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Variability and Characterization in Plant Microbes

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Abstract

Successful management of plant disease is mainly dependent on the accurate and efficient detection of plant pathogens, amount of genetic and pathogenic variability present in pathogen population, development of resistant cultivars and deploying of effective resistance gene in different epidemiological region. In case of most of the fungal and bacterial diseases, the main reason for frequent ―breakdown‖ of effective resistances is the variability that exists in the pathogen population, which necessitates a continual replacement of cultivars due to disease susceptibility. Molecular techniques are more precise tools for differentiation between species, and identification of new strains/ isolates. Biotechnological methods can be used to characterize pathogen populations and assess the genetic variability much more accurately. Molecular methods (RAPD, RFLP, AFLP, SSR, ISSR and rDNA markers) are being used to distinguish between closely related species with few morphological differences and to distinguish strains within species. These markers can detect differences at single base pair level and has been successfully used for detection of fungi and bacteria. In future, the development of simple PCR based protocols that can be used to detect the pathogen population present in the farmers‘s fields. So that we can use selective breeding lines with specific resistance to a particular pathotype.

Key words: Resistance, Species, Variability, Molecular Markers, Pathotype, PCR, Varieties, etc.

Introduction

Food losses due to crop infections from pathogens such as fungi, bacteria and viruses are major issues in agriculture at global level. In order to minimize the disease incidence in crop and to increase the productivity, advance disease detection and prevention in crop are

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necessary. In order to assist the breeding programs the evaluation of genetic diversity of pathogen and its molecular characterization are crucial. Genetic analysis of pathogen populations is fundamental to understanding the mechanisms generating genetic variation, host-pathogen co-evolution, and in the management of resistance (Aradhya *et al.*, 2001).

New pathotypes evolve with the introduction of new type of variety and hybrids to our crops. Rapid and accurate detection of new virulence will help formulate strategy for developing resistant cultivars in particular region and will also provide a base for breeding cultivars with durable resistance or designing strategies for the long-term management of major diseases. Understanding the role pathogens play in shaping the genetic structure of plant populations and communities requires an understanding of the pathogens' diversity, their origins, and the evolutionary interplay that occurs between pathogens and their hosts. Here we review sources of variation that contribute to the diversity of pathogen populations and some of the mechanisms whereby this diversity is maximized and maintained.

Mechanism of variability in Plant Pathogenic Fungi

Different pathogen develops different mechanism for generation of variability. Variability is essential for the survival of pathogen. Plant-pathogenic fungi are diversified group of organisms with significant importance in food and agriculture sector contributing to higher yield losses annually. They intact with their hosts in number of ways. These interactions range from species that establish perennial; systemic infections which kill their hosts rapidly that form discrete lesions whose individual effects are very limited (Burdon, 1993).

- **Migration and gene flow:** Migration of one pathogen population from one place to another leads to development of new species of which are either absent or not on many occasions (e.g., the introduction of *Cryphonectria parasitica* to North America, *Phytophthora infestans* to Europe, and *Puccinia striiformis* to Australia.
- **Recombination:** Recombination in plant pathogens is the similar process to that of sexual reproduction. It occurs either through a process of somatic hybridization, in which nuclear and cytoplasmic material get exchanged. In most of cases nuclear exchange may be followed by nuclear fusion and recombination also called as parasexual cycle. The exchange of cytoplasmic as well as nuclear fusion leads to increased genotypic diversity in a pathogen population, but their importance varies both within and among species. In sexual reproduction ploidy level of individual get altered. Haploid gametes which are carrying a

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single set of chromosome fuses to form a diploid zygote with a double set of chromosomes. The gametes are formed from diploid progenitor cells by meiosis, which involves genetic recombination—the key evolutionary aspect of sexual reproduction (Schoustra *et al*., 2007).

