDG that is of reversing TB incidence and which included: reduction in the burden of TB by cutting down its incidence rate, prevalence rate and the monitoring of TB patients with treatment success.\(^5\) The strategy also aimed to treat about one million MDR-TB patients between 2011 and 2015 and by 2015, about 75% of MDR-TB patients should complete their treatment.\(^9\) The aim of this strategy is to terminate the global TB epidemic, between 2015 and 2035, reducing TB deaths and new cases by 95% and 90% respectively. Meanwhile, the strategy has also set interim milestones for 2020, 2025, and 2030. Worldwide, the proportion of new cases with multidrug-resistant TB (MDR-TB) was 3.5% in 2013 and has not changed compared with recent years.\(^9\) On average, an estimated 9.0% of patients with MDR-TB developed extensively drug resistant TB (XDR-TB)\(^5\) remains one of the world’s deadliest communicable diseases.\(^6\)\(^9\)

**INDIAN SCENARIO**

It is also known as the disease of poverty. It is the single most common cause of death in the individuals at their reproductive age.\(^5\) In India, TB has been mentioned in the Vedas and the old Ayurvedic scriptures. Historically speaking, fight against TB in India can be broadly classified into three periods: early period, before the discoveries of x-ray and chemotherapy; post-independence period, during which nationwide TB control programs were
initiated and implemented; and the current period, during which the ongoing WHO-assisted TB control program is in place. India ranks second in harboring MDR-TB cases, i.e., about 99,000 cases. Among these, 50,000 cases are recorded in retreatment pulmonary TB cases and EPTB accounts for approximately 15-20% of all types of TB.4

Women experience different risk factors, social and economic consequences, and barriers to treatment than men. Yet little has been done to address the biological differences and gender disparities that present a unique challenge to the diagnosis and treatment of TB in women. TB affects women mainly during their economically and reproductively active years, causing a substantial burden on children and families. Although most TB cases and deaths occur among men; the burden of disease is also high among women.5,8

Early period of TB control was marked with non-availability of any chemotherapeutic agents, absence of diagnostic x-ray facilities and lack of any TB control program.

By 1925, chest radiology started playing diagnostic role in detecting deep-seated areas of TB consolidation. By 1945, the capability of this apparatus was enhanced to embody the MMR (mass miniature radiography) version.

The first genuine success against TB was in immunizing against TB. Developed from attenuated bovine (Mycobacterium bovis) strain of TB by Albert Calmette and Camille Guerin in 1906 was bacillus of Calmette and Guerin (BCG); it was first used on humans in France on July 18, 1921. In 1948, with support from WHO and UNICEF, a BCG vaccine production center in Guindy, Madras (now Chennai), was set up. In 1951, India started a mass BCG campaign to control TB, and for the first time in the history of India, message of health and prevention of disease was taken to the remotest parts of the country.5,6 9

HUMAN SOCIETY AND ORIGINS OF TB

Microbial infections played a key role in shaping life on earth and have been a major selector for the evolution of all present species. Evidences exist that demonstrate infectious diseases were already present in our remote ancestors.10 Considering the impact of MTB, in all probability it has had a greater influence on the genetic selection of the Homo sapiens population than any other infectious agent. The molecular identification of human pathogens in ancient human remains has recently opened new scientific fields that provide considerable insight into the history and evolution of host, pathogen and their interaction, which corresponds to the period subsequent to the expansion of Homo sapiens sapiens out of Africa.16,17 This allows us to track changes in the ancestral tubercle bacillus as it became more and more exposed to the internal environment and immune system of its human host. It is believed that the emergence of human infectious diseases is linked to population density transmitted from human to human living in close contact.11 However, the origin of the disease, the earliest hosts of MTB and its evolution remain unclear. The evolution of the bacteria cannot be considered in isolation.18 It is important to realise how TB has influenced the human development over the millennia, particularly our resistance/susceptibility genes. MTB experienced an evolutionary bottleneck when it became an obligate pathogen and has a colonial relationship with different human lineages.19 In past eras of low human population density, MTB adapted over time in response to host-adaptive changes and vice versa. This process, which can be defined as mutualism, is a biological interaction between individuals of two different species where both individuals derive a fitness benefit. As the host becomes more resistant, strains better able to colonize the resistant host will predominate, thus starting off another cycle. The development of antibiotics has shortened the mutualistic cycle significantly, but the combination of HIV co-infection, antimicrobial therapy and increased global human population density is leading to the emergence of some MTB strains19. TB does not respect anybody. Several important personalities,
statesmen and stateswomen, writers and poets were wiped out by TB (Table 1).10,11

Table 1: Well Known Victims of TB

<table>
<thead>
<tr>
<th></th>
<th>Name</th>
<th>Profession</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Srinivas Ramakrishnan</td>
<td>Mathematician</td>
</tr>
<tr>
<td>2</td>
<td>Alexander Pope</td>
<td>Writer and Poets</td>
</tr>
<tr>
<td>3</td>
<td>Percy Bysshe Shelley</td>
<td>Writer and Poets</td>
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<td>4</td>
<td>John Keats</td>
<td>Writer and Poets</td>
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<tr>
<td>5</td>
<td>Emily Bronte</td>
<td>Writer and Poets</td>
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<tr>
<td>6</td>
<td>Munshi Prem Chandra</td>
<td>Writer and Poets</td>
</tr>
<tr>
<td>7</td>
<td>Sir Walter Scott</td>
<td>Writer and Poets</td>
</tr>
<tr>
<td>8</td>
<td>Anton Chekhov</td>
<td>Writer and Poets</td>
</tr>
<tr>
<td>9</td>
<td>Smt. Kamla Nehru</td>
<td>Wife of Prime minister</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PT, Jawaharlal Nehru</td>
</tr>
<tr>
<td>10</td>
<td>Eleanor Roosevelt</td>
<td>Statesman</td>
</tr>
<tr>
<td>11</td>
<td>Mohd. Ali Jinnah</td>
<td>Statesman</td>
</tr>
<tr>
<td>12</td>
<td>Nelson Mandela</td>
<td>Statesman</td>
</tr>
<tr>
<td>13</td>
<td>Frederic Francois Chopin</td>
<td>Musician</td>
</tr>
<tr>
<td>14</td>
<td>Nicole Pagatini</td>
<td>Musician</td>
</tr>
<tr>
<td>15</td>
<td>Edward Livingstone Trudeau</td>
<td>Doctor</td>
</tr>
<tr>
<td>16</td>
<td>Vivian Leigh</td>
<td>Performing artists</td>
</tr>
<tr>
<td>17</td>
<td>Franz Kafka</td>
<td>Writer</td>
</tr>
<tr>
<td>18</td>
<td>Robert Louis Stevenson</td>
<td>Writer</td>
</tr>
<tr>
<td>19</td>
<td>Edgar Allen Poe</td>
<td>Writer</td>
</tr>
<tr>
<td>20</td>
<td>Amalthea Bachman</td>
<td>Actor</td>
</tr>
</tbody>
</table>

