**Programme for seminars on Methods on molecular biotechnology**

**The title of seminars**

**Module 1** Structure, feature and functions of nucleic acids

*Seminar* 1. Biochemical markers of disease resistance

*Seminar 2.* Cyclic adenosine monophosphate (cAMP). Mitogen-activated protein kinases (MAPKs

Recognition

* Initiates after the host and the pathogen come in contact with each other
* Indicates some kind of communication between the two
* Begins with onset of biochemical reactions in one or both the interacting units
* **Signal- sensor reaction**

**Singals**

* Host components acting as signals for recognition by
* and activation of pathogens are numerous e.g. cutin,
* galacturonans,

The perception of signals

* The perception of signals from plant surfaces by pathogenic fungi is the result of signaling pathways mediated by:

1. cyclic adenosine monophosphate (cAMP)
2. and mitogen-activated protein kinase (MAPK),

which have been implicated in regulating the development of infection-related phenomena in many different fungi.

*Seminar* 3. Biochemical markers related to disease resistance. Choice and application**Seminar 5.** Analysis of different types nuclear acids.

**Secondary Messengers**

Secondary messenger system exists in plants to transmit the primary elicitation signal of pathogen and/or host.

These are:

1. • Ca2+ ion influx
2. •Protein phosphorylation
3. •cAMP
4. •Active oxygen species
5. •Salicylic acid
6. •Methyl Jasmonic and Jasmonic Acid
7. •Ethylene
8. •Nitric Oxide

Seminar 4. Biochemical markers of disease resistance 4. Extraction DNA. Main principles.

. The resistance mechanisms involving the role of phenol, PAL, oxidative enzymes like peroxidase (PO), polyphenol oxidase (PPO), superoxide dismutase (SOD), catalase and PR-proteins like chitinase, β-1-3 glucanase were studied and they showed relatively higher activity in resistant hybrids than susceptible hybrids. Isozyme analysis of peroxidase (PO) and polyphenol oxidase (PPO) was also carried out in cultivars and hybrids, which revealed the induction of specific isoforms in the resistant hybrids upon challenge inoculation. This could be a useful tool for early identification of *F. oxysporum* f. sp. *cubense* resistance banana clones.

*Seminar* 5. Signal responses - massive changes in gene expression. Structural Defenses. Biochemical relationship in resistant and susceptible cultivars.

* Non-protein amino acids: Many plants also contain unusual amino acids called non- protein amino acids that incorporated into proteins but are present as free forms and act as protective defensive substances .
* For examples, canavanine and azetidine-2-carboxylic acid are close analogs of arginine and proline respectively.
* They exert their toxicity in various ways.
* Some block the synthesis of or uptake of protein amino acid while others can be mistakenly incorporated in to proteins.
* After ingestion, canavanine is recognized by herbivore enzyme that normally binds arginine to the arginine transfer RNA molecule and so become incorporated in to proteins in place of arginine.
* The usual result is a non-functional proteins because either its tertiary structure or it catalytic site is disrupted .
* Plants that synthesize non-protein amino acids are not susceptible to the toxicity of these compounds but gain defense to herbivorous animals, insects and pathogenic microbes.
* Also, a number of plants including Arabidopsis uses Arginine as a storage and transport form of N and proline as a compatible solute in the defense against abiotic stresses causing water deprivation

Seminar 6. Utilization of pigmented cells and phytoalexins as biochemical markers for screening resistance .

*Seminar.*7. Optimization and use of RFLP markers for comparative and synteny mapping

*Seminar* 8. To know and learn: application of Random Amplified Polymorphic DNA (RAPD) in plants breeding for disease resistance

# ***Seminar* 9.** **Role of secondary metabolites in defense mechanisms of plants**

* In all natural habitats, plants are surrounded by an enormous number of potential enemies (biotic) and various kinds of abiotic environmental stress.
* Nearly all ecosystems contain a wide variety of bacteria, viruses, fungi, nematodes, mites, insects, mammals and other herbivorous animals, greatly responsible for heavy reduction in crop productivity.
* By their nature, plants protect themselves by producing some compounds called as secondary metabolites.
* Secondary metabolites, including **terpenes**, **phenolics** and **nitrogen** **(N)** and **sulphur (S)** containing compounds, defend plants against a variety of herbivores and pathogenic microorganisms as well as various kinds of abiotic stresses.

They include

* glutathione (GSH),
* glucosinolates (GSL),
* phytoalexins,
* thionins,
* defensins and allinin

which have been linked directly or indirectly with the defense of plants against microbial pathogens

*Seminar* 10. DNA Separation Techniques for different types of DNA

## Seminar 11. Types of DNA microarrays

The Future of DNA arrays. Data standards and data exchange. DNA microarrays for transcription factor binding analysis.

