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Development of Plant-Protecting Biopesticides—Study and Application of New Pest-Resistance Protein VrCRP

While pesticides have certainly done much to improve yields of high-quality agricultural products, the long-term use of chemical pesticides has harmful effects on human beings, beneficial organisms, and the environment. One of the most important goals of sustainable agriculture is to find ways to continue the high-yield production of high-quality agricultural products while minimizing impact on human beings, beneficial organisms, and the environment. In this light, the growing need to develop pesticides that are not harmful to people and the environment has gradually increased interest in biopesticides.

Crop losses to insect pests may be very severe, and sometimes exceed 30%. While the use of selective breeding techniques to develop pest-resistant crop strains is an effective method of reducing pest losses, it cannot entirely replace the use of pesticides. Because many species of pests may attack crops, and plant breeding is very time-consuming, pest-resistant strains often cannot be developed in time to make a difference. In contrast, transgenic crop varieties can be created very rapidly using genetic technology, and are not subject to interspecific crossing restrictions. Because of this, growing attention is being paid to the development of transgenic varieties, and the regulations governing this work are also a matter of widespread concern. Advanced genetic technology has been used to isolate genes conferring pest-resistance from rice, soybeans, snap beans, cow peas, sweet potatoes, and mung beans, and some of these genes have been used to create pest-resistant transgenic varieties. Nevertheless, this genetic engineering has yet been widely used to make biopesticides. Pest-fighting gene products include phytocoagulin, amylase suppressors, trypsin suppressors, cysteine protinase suppressor, and pea globuloprotein. *Bacillus thuringiensis* toxin is currently the most successful and widely-mankind bioinsecticide; it is commonly applied by in-field spraying, and is produced by transgenic corn and soybeans. The *Bacillus thuringiensis* toxin has its drawbacks, however. Use of this toxin in the field over many years shows that pests can develop resistance. Moreover, the toxin has the structural defect of being easily broken down by ultraviolet radiation, causing it to lose its potency. A major issue in crop protection today is consequently finding a new *Bacillus thuringiensis* toxin or biopesticide from some other source.

The NSC has supported the "Integrated Project on Plant Reactions to Adverse Conditions" and "National Program on Agricultural Biotechnology" over the last few years, and phytopathology is one of the key research areas under these projects. Notably, the researcher Prof. Chen Cheng-san has screened Taiwan's indigenous organisms for genes conferring pest-resistance, and has isolated a new defensin known as VrCRP from bean weevil-resistant mung bean strains at the Asian Vegetable Research and Development Center (AVRDC).

VrCRP is an abbreviation of Cysteine-rich Protein of *Vigna radiata*. A defensin is a type of cysteine-rich peptide found widely in plants, animals, and microbes. It is commonly believed that this type of peptide is a major line of defense against pathogenic microbes. Apart from being able to kill pathogenic bacteria and fungi, the mung bean defensin VrCRP isolated by Prof. Chen's laboratory has been shown to suppress the suppress the development of larval bean weevils, a major mung bean pest, and prevent them from reaching adulthood. The first defensin discovered thus far that is able to protect against both pathogens and insect pests, VrCRP possesses considerable potential as biopesticide. After being expressed in large quantities by *E. coli* bacteria containing mung bean defensin cDNA from pest-resistant mung bean strains, purified mung bean defensin still possesses full potency against bean weevils. Genetic engineering techni-ques have been used to produce large amounts of VrCRP for use in the development of biopesticides, and Prof. Chen is performing this research work in cooperation with the AVRDC. If VrCRP, as described above, is also found to be active against pathogenic microbes, it may eventually become an important tool for protecting Taiwan's crops against bacteria, fungi, and actinomycetes molds.

Apart from providing protection against insects and microbes, because VrCRP is able to suppress protein synthesis (and can suppress cell growth in armyworms), it may also have potential applications as a human or animal antibiotic in addition to its agricultural uses. Use as an antibiotic is therefore worthy of further exploration and evaluation.