HISTORICAL TUBERCULOSIS

TB is an airborne communicable disease plaguing Human population since antiquity, as the signs of tubercular decay have been found in the bone fragments of 40000 year old Egyptian mummies.4 5 Several names have been used to refer TB. Pulmonary Tuberculosis (PTB) has been referred to as “tabspumonia”, Cutaneous TB has been called “lupus vulgaris”, Abdominal TB as “tabs Mesenterica” and Acute progressive TB has been called galloping tuberculosis.5

The Stigma of being a victim of TB was so much that it led to total isolation until death.11,11

In Ancient India the first reference to TB in Asian civilization is found in the Vedas. In Sanskrit, the disease has been called “Rajayakshma”, “Kahya” and “Susa”. In Yajurveda Samhita, there is a reference to how “Soma” (moon) had been affected by Yakshma. Since Soma the “King and Ruler” was affected by “Yakshma” it came to be known a “Rajayakshma”. The oldest of them (Rig-Veda, 1500 BC) calls the disease Yakshma. The Atharvaveda calls it another name “balasa.”5

TB was described by Hippocrates (400 B.C.) and was documented by Claudius Galen during the Roman Empire.2,3,5

PCR technique was employed to examine the sample of lung tissue from a Peruvian mummy (1000 years B.P), which was then cloned and the amplicon was sequenced.12 Although pathologists frequently used the term during the late 19th century; it did not fully replace “consumption” until well after Robert Koch discovered the causative microbe, Mycobacterium tuberculosis (MTB). In 1882, and Wilhelm Roentgen's 1895 discovery of X-rays which allowed physicians to diagnose and track the progression of the disease. It may be hard to believe but only a century ago many people considered TB to be a "romantic" disease. The ancient Greeks had a wonderful word to describe the ravages of TB: phthisis (from the root phthoe), which describes a living body that shrivels with intense heat as if placed on a flame. Hippocrates identified the illness as the most common cause of illness of his era.5,5 He thought the disease was hereditary but Aristotle argued that it was contagious in nature. Later, the Romans applied the Latin word consumere, to eat up or to devour, to the malady.3,5

During the period of early control, as no drug or treatments with combinations of drugs were available/ effective against TB, a sanatorium
movement originated in Europe and quickly spread worldwide. Popular rationale for sanatoria was that a regimen of rest, good nutrition, open fresh air and high altitude offered the best chance that the sufferer’s immune system would wall off pockets of pulmonary tuberculosis (PTB) infection. In 1863, for the treatment of TB, Hermann Brümmer opened the world’s first sanatorium named Brehmerschen Heilanstalt für Lungenkrank in the city of Górczyn (Sokolowsko), Silesia (now Poland). In India, the first open air sanatorium for treatment and isolation of TB patients was founded in 1906 in Tiluana, near Ajmer city of Rajasthan, followed by the first TB dispensary in Bombay in 1917. During 19th century bed rest, healthy diet and changed environment emerged as important forms of treatment of TB. A break in the chain of infection, the introduction of the sanatorium cure provided the first step against TB. In 1854, the development of sanatoria emerged as a powerful weapon in the battle against TB. The members of the MTBC are obligate pathogens. Today the principal cause of human TB is MTB. TB is an infection caused by slow-growing bacteria that grow best in areas of the body that have high supply of blood and oxygen. That’s why it is most often found in the lungs. This is called Pulmonary TB (PTB). But TB can also spread to other parts of the body, where it is called extra-pulmonary TB (EPTB). The pathogenic species are able to survive and grow within macrophages, which enables them to evade the host immune system. Treatment is often a success, but it is a long process. It usually takes about 6 to 9 months to treat TB. It is either latent or active.

Latent TB is asymptomatic; meaning that TB bacteria are present in the body, but body’s defenses (immune system) are keeping it from turning into active TB. People with latent TB don’t have symptoms unless the disease becomes active.

Active TB means that the TB bacteria are growing and causing symptoms. If the lungs are infected with active TB, it can easily spread to other organs. It is highly contagious.

**TB-Cause**

Robert Koch, a Prussian physician, discovered the cause of TB. The studies had confirmed the contagious nature of the disease and forced the medical community to accept that TB was indeed an infectious disease, transmitted by some etiological agent of an unknown origin.

TB develops when the bacteria are inhaled into the lungs. But the bacteria can travel through the bloodstream to other parts of the body (EPTB). TB may be recognized in human skeletal remains by characteristic vertebral lesions, leading to Pott’s disease. Bony joints are common sites of involvement, and the ribs may show lesions.

An initial or primary infection is very mild. In a person who has a healthy immune system, the body usually fights the infection by walling off the bacteria into tiny capsules called tubercles. If a person’s immune system is unable to prevent the bacteria from growing, the TB becomes active. Of people who have latent TB, 5% to 10% (1 to 2 people out of 20) will develop active TB at some point in their lives.

