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Review

CURRENT TOPICS IN MOLECULAR PLANT PATHOLOGY

TOPIK-TOPIK MUTAKHIR DALAM FITOPATOLOGI MOLEKULER

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INTISARI

Tulisan ini mengikhtisarkan topik-topik mutakhir mengenai teknologi molekuler dalam patologi tumbuhan. Untuk deteksi dan identifikasi patogen tumbuhan dipakai PCR *dengan bermacam-macam modifikasinya. Untuk pengelolaan penyakit, penelitian mengenai reaksi inang mempunyai arti penting dan mekanisme gen resisten juga dikaji. Dalam penelitian mengenai reaksi tumbuhan terhadap patogen banyak dimanfaatkan* gene silencing *dan dibandingkan dengan kematian sel secara terprogram pada hewan. Karena teknologi molekuler masih cukup mahal, para fitopatologiwan Asia diharap memanfaatkan teknik-teknik konvensionalnya agar proteksi tumbuhan yang benar secara ekologis menuju ke produksi tanaman yang berkelanjutan dapat direalisasikan.*

Kata kunci: PCR, *gen-gen resisten,* gene silencing, *perlindungan tanaman*

ABSTRACT

Some current topics on molecular technology in plant pathology are reviewed. For detection and identification of plant pathogens, PCR with various modifications is applied. Study of nucleic acids sequence of pathogens gives keys for classification. For disease management, investigation of host reaction is also significant and mechanism of resistant genes is studied. In the study of plant reaction against pathogens, gene silencing is focused and compared with programmed cell death of animals. Since cost of molecular technology is still expensive, plant pathologists in Asia are expected to utilize their conventional techniques to realize ecologically sound plant protection for sustainable crop production.

Key words: PCR, resistant genes, gene silencing, plant protection

INTRODUCTION

Molecular biology deals with aspects of organisms and their interactions by new molecular technology. Influenced by the success of molecular biologists, plant pathologists are also challenged to investigate their targets, *i.e.* virus, bacteria, fungi and phytoplasmas, and their interactions with the hosts by molecular tools. Thus molecular plant pathology is recognized as the field of study which covers a wide range of plant pathology, as well as taxonomy, genetics, physiology and ecology.

Techniques used in molecular plant pathology are various and Singh *et al.* (1995) classified them into 4 major groups: biophysical, biochemical, bio-molecular and biotechnological methods. Today we put more emphasis on bio-molecular and biotechnological methods to analyze nucleic acids and proteins of both pathogens and hosts and to detect and characterize pathogens and biological control agents. In this review, I present some of the current topics in molecular plant pathology and discuss what we can do with molecular tools to improve tropical agriculture.

DETECTION AND CHARACTERIZATION OF PATHOGENS BY VARIOUS PCR TECHNOLOGY

The detection and identification of pathogens are fundamental issues in plant protection. In production of healthy seedlings, accurate detection of any single pathogen is significant. For diagnosis, accurate and rapid detection and characterization of pathogens are essential. Most of the pathogens have been tested for detection and characterization by study of morphological, cultural, and other biological characters. When serological techniques have been applied, its capability for rapid and large-scale diagnosis was highly increased. After various modifications of serological or immunological techniques such as variation of ELISA, production of monoclonal antibodies, dot or tissue immuno-binding assay, immunological electron microscopy, polymerase chain reaction (PCR) was applied in plant pathology. This technology which brought Dr. K.B. Mullis the Nobel Prize in Chemistry in 1993 is now widely used for copying and amplifying the complementary strands of a target DNA molecule in plant pathogens and plants. There are many modified PCR methods such as RT-PCR for RNA viruses and LA-PCR with exonuclease for bigger DNA fragments. Nested PCR is suitable to samples with smaller quantity of target DNA. In nested PCR, two sets of primers are used and after the first amplification, the second set of primers, which are internal to the first set, is used for amplification to ensure high selectivity. Multiplex PCR (M-PCR) is used to detect multiple virus in a single PCR system. When combined with serological technique as multiplex immunocapture PCR (M-IC-PCR), it offers high sensitivities. Recently, Sharman *et al.* (2000) showed it useful to detect 4 viruses from bananas from different origin by M-IC-PCR with digoxigenin-labelled– virus specific probes.

