

**Presidential address : Some aspects of molecular genetics  
related to plant-microbe interaction**

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Distinguished members, Guests, Ladies and Gentlemen,

I consider it a great privilege, the honour you have conferred on me by electing me as the President of the Indian Mycological Society (IMS) which I deeply appreciate.

The year 1990 is of special significance to the World Mycologists since the Fourth International Mycological Congress (IMC 4) will be held in Regensburg Federal Republic of Germany from 28th August to 3rd September this year. In addition to scientific programme, excursions and Workshops are also being arranged. I hope that at least one or two members of our Society will participate in the ensuing Congress.

It is remarkable that the year began with the publication of the first issue of our new Journal entitled "Journal of Mycopathological Research" which I believe will attract the attention of International Mycologists. Our Society will also be benefitted by this publication. I would like to thank Prof. N. Samajpati, Editor of this Journal for the tremendous effort he has put into the preparation of the Journal.

Like previous years, this year also a few lectures have been arranged by the IMS in Calcutta. We invited Prof. R. S. Mehrotra, a distinguished Plant Pathologist and Head, Department of Botany, Kurukshetra University for delivering the Second Dr. S. R. Bose Memorial Lecture. We are indeed thankful to him for kindly accepting our invitation.

Since it is customary to deliver a formal discourse at the Annual General Meeting, I have decided to give a brief address on "Some aspects of Molecular Genetics

related to Plant-Microbe interaction" on the occasion of the 34th Annual Meeting of the Society. I have chosen this because this area of research has fascinated me for quite sometime now. It is not unreasonable to expect that this topic might create an interest to other Plant Pathologists also.

Why are Plant Pathologists interested in genetical studies? Because it is taken for granted that our growing knowledge of genetics of host and pathogen may help to design more effective control measures against crop diseases, new pathogens or new strains. Besides the study of plant microbe interaction sometimes provides new information which promises considerable practical benefit to agriculture.

The genetic interdependence of host and parasite was first demonstrated by Flor. According to the gene-for-gene hypothesis of Flor (1956) "for every gene that conditions resistance in the host there is a corresponding gene in the parasite that conditions pathogenicity". But very little is known about the nature of recognition reaction controlled by these interacting genes for avirulence in parasite and resistance in host. A few plant pathogens have been analysed at the gene level so far. It is expected that more studies in future on genes and their functions in plant pathogens will provide useful information in elucidating the role of specific gene products in pathogenicity and it would also help to locate and isolate gene products of host that encode for resistance genes. Once these genes are cloned and identified it would be possible to introduce these genes into the host by transformation system. By this process organisms take up naked DNA and subsequently acquire an altered genotype. Kerr (1987) suggested that transposable elements could be employed to locate resistance genes in plants, if present. Although it has now become possible to transfer transposable elements from maize to tobacco and other host plants, their use for the identification of resistance genes, remains problematical.

Genetics of many fungi including obligate biotrops, members of Deuteromycotina and higher plants is still unknown and hence several intricate problems pertaining to host-microbe interactions also remain yet to be solved.

The concepts and methods of molecular genetics could provide useful information for solving these intricate problems. For instance, DNA-transformation, gene fusion, restriction fragment length polymorphisms (RFLPs) and genetic manipulation are being used for the improvement and protection of plants and microbes. Some of the interesting findings will be discussed here.

In 1984, Staskawicz *et al.* identified a DNA sequence responsible for avirulence in a bacterium *Pseudomonas syringae* pv. *glycinea* race 6, a pathogen of soybean. The showed that when a normally virulent strain of the bacteria acquired an intact DNA sequence for avirulence, it also became avirulent. This discovery

may lead to the identification of the nature of the avirulence gene product, DNA sequence for resistance and also the nature of the product of resistance gene. The molecular genetic evidence supports the validity of the gene-for-gene hypothesis in bacteria plant interaction.

Kobayashi and Keen (1988) isolated several cosmid (i.e. plasmid which contains the cos site from bacteriophage  $\lambda$  and can be packaged *in vitro*) (Staskawicz, 1983), clones from a DNA cosmid library of *Pseudomonas syringae* pv. tomato. When these were introduced into *P. syringae glycinea*, they caused hypersensitive reaction (HR) in some but not in all soybean cultivars. It suggests that *P. syringae* pv. tomato contained avirulence genes which induced HR in soybean also in a rare specific manner when they were transferred to a related bacterium.

It has been recently demonstrated that cosmid clones isolated from a library of *Pseudomonas solanacearum* strain pathogenic to peanut rendered a tomato strain pathogenic which was originally avirulent to peanut (Daniels *et al.* 1988). Although attempt was made to detect specificity genes in the libraries of *Xanthomonas campestris* pv. *campestris* and *translucens* it was not successful.