TB spreads when a person who has active disease exhales air that contains TB-causing bacteria and another person inhales the bacteria from the air. These bacteria can float the air for several hours. Coughing, sneezing, laughing, or singing releases more bacteria than breathing. If TB is present in organs other than the lungs (EPTB), it does not spread easily (i.e., It is less contagious). The specific symptoms will depend on whether the infection is in the lungs or in another part of the body (EPTB). This includes people who:

- Have HIV or another illness that weakens the immune system.
- Care for a patient who has active TB.
- Live or work in crowded places, where other people may have active TB.
- Abuse drugs or alcohol.
- Travel to or were born in places where active TB is common.
SYMPTOMS OF ACTIVE TB
Symptoms of active TB in the lungs begin gradually and develop over a period of weeks or months. Common symptoms include:
- A cough that brings up thick, cloudy, and sometimes bloody mucus from the lungs (called sputum) for more than 2 weeks.
- Tiredness and weight loss.
- Night sweats and fever.
- A rapid heartbeat.
- Swelling in the neck (when in the neck are infected).
- Shortness of breath and chest pain (in rare cases). Skipping doses of medicine can delay the cure and cause a relapse. Relapses usually occur within 6 to 12 months after treatment. Without treatment, active TB can cause serious complications, such as formation of pockets or cavities or a hole that forms between nearby airways in the lungs. Symptoms of EPTB vary widely depending on which area of the body is infected. TB can be fatal if not treated properly.⁵ ¹³ ¹⁴ TB in certain groups of people like infants, children and people with HIV or AIDS need special care.

WHAT INCREASES RISK
People having latent TB infection are at risk of developing active TB, if they have a weakened immune system.⁵ ⁷ ¹¹ The immune system may be weakened in older adults, newborns, women who are pregnant or have recently given birth, and people who have HIV infection, some cancers (i.e. lung cancer, Cervical cancer etc), or poorly controlled diabetes.

TESTS: DIAGNOSING ACTIVE TB
Doctors diagnose TB by using a medical history, physical examination and by checking symptoms. Doctors will also look at the results of a:

AFB Smear Test or Rapid sputum test: This test can provide results within 24 hours. This test is done only when a person is strongly suspected of having TB.

Sputum culture: Testing mucus from the lungs (sputum culture) is the best way to diagnose active TB. But a sputum culture can take 1 to 8 weeks to provide results.

- Sputum Cytology
- Chest X-ray: A chest X-ray is done in suspected TB cases.
- Purified Protein Derivative (PPD test), or Montoux test: A tuberculin skin test.
- Biopsy: A sample of the affected area is taken out and looked for TB-causing bacteria in EPTB cases.
- Urine culture: This test looks for TB infection in the kidneys (renal TB).
- Lumbar puncture: A sample of fluid around the spine is taken to look for a TB infection in the brain (TB meningitis).
- CT scans: This test is used to diagnose TB that has spread throughout the body (miliary TB) and to detect lung cavities caused by TB.
- MRI: Looks for TB in the brain or the spine.
- Immunological: Interferon Gamma Assay (QuantiFeron assay), Enzyme-linked immunospot assay (ELISPOT)⁵¹
- Molecular Tests: Line Probe Assay (LPA), Nucleic Acid Amplification (NAA) Tests, PCR amplification,

TESTS DURING TB TREATMENT
During treatment:
- A sputum culture is done once a month or more often to make sure that the antibiotics are working.
- Have a chest X-ray at the end of treatment to use as a comparison in the future.

Specific tests to see the harmful effects of TB medicines:
- Liver function tests.
- Eye tests, especially if patients are taking Ethambutol for TB treatment.
- Hearing tests, especially if patients are taking streptomycin for TB treatment.

TREATMENT OVERVIEW ANTI-TUBERCULAR DRUGS
In India the first TB patient was found in 1906 in Tuluana, near Ajmer city of Rajasthan, followed by first TB dispensary established in Bombay in 1917.⁵ ⁸ ¹⁴
The anti-TB drugs currently used in first-line treatments are around 50 years old. Antibiotics were used against TB for the first time in 1944 after the discovery of streptomycin. Use of this agent alone led to antibiotic resistance that is still a major problem. Better results followed after the development of PAS (Para-aminosalicylic acid). PAS was an oral agent unlike streptomycin. Thereafter more effective drugs like INH (Isoniazid) came in 1950's and treatment with Rifampicin (RIF) followed. These antibiotics are given as pills or injections. These medicines are given to everyone who has TB, including infants, children, pregnant women, and people who have a weakened immune system.  

There are more than 20 drugs available for TB treatment. They are used in different combinations according to the circumstances. Treatment for TB will usually involve a long course of antibiotics. More than 90% of people who are not TB drug resistant can be cured in six months. Regimens for treatment of MDR-TB currently recommended by WHO entail at least 20 months of treatment with second-line drugs for most patients, and are associated with multiple (and sometimes serious) side effects and lower cure rates. EPTB usually is treated with the same medicines and for the same length of time as active TB. TB is a serious condition that can be fatal if left untreated; deaths are rare if treatment is completed. For most people, a hospital admission during treatment is not necessary.

**Directly Observed Treatment, Short-course (DOTS)** health professional, who watches every time to take medicine; visits to DOTS center are always fruitful because of the longer treatment course for TB. TB is highly efficacious, with cure rates of around 90% in HIV-negative patients. There are also interactions between anti-TB treatments and antiretroviral therapy (ART) for people living with HIV.

New drugs are required to shorten and simplify treatment, to improve the efficacy and tolerability of treatment for MDR-TB and to improve the treatment of TB among people living with HIV. There are other TB drugs that are only used for the treatment of drug resistant (DR) TB. MTB can persist in slow growing as well as in fast growing stages which makes treatment challenging. Almost all of the antibiotics that can be used to treat TB work when the bacteria are actively dividing. In the intensive phase (IP) of TB treatment, the antibiotics mainly kill rapidly growing bacteria, which causes rapid sputum conversion, and the eradication of clinical symptoms. However, in order to kill the persistent or slow growing strains of MTB, the continuation phase (CP) of the treatment is essential. TB can be treated effectively by using first line drugs (FLD). These are the TB drugs that generally have the greatest activity against TB bacteria and they are core to any TB drug treatment program. These TB drugs are particularly used for someone with active TB disease and who has not had TB drug treatment before. However, this FLD often fails to cure TB for several reasons. Relapse and the spread of the disease contribute to the emergence of DR bacteria.