Now that Taiwan has joined the WTO, its agricultural industry must keep pace with the development of the knowledge economy. Farmers must adopt forms of sustainable agriculture possessing international competitiveness, and agricultural biotechnology must play an important role in this transformation. On the positive side, Taiwan possesses many advantageous conditions and many talented agricultural technology and biotechnology specialists. If manpower is integrated in an effective manner, if Taiwan's wealth of germ plasma and microbial resources are put to use, and if research effort is channeled into function genomics, then it is likely that Taiwan's researchers can discover even more potent disease- and pest-resistance genes, as well as other useful genes that can improve the quality, yield, taste, and storage life of agricultural products. This research and development work can thus lay a solid foundation for the growth of an agricultural biotechnology industry in Taiwan.

NSC Signs "ROC-British Engineering and Physics Cooperation Agreement"

Prof. John O'Reilly, executive director of the British Engineering and Physics Research Committee, and NSC Vice Chairman Hsieh Ching-chih signed the "ROC-British Engineering and Physics Cooperation Agreement" on March 7, 2002.

This agreement will encourage researchers in Britain and Taiwan to engage in academic cooperation and interdisciplinary projects in technology, engineering, and physics, especially in the fields of electro-optics, electrical machinery, machinery, civil engineering, chemical engineering, computer science, environmental protection technology, nanotechnology, basic development, and systems integration. The two nations will provide grants to the participants in joint projects, and will fund two-way visits promoting the exploration of new technologies and new fields.

Prof. John O'Reilly was accompanied on his one-week visit to Taiwan by Dr. Nigel Birch, head of the British Engineering and Physics Research Committee's international cooperation office, and Ms Ling Thompson, head of the Royal Society's international cooperation office. The group's activities while in Taiwan included a lecture on the "Challenges of Global Telecommunications Development" to the National Taiwan University Department of Electrical Machinery on the morning of March 6, participation in the "ROC-British Micro-Electromechanics and Electro-

Optics Academic Interchange Conference" on March 7, and tours of the Academia Sinica, Industrial Technology Research Institute, National Health Research Institute, Hsinchu Science-Based Industrial Park, and NSC Center for High Performance Computing. In addition, the British visitors discussed strategic cooperation and resource sharing with the chairmen of the NSC's disciplines of telecommunications, nanotechnology, and biotechnology on the afternoon of March 6.

The signing of the agreement was witnessed by NSC Chairman Wei Cheho and British Technology Minister Lord Sainsbury.

Biotech Breakthroughs—Research on the Bamboo Mosaic Virus

Worldwide Crops worth US$30~50 billion are lost every year to pests and disease worldwide. Responding to this challenge, scientists are using a wide array of R&D methods to find ways of lessening plant losses, and among the most promising approaches are chemical agents, biological prevention, and improvement of the growing environment. Viruses are especially vicious enemies of crops. When a plant becomes infected with a virus, just as when humans are infected with the AIDS, polio, and influenza viruses, the virus uses the host's own cells to replicate itself, and any drug powerful enough to wipe out the virus is also likely to present a grave threat to the host. But thanks to mammals' immune systems, in many cases vaccination can prevent viral infection by stimulating the production of antibodies. Unfortunately, because plants have no equivalent mechanism, apart from the breeding of disease-resistant varieties, there are almost no ways of successfully preventing of viral diseases in plants. But thanks to the great strides made by biotechnology in recent years, scientists can now precisely manipulate viral genes, and have successfully prevented viral diseases through the creation of transgenic crops containing viral genes or genetic sequences. But since this prevention strategy is so highly selective, it cannot be used against a broad spectrum of viruses, and more effort is needed to gain a deeper understanding of the biology of viruses.

When the genetic engineering of plants got underway in the 1980's, most early work had the goal of making plants resistant to pests, diseases, and herbicides. As researchers targeted a more diverse range of genes, genetically-modified crops of different types emerged. Nowadays the use of plants as biological reactors has become a very hot field for the agricultural biotechnology industry, and plants have been used to produce oral vaccines and medical testing reagents, etc. Nevertheless, attempts increase output have found that plants usually can't match the unit output of bacteria or yeasts, and a major reason for this is that there are few effective vectors for plant systems. But because viruses can systematically replicate themselves and disperse themselves through plants, genetically-engineered viruses can potentially be used to bring exogenous genetic material into plants and enable it to be expressed. The development of a highly-efficient virus vector would thus make an enormous contribution to the genetic engineering of plants. While many examples of virus vectors appear in the literature, there is still tremendous room for improvement. The development of truly effective virus vectors will depend on more basic research on the molecular biology of viruses and a deeper understanding of viral replication.