A new phase of research began on the structure and function of plasmid DNA after the discovery of Ti plasmid as tumour inducing principle (TIP) in *Agrobacterium*.

A good deal of information is now available regarding molecular mechanisms involved in transformation of plants by *Agrobacterium*. Usually bacteria bind at specific sites of wounded plant cells. Exudation of phenolic compounds from wounded cells activate the Ti plasmid virulence (*vir*) genes and also control the excision and perhaps the export of T-DNA from the bacterium to the plant cell. Finally, the T-DNA becomes integrated into chromosomal DNA of host and causes its genetic transformation. The signal molecules that trigger the induction of *vir* genes have been identified as two phenolic substances, namely acetosyringone (AS) and  $\alpha$ -hydroxy-acetosyringone (OH-AS). It is interesting that in monocotyledonous plants, wounded cells do not exude the phenolic inducers of *vir* genes and the plants are also not readily infected by *Agrobacterium*.

Another example is *Agrobacterium rhizogens* which causes hairy root disease on dicotyledonous plants. This bacterium contains a large oncogenic plasmid, called Ri plasmid. When the T-DNA portion of the Ri-plasmid is transferred to the plant, transformation takes place and hairy roots are produced at the site of infection. However, very little information is available so far about the organization of the Ri plasmid T-DNA genes.

Gabriel *et al.* (1986) cloned five avirulence genes from *Xanthomonas campestris* pv. *malvacearum* race H (a pathogen of cotton). Each of five exhibited incompatible reaction with plants carrying the corresponding R gene. Another race of the same

bacterium, however, appeared to contain recessive (virulence) alleles with homology to two of the cloned *avr* genes. Cloning of presumptive avirulence genes from *P. syringae* pv. *phaseolicola* race 3 (Hitchin *et al.*, 1988), *P. syringae* pv. *pisii* (Vivian *et al.*, 1988) and *Erwinia amylovora* (Norelli *et al.*, 1988) was also reported by other workers.

Expression of specific bacterial genes may be required for the establishment of plant-microbe interaction. These genes are induced when the bacteria come in contact with their respective hosts. It has been observed (Firmin *et al.*, 1986; Redmond *et al.*, 1986; Peters *et al.*, 1986) that extract and exudates of legume cultivars induce *nod* gene expression specifically in suitable *Rhizobium* species. The active components in extracts/exudates have been identified as flavones or flavanones. Luteolin was detected in alfalfa, 7,4'-dihydroxyflavone in clover and eriodictyol and epigenin 7-O-glucoside in peas. Most potent inhibitors of *nod*-gene activation are structurally similar to inducing compounds. Certain structurally related coumarins and other compounds function as inhibitors.

Another interesting observation was made by Horvath *et al.* (1987). They found that *nod D*-genes from two *Rhizobium meliloti* strains varied significantly in structure and plant factors which activated them. For example, *nod D*-gene from a narrow host range strain was activated by flavone and luteolin only while the *nod D*-gene from wide host range strain (M PIK 3030) was activated by other unknown plant metabolites also.

Recent evidence suggests that in *P. syringae* pvs. *atropurpurea*, *glycinea* and tomato, some of the genes involved in toxin biosynthesis are plasmid borne. Peet *et al.* (1986) reported that phaseolotoxin production by the bacterium is governed by clustered genes. The cluster also contains a gene for a toxin-insensitive ornithine carbamoyl-transferase (i.e. OC Tase). This may be a reason for the immunity of *P. syringae* pv. *phaseolicola* to the toxin.

It was conclusively demonstrated by Nachmias (1987) that *Verticillium dahliae* race 2 isolates caused severe symptoms on tomato plants carrying the *Ve* gene (*Verticillium* resistance) but the race 1 isolates caused little or no damage. Phytotoxic peptides were isolated from the culture fluids of both the races but they differed in amino acid composition and phytotoxicity to tomato leaves, root tips and suspension cells. The peptide isolated from race 1 exhibited severe symptoms on tomato lacking *Ve* gene while peptide from race 2 isolates showed severe symptoms on both tomato genotypes. It is not clear whether *Ve* gene in tomato confers resistance to race 1 solely by conferring tolerance to the race 1 peptide toxin or whether the gene is pleiotropic. It requires further investigation.