**Table 2: List of anti tuberculous drugs under first and second line**

<table>
<thead>
<tr>
<th>First line drugs</th>
<th>Second line drugs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoniazid (INH)</td>
<td>Ofloxacin (OFX)</td>
</tr>
<tr>
<td>Rifampicin (RIF)</td>
<td>Moxifloxacin (MOX)</td>
</tr>
<tr>
<td>Ethambutol (EMB)</td>
<td>Levofloxacin (LEV)</td>
</tr>
<tr>
<td>Streptomycin (SM)</td>
<td>Ciprofloxacin (CIP)</td>
</tr>
<tr>
<td>Pyrazinamide (PZA)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Fluoroquinolones</th>
<th>Injectable drugs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ofloxacin (OFX)</td>
<td>Kanamycin (KAN)</td>
</tr>
<tr>
<td>Moxifloxacin (MOX)</td>
<td>Amikacin (AMK)</td>
</tr>
<tr>
<td>Levofloxacin (LEV)</td>
<td>Capreomycin (CAP)</td>
</tr>
<tr>
<td>Ciprofloxacin (CIP)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Less effective second line antituberculosis drugs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethionamide (ETH)/ Prothionamide (PTII)</td>
</tr>
<tr>
<td>Cycloserine (CS)/ Terizidone,</td>
</tr>
<tr>
<td>P-amino salicylic acid (PAS)</td>
</tr>
</tbody>
</table>
All the other TB drugs are generally referred to as “second line” or “reserve TB drugs”. For the treatment of DR-TB, the current TB drugs are grouped according their effectiveness, experience of use, and drug class. There are two major indications for the use of second-line therapy in the treatment of TB: resistance of the MTB isolates to few first-line agents and/or patient intolerance (including hypersensitivity reactions) to FLDs.

**New anti-TB drugs:**

Despite of the drugs available to cure drug susceptible as well as DR TB; there is a great demand for the new drugs. This is because patients have started showing resistance to the already available drugs. Therefore, for such patients, alternative treatment options are much needed. The characteristics of new drugs should include:

- New drugs should be affordable, shorter in duration as well as simpler.
- More effective than the old (current) drugs, more effective and less toxic.
- New drugs should be safe to consume by patients with latent TB.
- There should be less drug drug interactions, thereby, beneficial to HIV patient.

The US Food and Drug Administration (FDA) on 28 December 2012 gave approval for including Bedaquiline as part of combination therapy to treat the DR cases, only when patient is left with no other single option. Scientists at Johnson and Johnson discovered the drug (Sirturo), which aims at treating the drug resistant strain of MTB. Various new drugs are on their trial basis and some are under development.

**MULTIDRUG-RESISTANT TB (MDR-TB)**

TB organisms resistant to the antibiotics used in its treatment are widespread and occur in all countries surveyed. Drug resistance (DR) emerges as a result of inadequate treatment and once TB organisms acquire resistance they can spread from person to person in the same way as drug-sensitive TB. MDR-TB is caused by organisms that are resistant to at least the two most effective anti-TB drugs, Isoniazid and Rifampicin. The emergence of MDR-TB is of great concern, because it requires the use of second-line drugs that are difficult to procure.

Therefore, the detection and treatment of drug susceptible or single drug resistant TB is an important strategy for preventing the emergence of MDR-TB.

Extensively drug-resistant TB (XDR-TB) is a form of TB caused by organisms that are resistant to Isoniazid and Rifampicin (i.e. MDR-TB) as well as any fluoroquinolones and any of the second–line anti-TB injectable drugs.

Rifampicin-resistant TB (RR-TB) is caused by organisms that are resistant to Rifampicin, with or without resistance to other drugs. MDR-TB, XDR-TB/TDR-TB are forms of RR-TB. These forms of TB do not respond to the standard six month treatment with first-line anti-TB drugs and can take more years to treat with drugs that are less effective, more toxic and more expensive. Resistance to anti-TB drugs can occur when:

- Patients do not complete their full course of treatment.
- Healthcare providers prescribe the wrong treatment, dose, or length of time for taking the drugs.
- The supply of drugs is not always available, or
- The drugs are of poor quality.

**SUPPORTIVE TREATMENT**

Because TB treatment takes so long, it is normal to be embarrassed about having TB, feel isolated and alone, be worried about losing income or job during treatment, feel guilty about the stresses or feel depressed. Hence, in order to provide psychological support and quality care to the patients, Family members, friends and health professionals need to
recognize these factors. Fears, anxieties, other social and emotional problems associated with TB are genuine and should be incorporated for clinical management. TB control policies need to focus on the provision of psychosocial health services as an integral part of TB care.

As a unique attempt anti-TB agent from natural sources, including microbial metabolites and traditional Chinese medicines are being designed.

**SURGERY**
Surgery is rarely used to treat TB. Surgery has a high success rate, but it also has a risk of complications.7,15,16

**HOME TREATMENT**
Home treatment for TB focuses on taking the medicines correctly to reduce the risk of developing multidrug-resistant TB. (Table 3)

Table 3 : Do’s and Don’ts

<table>
<thead>
<tr>
<th>SN</th>
<th>Do’s</th>
<th>Don’ts</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Have 2 sputum examinations done if you have cough of three weeks or more. These tests are done free of cost at Government sputum microscopy centers.</td>
<td>Don't avoid medical care if you have cough of three weeks or more.</td>
</tr>
<tr>
<td>2</td>
<td>Take all the medicines for the full prescribed period on a regular basis.</td>
<td>Don't rely only on X-ray for diagnosis of TB.</td>
</tr>
<tr>
<td>3</td>
<td>Understand that TB can be cured.</td>
<td>Don’t stop medicines before your physician discontinues them. This to antibiotic-resistant infections much harder to treat.</td>
</tr>
<tr>
<td>4</td>
<td>Cover your mouth when you sneeze or cough.</td>
<td>Don't discriminate against TB patients.</td>
</tr>
<tr>
<td>5</td>
<td>Spit in spittoons containing house-hold germicides.</td>
<td>Don't spit indiscriminately.</td>
</tr>
<tr>
<td>6</td>
<td>If you live with someone who has active TB, help and encourage the person to follow treatment instructions.</td>
<td>Don't spend long periods of time in stuffy, enclosed rooms with anyone who has active TB.</td>
</tr>
<tr>
<td>7</td>
<td>Report any side effects of the medicines, especially vision problems.</td>
<td>--------</td>
</tr>
<tr>
<td>8</td>
<td>During treatment for TB, eat healthy foods and some exercise to help your body fight the infection.</td>
<td>Don't feel guilty about the stress this is causing to family members or friends who are worried about getting TB or already have it.</td>
</tr>
<tr>
<td>9</td>
<td>Try to walk as often as you can.</td>
<td>Don't feel depressed.</td>
</tr>
<tr>
<td>10</td>
<td>Sleep in a bedroom by yourself until you can no longer infect other people.</td>
<td>Don't go to work or school while you can spread the TB infection.</td>
</tr>
<tr>
<td>11</td>
<td>Open windows in a room where you stay. This can help get rid of TB bacteria from the air in the room.</td>
<td>Don't dispose of the soiled tissue in an uncovered container after coughing.</td>
</tr>
</tbody>
</table>
Healthy eating, get enough sleep and exercise.
Keep all your medical appointments.
Take your medicines as prescribed.
Report any side effects of the medicines, especially vision problems.
If you plan to move during the time that you are being treated, let your doctor know so that arrangements can be made for you to continue the treatment.