Since PCR has been used mainly in virus detection and identification, these molecular techniques are also capitalized in detection and diagnostics of plant pathogenic fungi, bacteria, and phytoplasmas In fungal study, the ITS regions in ribosomal DNA are widely used to design PCR primers that are universal in wide range of fungi. According to the purpose of detection, sets of primers with the universal sequences, which are strongly conserved among the species in the same family, for pathogen family detection, or specific set primers which can distinguish different isolates/strains in one species.

Consequently PCR and sequencing technology are now used for comparison of various isolates in the same species, family or genus in phylogenetic analysis in the classification of plant pathogens. After the construction of phylogenetic trees or dendrogram with the help of computer software, the target pathogen is analyzed to estimate evolutionary distances among other pathogens. DNA data banks are accessible via internet. For example, DDBJ (DNA Data Bank of Japan, http://www.ddbj.nig.ac.jp/Welcome-e.html) offers English information for retrieval and analysis such as FASTA, BLAST, and CLUSTAL-W for homology search or multiple alignment. DDBJ together with

NCBI (National Center for Biotechnology Information, USA) and EBI (European Bioinformatics Institute) supplies over 900 milion nucleic acid sequences as well as many amino acid sequence data. It is also possible to compare the target plant pathogen with not only other plant pathogens but also with non-plant pathogenic organisms.

PCR and related techniques require costly chemicals, reagent and apparatus. However, high sensitivity and reduction in test time will justify these costs and the costs are expected to decrease in the future if their use become more frequently. Moreover, for many virus families and some bacteria and phytoplasmas, PCR detection kits are now available from some companies commercially since the price is not cheap yet.

Recently, molecular detection expands to microarray techniques (Schena *et al.*, 1995, Brown *et al.*, 1999). With DNA microarray based upon genetic and biochemical information, we can observe the expression pattern of each of over thousands genes simultaneously. This technology provides large-scale gene information with high sensitivity and accuracy.

RESISTANT GENES OF HOST PLANTS

Molecular plant pathology deals with not only nucleic acids of plant pathogens but also genes of host plants. Now, more and more plant genes, which show resistance against plant pathogens have been detected and some have been already cloned. Molecular mechanisms of plant resistance is focused to find the way to enhance plant resistance to the attack of plant pathogens.

N gene in tobacco plants is long known to be responsible for resistance against tobacco mosaic virus. N gene dependent hypersensitive reaction (HR) was studied as a model of gene to gene theory. The gene was cloned as the first virus resistance gene by Baker and her group (Dinesh-Kumar *et al.*, 2000). Now it has been demonstrated that a small portion (50 KDa) at C-terminal of the replicase could trigger HR. It is also shown that the N gene shares very similar sequence with other resistance genes (R genes) which govern gene-to-gene theory type resistant reaction. Their predicted proteins are similar to some proteins of the drosophila protein and interleukin-1 receptor (Whitham *et al.*, 1994). In this regard, N gene encodes receptors to recognize pathogens like bacteria, fungi and viruses.

Pto gene is another well-investigated gene by Tanksley (1992) and others. Pto gene, which is shown as a serine/threonine protein kinase, confers resistance to *Pseudomonas syringae* pv. *tomato* and tomato leaves with Pto gene show necrotic lesions. Bacteria has gene for avirulence named avrPto and produce signal protein. Then signal protein is bound in plant cytoplasm by Pto kinase. As for potato, which is one of the major food crops, Gebhardt *et al.* (2001) reviewed the nearly 20 dominant resistant genes against viruses, nematode, viruses and fungi. They are detected in several different places which are close each other in potato gene. Quantitative trait loci (QTL) was recognized in potatoes for resistance against *Phytophthora infestans* and others including plant pathogenic bacteria.