- **Mutation:** Gassner and Straib (1993) were the first to suggest mutation as a mechanism for formation of new races in *P. striiformis*. Mutation is a process in which there is a change in the genetic material of an organism occur either through naturally or through induced factors, which is then transmitted in a hereditary fashion to the progeny. Mutations represent changes in the sequence of bases in the DNA either through substitution of one base for another or through addition or deletion of one or many base pairs.
- **Heterokaryosis:** Heterokaryosisis a condition in which cells of fungal hyphae or parts of hyphae contain two or more nuclei those are genetically different. For example, in Basidiomycetes, the dikaryotic state is found to be completely different from the haploid mycelium and spores of the fungus.In *P. graminis* f. sp. *tritici*, the dikaryotic mycelium can grow in both barberry and wheat but the haploid mycelium can grow only in barberry not on wheat. Similarly, the haploid basidiospores can infect barberry but not wheat. However, the dikaryotic aeciospores and uredospores can infect wheat but not barberry.
- **Para sexuality ;** A process in which plasmogamy, karyogamy and haploidization takes place in sequence but not at specified points in the life cycle of an individual. First discovered in 1952 by Pontecorvo and Roper in the University of Glasgow in *Aspergillus nidulans*, the imperfect stage of *Emericella nidulans*. The sequences of events in a complete parasexual cycle are as follow: Formation of heterokaryotic mycelium. Fusion between two nuclei. Fusion between like nuclei. Fusion between unlike nuclei. Multiplication of diploid nuclei Occasional mitotic crossing over during the multiplication of the diploid nuclei. Sorting out of diploid nuclei Occasional haploidization of diploid nuclei. Sorting out of new haploid strains.
- **Formation of heterokaryotic mycelium:** There are several ways in which a heterokaryotic mycelium may be formed. Anastomosis of somatic hyphae of different genetic constitutions is the most common method in which the foreign nucleus or nuclei introduced into a mycelium multiplies and its progeny spread through the mycelium, rendering the latter heterokaryotic. Another way is, in which a homokaryotic mycelium may change into

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heterokaryotic is by multiplication in one or more nuclei, as has been shown to occur on some ascomycetes. Third way is by the fusion of some of the nuclei and their subsequent multiplication and spread among the haploid nuclei. This would result in a mixture of haploid and diploid nuclei.

- **Karyogamy and multiplication of diploid nuclei:**When a mycelium has become heterokaryotic, nuclear fusion takes place between haploid nuclei of different genotypes as well as between nuclei of same type. The former results in a heterozygous diploid nucleus and the latter in a homozygous diploid nucleus. At this stage the mycelium may contain at least five types of nuclei. Two types of haploids, two types of homozygous diploid, heterozygous diploid nuclei. All these nuclei multiply at about same rate, but diploid nuclei are present in much smaller number then the haploidmitotic crossing over Crossing over give rise to new combinations and new linkages. It occurs not by a reduction division but by aneuploidy a phenomenon in which chromosomes are lost during mitotic division. e.g., in *Penicillium chrysogenum* and *Aspergillus niger*, the frequency of mitotic crossing over is as high as during meiosis in sexual reproduction; both lack sexual reproduction.
- **Sorting out of diploid strains:** In the fungi which produce uninucleate conidia, sorting out of the diploid nuclei occurs by their incorporation into conidia which then germinate and produce diploid mycelia. Diploid strains of several imperfect fungi have been isolated.
- **Haploidization: Diploid** colonies will often produce sectors which may be recognized by various methods. It produces haploid conidia which may be isolated and grown into haploid colonies. It means that some diploid nuclei undergo haploidization in the mycelium and are sorted out. Some of these haploid strains are genotypically different from either parent because of mitotic recombinations producing new linkage groups, which are sorted out in the haploid conidia. In fungus *A. nidulans*, during somatic growth, mitotic recombination occurs at a sufficiently high rate to allow an acceleration of the adaptation to novel environmental conditions. Because fungi (unlike animals) lack a clear soma-germline distinction, nuclei with a novel recombinant genotype in the somatic tissue (the mycelium) can give rise to progeny in the form of asexual spores. The results show that recombination at the somatic level (so-called parasexual recombination) appears to be of evolutionary relevance (Schoustra *et al*., 2007).

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Mechanism of variability in Plant Pathogenic Bacteria