PREVENTION AND VACCINATION
The battle against the TB is very challenging. TB prevention and control takes two parallel approaches: identifying and treating those with TB and their contacts. Unfortunately, no vaccine is available that provides reliable protection for adults. Many countries use BCG vaccine as part of their TB control programs, especially for infants.

SECRETS OF TB DRUG RESISTANCE UNCOVERED
Researchers have uncovered genetic secrets of the TB bacterium that may be contributing to the spread of multidrug resistance to the disease:
The study investigates the global diversity of TB bacterial.
The researchers examined genes in 99 strains of MTB, which causes TB.
The researchers found that human TB falls into two distinct groups: 'ancient' strains found only in West Africa and 'modern' lineages that dominate Europe, the Americas, East Asia, East Africa and India.
Researchers have uncovered that the bacteria undergo low rates of genetic selection by the mutations in their DNA by a process known as genetic drift and because of this mutations that confer drug resistance are not removed from the gene pool and persist in populations.

TB AND HIV
TB is a leading cause of HIV-related deaths worldwide. TB resurgence was started in late 80s mainly due to HIV. TB and AIDS are the double edged weapon and cause most common opportunistic infection in people living with HIV virus. Globally more and more Acquired Immune Deficiency Syndrome (AIDS) patients are getting infected with TB bacilli. According to the National AIDS Control Organization (NACO), 60% of AIDS patients die of TB. Due to damaged defense machinery of the host TB progresses from a harmless infection to a life threatening condition. The worse happen when the MDR TB strains join hands with HIV. In this lethal partnership with HIV both deadly partners speed up each other's progress. However, even among HIV-infected people, TB can be cured. DOTS is as effective among HIV-infected TB patients as among those who are HIV negative.

EFFORTS FOR TB CONTROL-TB AND PHARMACOVIGILANCE
Pharmacovigilance (PV) is very relevant today for the TB practitioner. WHO defines PV as "the science and activities relating to the detection, assessment, understanding and prevention of adverse effects or any other drug-related problem. The scaling up of treatment among populations with varied demographic profile, nutritional status and background co-morbidity (e.g. HIV-TB) may influence the form and frequency of adverse drug reactions."

TB is more than just a biomedical phenomenon. It maintains its grip on those human populations already suffering from poverty, overcrowded living conditions, inadequate housing, malnutrition, and lack of access to medical care. Thus, any TB control programme needs to therefore move beyond 'medicalization of the disease', and include the socio-cultural and psychological dimensions that impact the disease and its treatment. Health policy planners' and health care workers need to recognize that understanding the psychosocial world-view of patients, are important inputs for any effective treatment and control of the disease.

The Directly Observed Treatment, Short-course (DOTS) remains the heart of the Stop TB Strategy. It is cost-effective and a systematic strategy, having international standard for TB control programmes.
India has adapted and tested the DOTS strategy in various parts of the country since 1993, with excellent results having five components:
1. Pursue quality DOTS expansion and enhancement, Address TB-HIV, MDR-TB and other challenges.
2. Contribute to health system strengthening.
3. Involve all health care providers.
4. Engage people with TB, and affected communities.
5. Enable and promote research.

In the first phase of Revised National Tuberculosis control Programme (RNTCP) (1998-2005), the programme's focus was on ensuring expansion of quality DOTS services to the entire country. RNTCP has introduced second line anti-TB treatment for MDR-TB cases, started in 2007.5,7,9

Another initiative is the Foundation for Innovative New Diagnostics (FIN Diagnostics), working with nearly 40 companies, academic institutions and government bodies to develop simple diagnostic tests.

**CURRENT CHALLENGES**

Even today in India, two deaths occur every three minutes from TB.6,7,9 The greatest challenge in treating TB is that organisms persist for extended periods of time despite antibiotic therapy. Surprisingly, in India, people are still under the impression that TB is a disease of poor people. The rich and affluent persons need to know that their cooks/servants/drivers can be asymptomatic carriers of this deadly disease and hence they can potentially get infected with TB even without stepping into slum areas. The consumption of unpasteurized milk or dairy products made from milk is another potential source of TB for humans, as there is ample evidence that bovine TB (Mycobacterium bovis) gets transmitted to humans.3,4 Major challenges to control TB in India include poor primary health-care infrastructure in rural areas, unregulated private health care, irrational use of first-line and second-line anti-TB drugs; spreading HIV infection and poverty. A collaborative effort is in progress between National tuberculosis control program (NTCP) and National Rural Health Mission (NRHM), to improve primary health care in rural areas. In addition to this, NTCP has established several initiatives to improve TB care.

**CONCLUSION**

TB has been a major public health problem for centuries and creating enormous burden of morbidity and mortality around the world. After more than a century since its discovery and establishment as a pathological agent, we are still trying to understand the different ways used by the MTB to evade host defense system. We still have miles to go before we will make this planet TB free. WHO with its “STOP TB” strategy has given a vision to eliminate TB as a public health problem from the face of this earth by 2050.5,6,8,12

In order to intensify our fight against this deadly disease, we need to further strengthen our surveillance programs to accurately estimate the burden of all kinds of TB (childhood, HIV/TB, MDR-TB).11,17 There is need to regulate the rational use of first- and second-line anti-TB drugs. Working association between physicians; private sector; religious bodies; and other local nonprofit organizations should be strengthened for better dissemination of awareness about diagnosis, management and control of this disease. Better diagnostic tests for quick screening of this disease at field level should be developed and made available at the grass-root level. Hence, much effort is needed with all advanced practices and techniques for useful product(s) to fight with the disease to save lives among the masses.