*Seminar* **12.** Strategies for SNP detections strategies for arrays

**Seminar 13.** Preparation of DNA chip and the experiment

**Seminar 14.** Marker-assisted backcrossing (MABC). Marker assisted pyramiding

Backcrossing is used in plant breeding to transfer favourable traits from a donor plant into an elite genotype (recurrent parent). In repeated crossings the original cross is backcrossed with the recurrent parent until most of the genes stemming from the donor are eliminated (Becker1993). However, the donor segments attached to the target allele can remain relatively large, even after many backcrossing generations. In order to minimize this linkage drag, marker assays can be of advantage.

There are three levels of selection in which markers may be applied in backcross breeding. Markers can be used in the context of MABC to either control the target gene (foreground selection) or to accelerate the reconstruction of the recurrent parent genotype (background selection) and to select backcross progeny having the target gene with tightly- linked flanking markers in order to minimize linkage drag (recombinant selection).

According to Frisch et al. (1999) in a computer simulation MAS can reconstruct a

level of recurrent parent genome in BC3 which would only be reached in BC7 without the use of markers.

However, the authors also state that large numbers of marker data points are required to achieve such results. MABC is especially efficient if a single allele is to be transferred into a different genetic background, for example, in order to improve an existing variety for a specific trait. To overcome the limitation of only being able to improve existing elite genotypes, other approaches like marker-assisted recurrent selection(MARS) have to be considered.

**Marker-assisted recurrent selection (MARS):** The improvement Of complex traits Via phenotypic recurrent selection is generally possible, but the long selection cycles impose restrictions on the practicability of this breeding method. With the use of markers, recurrent selection can be accelerated considerably and several selection-cycles are possible within one year, accumulating favourable QTL alleles in the breeding population.

* Pyramiding may involve combining genes from more than two parents. For example, combined genes originating from three parents for rice blast and stripe rust in barley, respectively. MAS pyramiding was also proposed as an effective approach to produce three-way F1 cereal hybrids with durable resistance.

The most widespread application for pyramiding has been for combining multiple disease resistance genes (i.e. combining qualitative resistance genes together into a single genotype). The motive for this has been the development of ‘durable’ or stable disease resistance since pathogens frequently overcome single-gene host resistance over time due to the emergence of new plant pathogen races. Some evidence suggests that the combination of multiple genes (effective against specific races of a pathogen) can provide durable (broad spectrum) resistance. The ability of a pathogen to overcome two or more effective genes by mutation is considered much lower compared with the ‘conquering’ of resistance controlled by a single gene. In the past, it has been difficult to pyramid multiple resistance genes because they generally show the same phenotype, necessitating a progeny test to determine which plants possess more than one gene. With linked DNA markers, the number of resistance genes in any plant can be easily determined. The incorporation of quantitative resistance controlled by QTLs offers another promising strategy to develop durable disease resistance.

**Seminar 15.** Advantages of MAS over conventional methods.QTL applications in breeding

a- Gene stacking for a single trait: MAS allows breeders to identify the presence of multiple genes/alleles related to a single trait, when the alleles do not exert individually detectable effects on the expression of the trait. E.g: when one gene confers

resistance to a specific disease, breeders would be

unable to use traditional phenotypic screening to add another gene to the same cultivar in order to increase the durability of resistance. In such cases, MAS would

be the only feasible option, provided markers are available for such genes.

b- Early detection:MAS allows alleles for desirable traits to be detected early i.e in the seedling stage itself well before the trait is expressed phenotypically. This benefit can be particularly important in slow growing and long duration crops.

c- Recessive genes: MAS allows breeders to identify heterozygous plants that carry a recessive allele of interest whose presence cannotbe detected phenotypically. In traditional breeding approaches, an extra step of selfing is required to detect phenotypes associated with recessive genes

d***- Heritability of traits:*** MAS is mainly useful in selection for traits with low heritability up to a point, gains from MAS increase with decreasing heritability.

e- Seasonal considerations: MAS offers potential savings compared with conventional selection when it is necessary to screen for traits whose expression depends on seasonal parameters. Using molecular markers, at any time of the year, breeders can screen

for the presence of an allele (or alleles) associated with traits that are expressed only during certain growing seasons. For example, CIMMYT’s wheat breeding station in northern Mexico is usually used for screening segregating germplasm for leaf rust resistance.

However, expression of leaf rust is not uniform in all growing seasons. When there are seasons with low expression of leaf rust, markers, if available, can be a valuable alternative as a tool for screening.

***f- Geographical considerations***: MAS is necessary to screen

for traits whose expression depends on geographical considerations. Using molecular markers,

breeders in one location can screen for the presence of an allele (or alleles) associated with traits expressed only in other locations.

***g-Multiple genes***, multiple traits: MAS offers

potential savings when there is a need to select for multiple traits simultaneously.

With conventional methods, it is often necessary to conduct separate trials to screen for individual traits.

***h- Biological security considerations***: MAS provides a potential advantages over selection based on the use of

potentially harmful biological agents (e.g. artificial viral infections or artificial infestations with pathogens), which may require specific security measures.