- **Bacterial conjugation:** Bacterial conjugation is the transfer of genetic material between bacteria through direct cell to cell contact, or through a bridge-like connection between the two cells. Bacterial conjugation is often incorrectly regarded as the bacterial equivalent of sexual reproduction or mating since it involves some genetic exchange. In order to perform conjugation, one of the bacteria, the donor, must play host to a conjugative or mobilizable genetic element, most often a conjugative or mobilizable plasmid or transposon (Ryan and Ray, 2004). Most conjugative plasmids have systems ensuring that the recipient cell does not already contain a similar element. There are two categories of conjugative plasmids with respect to transfer: (1) self-transmissible plasmids, which encode all the genes necessary to promote cell-to-cell contact and transfer of DNA, and (2) mobilizable plasmids, which do not promote conjugation, but can be efficiently transferred when present in a cell that contains a self-transmissible plasmid. The self-transmissible plasmids are usually large. They code for 20- 30 proteins specifically required for bacterial cells to form a mating pair, develop a small pore, and transfer plasmid DNA through the pore from one cell to the other. The genetic information transferred is often beneficial to the recipient cell. Benefits may include antibiotic resistance, other xenobiotic tolerance, or the ability to utilize a new metabolite. Such beneficial plasmids may be considered bacterial endosymbionts. Some conjugative elements may also be viewed as genetic parasites on the bacterium, and conjugation as a mechanism that was evolved by the mobile element to spread itself into new hosts.
- **Transformation:** The uptake of naked DNA molecules and their stable maintenance in bacteria is called transformation. The phenomenon was discovered in 1928 by Griffith. Bacteria have developed highly specialized functions that will bind DNA fragments and transport them into the cell. Competence refers to the state of being able to take up exogenous DNA from the environment. There are two different forms of competence: natural and artificial. Some bacteria (around 1% of all species) are naturally capable of taking up DNA under laboratory. Such species carry sets of genes specifying the cause of the machinery for bringing DNA across the cell's membrane or membranes. Artificial competence is not encoded in the cell's genes. Instead, it is

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induced by laboratory procedures in which cells are passively made permeable to DNA, using conditions that do not normally occur in nature (Kunik *et al*., 2001).

- **Transduction:** Bacteriophages have the ability to transfer genes from one bacterial cell to another, a process known as transduction. There are two varieties of bacteriophage-mediated gene transfer: generalized transduction and specialized transduction.

Generalized transduction occurs as a result of the lytic cycle. In the process of packaging bacteriophage DNA, the head structures of some bacteriophages will package random fragments of the bacterial chromosome. Most of the particles contain viral DNA. When these inject their DNA, the lytic cycle will repeat and new bacteriophage particles will be produced. A small fraction of the particles, possibly as high as 1%, contain fragments of the bacterial chromosome in place of the bacteriophage DNA. When one of these particles injects its DNA into the cell, the cell is not killed. The newly introduced DNA contains only bacterial genes and is free to recombine with the chromosome. Some transducing bacteriophages can introduce 100-200 kilobases of DNA.

Specialized transduction requires a temperate bacteriophage. In this class of transduction, a bacterial gene becomes associated with the bacteriophage genome (e.g. by recombination). When such a bacteriophage lysogenizes a new bacterial host, it brings with it the associated bacterial gene. Specialized transduction leads to three possible outcomes: DNA can be absorbed and recycled for spare parts. The bacterial DNA can match up with a homologous DNA in the recipient cell and exchange it. DNA can insert itself into the genome of the recipient cell as if still acting like virus resulting in a double copy of the bacterial genes.

Characterization of variability among pathogen population

Successful management of plant diseases is mainly dependent on the accurate and efficient detection of plant pathogens, amount of genetic and pathogenic variability present in pathogen population, development of disease resistant cultivars and development of effective resistant gene in different epidemiological regions. Assessment of variability provides a basis of breeding cultivar with durable resistance and designing strategies for long term management of major diseases. All the disease management strategies based on host resistant require the knowledge of variability in pathogens (Sharma, 2003). The choice of method for characterization of pathogen isolates should be based upon simplicity, reproducibility and cost

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effectiveness. Dynamics of pathogen variability can be used to develop resistance gene pyramiding or gene development strategies. Methods of characterization of genetic variability: traditional methods and molecular or biotechnological methods.

Molecular or biotechnological methods for characterization of variability

Different molecular markers are used for the characterization of genetic variability in plant pathogens (Sharma et al., 1999). Molecular techniques are most precise tools for differentiation between species, and identification of new strain/ isolates collected from infected samples. The molecular methods vary with respect to discriminatory power, reproducibility, ease of use and interpretation (Lasker, 2002). DNA fingerprinting has been successfully used for *Fusarium* in characterization of individual isolates and grouping them into standard racial classes Lal and Dutta, 2012). This is also particularly useful when any unknown fungal sample is to be identified. Comparison at the DNA sequences level provides accurate classification of fungal species; they are beginning to elucidate the evolutionary and ecological relationships among diverse species. Molecular biology has brought many powerful new for rapid identification of isolates and methods for rapid determination of virulence or toxicity of strains.