**KEY FACTS**

- TB caused by a bacterium that is spread through the air from one person to another.
- TB is a leading killer of people living with HIV causing one fifth of all deaths.
- The burden of disease is high among women. Women account for 37% of the total of 9.0 million incident cases in 2013.
- Over 95% of TB deaths occur in low- and middle-income countries, and it is among the top three causes of death for women aged 15 to 44.
- Worldwide, one in every 3 persons gets infected with TB infection and approximately new cases of TB infection gets reported every second
In 2013, an estimated 510,000 women died from TB (250,000 among HIV-negative women and 160,000 HIV+ women).

In 2013, an estimated 550,000 children became ill with TB and 80,000 HIV-negative children died of TB.

MDR-TB is present in virtually all countries surveyed.

An estimated 22 million lives saved through use of DOTS and the Stop TB Strategy recommended by WHO.

In many countries, diagnosis has depended largely on one archaic test for the last 120 years.

In 2013, 6.1 million TB cases were reported to WHO. Of these, 5.7 million were people newly diagnosed and another 0.4 million were already on treatment.

More than one half of these cases occurred in China, India, and the Russian Federation.

Globally, 3.5% of new and 20.5% of previously treated TB cases was estimated to have had MDR-TB in 2013.

For the first time in four decades, two new drugs have been approved for the treatment of MDR-TB under specific conditions: Bedaquiline and Delamanid.

The Millennium Development Goal (MDG) framework includes five indicators: TB incidence, TB mortality, TB prevalence, the case detection rate for new TB cases and the treatment success rate for new TB cases.

By 2013, the global TB mortality rate had fallen by 45% compared with a baseline of 1990.

There are 10 new anti-TB drugs currently in the late phases of clinical development.

There are currently 15 vaccine candidates in clinical trials.

**TIMELINE**

1882 - German physician Robert Koch identifies the bacterial strain as Mycobacterium tuberculosis and new staining method for the sputum of TB patients.

1900s - Tuberculosis causes one-quarter of all deaths in Europe throughout the 19th century and early 20th century.

1921 - French bacteriologists Albert Calmette and Camille Guerin use a live but controlled bovine tubercle bacillus called M. bovis responsible for causing TB in cattle, to create a vaccine named bacilli Calmette-Guerin (BCG). Well and Halle made the first attempt.

1930 - The BCG vaccine is widely used to prevent TB after being used to vaccinate children in Europe and South America.

1939 - TB Association of India was established.

1943-1944 - Microbiologist Selman A. Waksman and his associates at Rutgers University discover an antimicrobial agent, Streptomycin.

1944-1945 - Veterinarian W.H. Feldman and physician H.C. Hinshaw experiment with Waksman's streptomycin and discover its effect on inhibiting TB in animals and people.

1946 - Discovery of Para-amino Salicylic Acid (PAS)

1948 - BCG vaccine laboratory established in King Institute of Guindy, Madras. 1950 - Tuberculosis Seal Sale campaign started.

1951 - Mass BCG vaccination launched.

1952 - Discovery of Isoniazid (INH) after successful tests in the United States and Germany. The antibiotic is used in combination with other drugs to treat TB, which are released the following years.

1953 - Publication of Indian Journal of Tuberculosis.


1956 - Establishment of TB Chemotherapy Centre (TCC), Madras.

1959-60 - Establishment of National TB Institute (NTI), Bangalore.

1962 - Involvement of District TB Programme (DTP) as a part of the National TB programme.


1967 - Discovery of Rifampicin.
March 24, 1982 - The first World TB Day is sponsored by the WHO and the International Union against TB.

Mid-1980s - Tuberculosis make a sudden resurgence, resulting in deaths in developing countries. Scientists attribute this to inadequate health care systems, immigration from countries where tuberculosis is prevalent, and the spread of HIV.


1993 - First direct detection of microbial pathogenic DNA in archaeological material using PCR technique showed that TB was undoubtedly present in the Americas before Columbus.

1993 - The WHO declares TB, a global emergency.

1995 - The WHO launches Directly Observed Therapy Short-Course (DOTS). The program requires doctors to ensure TB patients take their medications while also monitoring their treatment.

Early 2000s - The number of reported TB cases drops in Africa due to programs launched by the WHO.

May 2007 - American Andrew Speaker causes an international health scare after coming into contact with various passengers on international flights. Doctors later confirm Speaker's test results for a drug-resistant form of TB (XDR-TB) are negative.

2008 - The WHO reports the highest rates of MDR-TB worldwide.

2009 - Foreign-born persons reported higher rates of TB cases compared to those born in the United States.

December 8, 2010 - The WHO endorses a new test that diagnoses tuberculosis in 100 minutes instead of three months.

May 26, 2011 - Nearly 700 patients and 100 employees are exposed to TB at Emory University Hospital in Atlanta after interacting with a hospital employee carrying the disease.

REFERENCES

14. www.TBfacts.org
INSTITUTE OF FOREST PRODUCTIVITY, RANCHI

Institute of Forest Productivity, Ranchi, is one of the eight institutes under Indian Council of Forestry Research and Education, Dehra Dun, which is an autonomous council of Union Government of India Ministry of Environment and Forests, New Delhi. The institute has a long glorious history, which commenced with its establishment as erstwhile Directorate of Lah Research under Indian Council of Agriculture in 1966. The directorate was subsequently transferred as subsidiary to Forest Research Institute (FRI), Dehra Dun in 1985 under Ministry of Agriculture, New Delhi. In a turn of events, Directorate of Lah Research and also FRI, Dehra Dun was shifted to newly created Ministry of Environment and Forests, New Delhi in 1987. Subsequently in 1988, the directorate with the merger of three small research units, viz., Forest Soil and Vegetation Survey and Eucalyptus Research Centre, Midnapore (WB), Environmental Research Station, Sukna (WB) and Cash Crop Centre, Mandar (Bihar), was rechristened as Institute of Forest Productivity in 1993. As a result, the institute was conferred with enlarged and focused research mandate of augmenting forest productivity and quality vis-à-vis enhancing livelihood of tribals and forest fringe villagers of eastern part of the country, i.e. Bihar, Jharkhand and West Bengal. The institute caters to the R & D needs of > 50,000 square km of forests spread over eight forest types and six agro-ecological zones. Additionally, the institute also focuses on eco-restoration of vast area of diverse mined over burdens. Thus, the jurisdiction of the institute encompasses the picturesque eastern Himalayas in Sikkim and North Bengal, the fertile alluvial expanse of indo-gangetic plains in Bihar and West Bengal, deltaic and coastal mangroves of the world famous Sunderbans, a pocket of Terai sal forest in the north west corner of Bihar and tropical deciduous forests of Kaimur and Chotanagpur plateau overlaying rich and enticing mineral resources.