Mechanism of resistance has not been always clearly studied. We, however, can use these genes as the source of transgenic or genetically modified organisms. Production of transgenic plants with resistant gene introduced form different plant species will increase in number in the future.

GENE SILENCING

Cross protection is a well-known phenomenon. This is the phenomenon in which plants already infected by a virus was protected from second similar virus (virus strain) infection. The mechanism was not clearly explained for a long time, it seems the first virus infection induces a kind of resistant response against the second virus with similar or almost same nucleic acid sequence. Gene silencing is a phenomenon to suppress the expression of genes of infected pathogens and invaded alien genes. The mechanism of this interesting phenomenon which is applied for attenuated virus inoculation, has been studied as one of the anti-viral reaction of virus infected plants in plant pathology. Since gene silencing is categorized into several types, post-transcriptional gene silencing (PTGS) is the type most focused. This phenomenon was first noticed in transgenic plants which was created to express target gene but did not express sufficient transgene products. In this case, after normal transcription in nuclei, messenger RNA (mRNA) of transgene was degraded before they can be translated into proteins in cytoplasm. After infection of RNA viruses, double stranded RNA (dsRNA) or abnormal small RNA are synthesized as a systemic signal and cause methylation of target DNA. This target DNA is then degraded by dsRNA specific RNase. In transcriptional gene silencing (TGS), another type of gene silencing, mRNA synthesis is reduced very much. In gene silencing, as the target genes and the expressed sequences are suppressed, it called co-suppression.

PTGS can be a promising tool to produce resistant plants against specific viruses when the expression is appropriately controlled. PTGS signals are transmitted systemically and last long. The use of small antisense RNA in transgenic plants can be also a good method for protection of plants.

Since plants have no immune system, gene silencing is a very effective protection manner against invaders. Interestingly, similar syndrome is also found in nematodes, fungi, insects and others. The synthesis of abnormal small RNAs which causes gene silencing is also found in *Drosophila* and others and this indicates that gene silencing is phenomena common in many living organisms. Relationship with apoptosis, programmed death of cells, is also focused.

ROLE OF MOLECULAR PLANT PATHOLOGY IN ASIA

I reviewed and introduced some of the recent important technology in molecular plant pathology. Some readers, however, may think that those technologies are beyond reach under their research environment. I would like to emphasize that it is necessary to know what we can do with the latest technology but not always necessary to study molecular plant pathology. Significance of research by conventional technology should not at all ignored. Without basic information, molecular study will not give plant pathologists any superficial information. As we enjoy rich bio-diversity in Asia, we should notice even plant pathogens are also bio-resources both for future utilization in various range of practical fields and for future research. Some pathogens may play an important role in classification. Some pathogens may be used for biological control, bio-herbicide for example. Or we may be able to find useful antagonists through the study of plant pathogens and diseases. Recently, Zeigler (2001) reviewed agricultural biotechnology as a tool to reduce poverty in developing countries. As Asian plant pathologists, priority is given to study plant protection in an environmentally sound manner in agricultural production in Asia and molecular plant pathology will play a bigger role for it.

As regards to the economy of molecular plant pathology, cost of useful techniques tends to be cheaper as increasing number of people and institutions use them. Besides such technology is, if it is really useful, improved and will become universal for general users very quickly. Thus, approach to such technology become easier for researchers those who first see them difficult to use in their research because of economical or technical reasons. Then we can integrate data from both by conventional methods and by molecular technology. In this regard, it is also necessary to proceed and publish our research results in internationally acceptable way.

In this review the significance of transgenic plants for Asian countries was not discussed. This issue is discussed mainly from the viewpoint of environment and food safety. For Asian farmers and plant pathologists, however, it is not an issue of consumers' choice but an issue of sustainable food production to combat hunger in Asia. To discuss transgenic plants, we also need knowledge and information of molecular plant pathology. Thus, plant pathology through conventional and molecular technology is requested to contribute stable and sustainable agricultural practice.

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