Restriction fragment length polymorphisms (RFLPs) analysis is now being used for diagnosis of plant diseases and genetic disorders. This analysis may help to

identify regions of genomic or mt DNA that could be used in the development of species or race specific probes. Usually each restriction enzyme recognises a specific nucleotide sequence and cuts the DNA specifically when the sequence for particular enzyme. Subsequently, the fragmented DNA is separated by gel electrophoresis and the banding pattern may be seen by staining with ethidium bromide or by autoradiography. Any difference in size and number of restriction fragments can be detected. This difference may be due to inserts or deletions. RFLP analysis could also be used to compare the products from a resistant plant with products from a plant known to have a genetic deletion of the resistance gene. The differences in restriction fragments are known as restriction fragment length polymorphisms (Miller and Martin, 1988). This type of analysis is imperative in the study of resistance genes.

Although application of molecular genetics to study plant microbe interaction is developing slowly, there is no doubt that its future holds great promise.

#### REFERENCES

- Daniels, M. J., Dow, J. M. and Osborn, A. E. (1988). Molecular genetics of pathogenicity in phytopathogenic bacteria. *Ann. Rev. Phytopathol.*, 26 : 285-312.
- Firmin, J. L., Wilsons, K. E., Rossen, L., and Johnston, A.W.B. (1986). Flavonoid activation of nodulation genes in *Rhizobium* reversed by other compounds present in plants. *Nature*, 324 : 90-92.
- Flor, H. H. (1956). The complementary genic systems in flax and flax rust. *Advances in Genetics*, 8 : 29-54.
- Gabriel, D. W., Burges, A. and Lazo, G. R. (1986). Gene-for-gene interactions of five cloned avirulence genes from *Xanthomonas campestris* pv. *malvacearum* with specific resistance genes in cotton. *Proc. Natl. Acad. Sci., USA*, 83 : 6415-19.
- Hitchin, F. E., Harper, S., Jenner, C. E., Mansfield, J. W. and Daniels, M. J. (1988). Cloning of an avirulence determinant from *Pseudomonas syringae* pv. *phaseolicola* race 3. Proc. 3rd Int. Working Group on *Pseudomonas syringae* pathogens, Lisbon.
- Horvath, B., Bachem, C W. B., Schell, J. and Kondorosi A. (1987). Host-specific regulation of nodulation genes in *Rhizobium* is mediated by a plant signal, interacting with the *nod D* gene product. *EMBO J.*, 6 : 841-848.
- Kerr, A. (1987) The impact of molecular genetics on plant pathology. *Annu. Rev. Phytopathol.*, 25 : 87-110.
- Kobayashi, D. K. and Keen, N. T. (1988). The cloning of avirulence genes from *Pseudomonas syringae* pv. tomato which function in *P. s* pv. *glycinea* to elicit a hypersensitive reaction in soybean. (In press).
- Miller, S. A. and Martin, R. R. (1988). Molecular diagnosis of plant disease. *Annu. Rev. Phytopathol.*, 26 : 409-432.

- Nachmias, A., Buchner, V., Tsrur, L., Bursteen, Y. and Keen, N. (1987). *Phytopathology*, 77 (3) : 506-510.
- Norelli, J. L., Aldwinkle, H. S., Steinberger, E. M. and Beer, S. V. (1988). Virulence of *Erwinia amylovora* altered by plasmid DNA. *Phytopathology*. (In press).
- Peters, N. K., Frost, J. W., Long, S. R. (1986). A plant flavone luteolin induces expression of *Rhizobium meliloti* nodulation genes. *Science*, 233 : 977-79.
- Peet, R. C., Lindgren, P. B., Willis, D. K. and Panopoulos, N. J. (1986). Identification and cloning of genes involved in phaseolotoxin production by *Pseudomonas syringae* pv. *phaseolicola*. *J. Bacteriol.*, 166 : 1096-1105.
- Redmond, J. W., Bailey, M., Djordjevic, M. A., Innes, R. W., Kuempel, P. L. and Rolfe, B. G. (1986). Flavones induce activation of nodulation of genes in *Rhizobium*. *Nature*, 323 : 632-635.
- Staskawicz, B. J., Dahlbeck, D. and Keen, N. T. (1984). Cloned avirulence gene of *Pseudomonas syringae* pv. *glycinea* determines race specific incompatibility on *Glycine max* (L) Merr. *Proceedings of the National Academy of Sciences, USA*. 81 : 6024-6028.
- Vivian, A., Atherton, G. T. and Taylor, J. D. (1988). Isolation and characterization of a clone conferring race-specific avirulence in *Pseudomonas syringae* pathovar *psi*. Proc. 3rd Int. Working group on *Pseudomonas syringae* pathovars hisbon. (In press).