Infrastructure

The institute houses two blocks, i.e. R & D Block and Administrative Block and one guesthouse along with adjacent campus of Lead Botanical Garden and Silviculture Nursery and is situated at 15km on Gumla NH-23 from Ranchi city on a picturesque hillock. The institute has excellent infrastructure and experimental area. These include state of the art plant tissue culture laboratory, biochemistry and molecular biology laboratory, soil testing laboratory, mist propagation and hardening facilities and experimental field of 16.64ha (Mandar, Ranchi) and
26ha (Nagri, Ranchi) in Jharkhand and 6.42ha (Udai Jote Singh, Darjeeling) in West Bengal. Of the 125 indigenous bamboo species, the institute houses a bamhuscum of 39 species, which is the largest collection in the eastern part of India. Also, there is a Lead Botanical Garden comprising 200 species of medicinal plants. The institute has received/ is getting research grants from international agency like UNDP and national agencies such as DST, DBT, NMPB, Planning Commission and state forest departments of Bihar, Jharkhand and West Bengal. The institute implements R & D program through six research divisions, viz., Silvicultural & NTFP Management Div., Agro-forestry & Extension Div., Information Technology & GIS Div., Forest Physiology & Molecular Biology Div., Forest Ecology & Land Management Div. and Forest Genetics & Breeding Div.

Research Mandate

The institute conducts R & D activities in order to realize the following mandates:

- To setup, undertake and coordinate research, development and extension in forestry sector in the states of Bihar, Jharkhand, Sikkim and West Bengal.
- To enhance the productivity of floral and faunal resources, conservation of biodiversity, eco-restoration of degraded lands and protection of fragile ecosystem unique to the region.
- To develop and maintain information on forestry and allied sciences.
- To act as a clearing-house for region specific research and general information relating to forests and wildlife.
- To undertake research and demonstration of agro-forestry models.
- To establish model nursery, germplasm bank and identify suitable seed production areas for improving forest productivity.
- To develop extension programmes and propagate the research results from lab to land.
- To provide consultancy services in the field of forestry research, training and in allied sciences.
- To develop and extend Lac cultivation and dissemination of market data on lac production in the country.
- To carry out the related activities considered necessary to attain the above activities.

Academic and Training Activities

The institute provides graduate and post-graduate summer academic trainings to university students of forestry, molecular biology, soil science, biotechnology, vocational trainings to artisans and farmers for skill up-grading and livelihood enhancement and orientation and refresher trainings to officers and staff of forest departments. The institute also offers doctorate program in Forest Genetics, Biotechnology, Environment & Climate Change through FRI Deemed University, Dehra Dun or in affiliation with national / state universities.

R & D Achievement

The institute has made noteworthy R & D contribution on various aspects of forestry sector and improvement of livelihood of tribals, which are briefly enlisted below:

- Standardization of clonal propagation techniques for eastern Indian genotypes / populations of Anthocephalus chinensis, Bambusa nutans, Ceiba pentandra Dendrocalamus asper, Embelia ribes, Pongamia pinnata, Raouwolfia serpentina, Schleichera oleosa.
- Standardization of quality seedling production system for Buchania lanzan, Anthocephalus chinensis and Bombax ceiba.
- Bio-reclamation of problem soils and mined overburdens, including lateritic soils and fly-ash.
- Selection and improvement of oil-yielding

- Revival and promotion of lac cultivation in the region through popularization of short rotation and small sized shrub (*Flamingia* spp.) in place of conventional long rotation and large sized conventional host trees for rearing of lac insects and upliftment of economic conditions of villagers.

- Revival of conventional chuan system (natural groundwater springs and their management) as a perennial water resource for irrigation and other purpose vis-à-vis increasing numbers of annual crops and augmenting economic conditions of regional farmers of small holdings.

- Introduction of poplars on bunds or as tree crop in the fields of farmers of Bihar.

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International Conference on Recent Innovations in Ayurvedic Sciences and Technology (ICRIAST), 24-25 October, 2015, Varanasi

Themes:

- Herbal Drug Research: Challenges and Opportunities
- Drug Discovery & Development
- Anti-Inflammatory Drug Research and Therapeutics
- Infections of the various systems: Challenges and Opportunity
- Interrelationship between Inflammation, Cancer and Related Diseases
- Innovation in Medicine and Healthcare
- Evidence-Based Lifestyle Factors that Enhance Cardiovascular
- Cognitive, Metabolic and Hormone Function
- Integration of Pharmacogenomics in clinical decision making
- Nano-medicine and Ayurveda
- Clinical trials and pharmaco-vigilance in Ayurveda
- Biochemistry & microbiology in Ayurvedic Research
- Biotechnology and Ayurveda
- Pharmaceutical science & Pharmacology in Ayurvedic drug science
- Stress & Psychosomatic Disorders
- Auto Immune Disorders
- Metabolic Disorders
- Genetic Disorders
- Life style Disorders

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International Conference on Frontiers of Plant Sciences & Developing Technologies (ICFPSDT), 7-8 November, 2015 Varanasi

Themes:

- Indigenous Technical Knowledge in Plant Sciences
- Information and Communication Technologies for Development
- Soil Health & Plant Nutrients
- Soil contamination, phytoremediation techniques
- Recent advances in integrated pest management
- Prediction of migration pattern from rural areas and its effect on agriculture
- Recent advances in horticulture
- Carbon Cycle Science
- The Need for Accurate Atmospheric Transport in Carbon Cycle Research
- Toward Development of an Integrated Earth System Analysis
- Innovations in Plant Biotechnology
- Natural Resources and Sustainable Agriculture
- Abiotic Stresses: Physiological and Molecular Approaches for Mitigation
- Pharmaceutical Biotechnology
- Targeting Global Sustainability-Food Security, Biodiversity and Climate Change
- Organic crop production and animal husbandry.
- Floods, drought, forest fires, hurricanes and other sporadic events
- Impacts on humans: agriculture, fish stocks, food supply, health, water resources
- Improvement of water-nitrogen and carbon management to increase crop production
- Modern farming of vegetables
- Expansion of knowledge and sustainable use of biodiversity
- Performance of hybrids in cultivation of crops
- Provision of ecological services, such as crop protection, yield stability and system resilience
- Sensor technology and modern agronomic service