Molecular methods have also been used to distinguish between closely related species with few morphological differences and to distinguish strains (or even specific isolates) within a species. Molecular markers monitor the variations in DNA sequences within and between the species and provide accurate identification. In recent years, different marker system such as Restriction Fragment Length Polymorphisms (RFLP), Random Amplified Polymorphic DNA (RAPD), Sequence Tagged Sites (STS), Amplified Fragment Length Polymorphisms (AFLP), Simple Sequence Repeats (SSR) or microsatellites, Single Nucleotide Polymorphism (SNPs) and others have been developed and applied to different fungus species. The ribosomal DNA (rDNA) based classification is also the method of choice especially when classifying the related species. The nucleotide sequence analysis of rDNA region has been widely accepted to have phylogenetic significance and is therefore useful in taxonomy and the study of phylogenetic relationships.

Conclusion

For breeding of resistant crop varieties, knowledge about the pathogen races in that particular crop area is very important. This is more so when the breeding objective is to provide resistance against multiple races or to pyramid several resistance genes in an elite genotype.

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Variability serve as survival source of pathogen. The selection pressure leads to development of the variation in pathogen which is necessary for their survival. Resistance is break down with the due course of time due to presence of variation which leads to development of virulent strains which are previously not known. General and specialized mechanisms are developed by different pathogens to produce variability. Molecular methods are more efficient, accurate and less laborious with higher degree of precession. The choice of the method for characterization of pathogen isolates should be based on simplicity, reproducibility and cost-effectiveness. The development of simple PCR based protocols that can be used to detect the pathogen population present in the farmer ‘s fields. The complementarity of the disease resistance gene [R] present in the plant and the = avirulence‘(avr) gene in the pathogen forms the basis of plant -pathogen recognition and ultimately leads to resistance or susceptibility of a plant to a specific disease. So the information on the prevalent pathotypes in a farmer ‘s field can be made use of in selecting breeding lines with specific resistance to a particular pathotype. This resistance (QTL) can be utilized in developing varieties and hybrid cultivars with higher levels of disease resistance. This study also will lead to an understanding of the dynamics of pathogen variability that can be used to develop resistance gene pyramiding or gene deployment strategies.

References

Aradhya, M.K., Chan, H.M. and Parfitt, D. E. (2001). Genetic variability in the pistachio late blight fungus, *Alternaria alternata*. *Mycological Research*, 105: 300–306.

Burdon, J. J. (1993). The structure of pathogen populations in natural plant communities. *Annual Review of Phytopathology*, 34: 305-323.

Gassner, G. and Straib, W. (1993). Uber mutationen in einerbiologischenrassevon ―*Puccinia glumarumtritici*” (Schmidt) Erikss. and Henn. *Molecular Genetics and Genomics*, 63:154–180.

Kunik, T., Tzfira, T., Kapulnik, Y., Gafni, Y., Dingwall, C. and Citovsky V. (2001). Genetic transformation of HeLa cells by *Agrobacterium*. *Proceedings of the National Academy of Science of the United States of America*, 98: 1871– 1876.

Lal, N. and Dutta, J. (2012). Progress and perceives in characterization of genetic diversity in plant pathogenic *Fusarium*. *Plant Archives*, 12: 557-568.

Lasker, B. A. (2002). Evaluation of performance of four genotyping methods for studying the genetic epidemiology of *Aspergillus fumigates*. *Journal of Clinical Microbiology*, 40: 2886-2892.

Ryan, K. J. and Ray, C. G. (2004). Sherris Medical Microbiology. *4th ed. McGraw Hill Medical*, 992.

Schoustra, S. E., Debets, A. J. M., Slakhorst, M. and Hoekstra, R. F. (2007). Mitotic Recombination Accelerates Adaptation in the Fungus *Aspergillus nidulans*. *PLoS Genetics*, 3: 648-653.

Sharma, T. R. (2003). Molecular diagnosis and application of DNA markers in management of fungal and bacterial plant diseases. *Indian Journal of Biotechnology*, 2: 99-109.

Sharma, T. R., Prachi, and Singh, B. M. (1999). Application of polymerase chain reaction in phytopathogenic microbes. *Indian Journal of Microbiology*, 39: 79- 91.