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**DAE BRNS National Laser Symposium (NLS-24), December 2–5, 2015, Indore (M.P.)**

**Topics:**
- Physics and technology of Lasers
- Lasers in Nuclear Science & Technology
- Laser Materials, Devices and Components
- Nonlinear, Quantum and Atom Optics
- Ultrafast Lasers and Applications
- Lasers in Materials Science
- Lasers Plasma Interaction
- Lasers in Industry and Defense
- Lasers Spectroscopy and Applications
- Lasers in Chemistry, Biology & Medicine
- Laser Based Instrumentation
- Electronics and Instrumentation for Lasers

Contact: Website http://www.ila.org.in/nls24/, Contact Person – H. S. Vora, Event enquiries email address – vora@rrcat.gov.in
BRAZIL : COMBATING DENGUE BY USING COLORANT EXTRACTED FROM TURMERIC

A compound extracted from the roots of turmeric (Curcuma longa L.), a plant that belongs to the ginger family (Zingiberaceae) and is also known as Indian saffron, has been successfully tested by researchers in the city of Sao Carlos, Sao Paulo State, as a weapon against larvac of the mosquito species that transmits dengue virus. The research is being conducted at the Optics and Photonics Research Center (CePOF), one of the Research, Innovation and Dissemination Centers (RIDCS) funded by FAPESP. Curcumin, one of the substances that give turmeric its orange color, has natural photodynamic properties. In the presence of light, it stimulates the production of reactive oxygen species, which are highly toxic. The larvae of Aedes aegypti are transparent and are therefore particularly sensitive to photodynamic effects. The pigment accumulates in the insect’s gut after being swallowed with the water in which the larvae breed. When activated by light, it stimulates the production of singlet oxygen molecules, which cause fatal damage to digestive tract tissue. A similar principle has been used in experimental applications of photodynamic therapy designed to target tumor cells and infectious agents. The researchers are comparing the effects of photodynamic therapy using sunlight, ordinary white light, and blue LED light. In the most successful trial, 100% of the larvae that were present in the sample died after eight hours of exposure to sunlight. Mortality rates began to increase after the first two hours. The colorant was used at a concentration of 15 micrograms per milliliter of water. An important result is that mortality was high even on overcast days, which means that the breeding ground does not have to be in direct sunlight for the method to work. CEPOF group is also conducting three separate clinical trials to evaluate the effectiveness of the curcumin-based colorant to combat nail fungus, for oral decontamination, and in the treatment of venous ulcers. In vitro experiments performed at the University of Sao Paulo's Sao Carlos Institute of Physics (IFSC-USP) have already shown that the compound is effective at killing microorganisms. The process used to extract curcumin pigment from turmeric power was developed in partnership with researchers at UFSCar, who belong to the CePOF group and PDT Pharma. However, the extraction and purification of the natural product would be too costly for use on a large scale. To address this problem, UFSCar's Photosensitizing Heterocyclic Compound Synthesis Group developed a method of producing synthetic curcumin with the same chemical structure as the natural pigment, which can be produced on a large scale and is also more sustainable. The absence of other curcuminoids does not significantly diminish the molecule's activity in our ongoing studies. On the contrary, the use of synthetic curcumin enhances the experiments' dynamism, breadth, versatility and reproducibility.

(Source: Agencia FAPESP, 6th May 2015)

CHINA : HEAT TOLERANCE GENES FROM AFRICAN RICE VARIETY

A team of scientists from the Chinese Academy of Sciences successfully isolated and cloned heat tolerance genes from African rice strains, which could be used to develop rice varieties that can resist the effects of global warming. The temperatures over 35 degrees Celsius decrease the productivity of rice plants. Heat stress destroys rice proteins, causing the plants to wither. Under heat stress, the heat tolerance gene from African rice variety is activated, and gets rid of the toxic proteins that may cause death to the rice plant. The researchers have tested Asian rice varieties with the transplanted gene in field conditions. The results showed that the gene's dominant traits enabled to transformed plants to withstand heat stress. Furthermore, the cloned gene may also be used to develop heat tolerant varieties of wheat and cruciferous vegetables such as Chinese cabbage.

(Source: Crop Biotech Update, 27th May 2015)
INDIA: GRAPHENE NANORIBBONS FOR SENSORS, SUPERCAPACITORS

Researchers of the TIFR Centre for Interdisciplinary Sciences, Hyderabad have used three-dimensional graphene nanoribbons to produce and electrode that could potentially be used in efficient sensors and supercapacitors. They synthesized two-dimensional graphene nanoribbons by chemically cutting multiwalled carbon nanotubes with a strong oxidizing agent such as potassium permanganate in an acidic medium and deposited these on a glassy carbon electrode, converting them into three-dimensional graphene nanoribbons using a chemical called glutaraldehyde. The scientists performed electrochemical measurements to probe the efficacies of the three-dimensional nanoribbon-modified electrodes to sense biochemicals and store charge and compared the results with those for an electrode modified with two-dimensional graphene nanoribbons. It was found that the electrode with three-dimensional nanoribbons exhibited a high peak current and fast charge transfer due to large surface area of the nanoribbons. When dipped in solutions containing ascorbic acid and the neurotransmitter dopamine, the three-dimensional nanoribbon-coated electrode showed a large increase in peak current. The researchers attribute this current response to the high surface area and fast electron transfer through the network of three-dimensional graphene nanoribbons. The electrode coated with three-dimensional nanoribbons could also store charge, indicating its potential for use in supercapacitors. The three-dimensional graphene nanoribbons have been found to aid the oxygen reduction reaction, which is important for triggering electrochemical energy conversion processes in fuel cells and metal-air batteries.

(Source: Nature India update, 29th April 2015)
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