This integrated research project brought together specialists in such diverse fields as plant pathology, virology, cellular biology, protein engineering, nucleic acid chemistry, and plant tissue culture for the sake of probing the mechanism of viral RNA replication and movement. This research team explored the function and characteristics of RNA replication enzymes, the distinctive structures formed when the 3' end of the viral genome and subgenomic RNA initiators bind with RNA replication enzymes, and the functions of viral proteins that participate in inter-cellular or long-distance movement. The results of this basic research will be put to use in the development of strategies for preventing viral diseases and the application of viruses or sub-viral RNA molecules as genetic vectors in plants.

The Bamboo mosaic virus (BaMV) was chosen as the subject of this research because of its importance and unique characteristics:

1. BaMV is universally distributed throughout bamboo-growing areas of Taiwan: Taiwan suffers the world's most severe outbreaks of this virus, and more than 90% of the plants are infected in most stands of *Dendrocalamus latiflorus* and green bamboo. This virus strongly affects the growth of bamboo plants, and causes the quantity and quality of bamboo shoots and bamboo stalks to deteriorate.
2. Retrovirus research is extremely challenging: The BaMV genome composed of single-strand positive RNA, and the virus creates the most basic three types of genetic products characteristic of ordinary plant viruses: RNA replication enzymes, viral movement proteins, and viral coat proteins. By understanding the structure, expression, regulation, and function of the BaMV genes making these products, it will be possible to greatly advance human knowledge of retroviruses.
3. BaMV possesses associated defective RNA and satellite RNA: These sub-viral RNA molecules will make a great contribution to the study of nucleic acid sequences connected with viral replication, the viral coating, systematic viral movement, and expression of symptoms.
4. BaMV is indigenous to Taiwan and is a very characteristic local virus: BaMV used for isolation of strains and bamboo tissue culture is obtained locally in Taiwan. While it is mentioned in some scattered reports from Brazil, Hawaii, and the Ryukyu islands, Taiwan is the most convenient place in which to collect this virus.

After many years of hard work, the research team has published 35 academic papers cited in *SCI* and been granted one international patent. It is especially noteworthy that eight papers concerning the BaMV have been published in the most highly reputed periodical in the field—the *Journal of Virology*—showing that Taiwan's virus research has begun to achieve international recognition. Ten years ago, when the team was just starting to probe the mysteries of the BaMV, Taiwanese researchers had published only two *SCI*-cited papers on the subject in international journals. Today at least 35 such papers have been published. In fact the research team has been able to make many striking breakthroughs, such as the *in vitro* determination of the activity of recombinant RNA replication enzymes, the use of RNA replication enzymes to identify the sequence and structure of the 3' end of the viral genome and of RNA initiators, and the discovery of genomic replication starters. All of these discoveries can be broadly applied to general retrovirus replication models. Sub-viral molecules discovered in research of this virus possess unique biological characteristics; the team has successfully studied the use of satellite nucleic acids to suppress viral revenue replication, and the role of viral/satellite nucleic acids in intercellular and systematic movement. This work has opened up whole new research vistas. Able to infect such monocots as bamboo, rice, and wheat, BaMV possesses a unique satellite nucleic acid system. The team has successfully developed of viral and satellite nucleic acid vectors able to express non-homologous proteins, and these vector systems can be applied to the analysis of functional genes. Since the vectors can also be used to produce compounds and vaccines with economic importance, their potential is great. Cellular and tissue culture regeneration systems have been developed for many species of bamboo. For instance, bamboo plants can be propagated by means of their reproduce organs, such as flowers, or their nutritive tissues, such as stems and roots, and many can be propagated by means of their somatic embryos. These propagation approaches can be used by future genetic engineering efforts. Furthermore, meristem-tip culture has been used to grow many virus-frees green bamboo seedlings. The Tainan Agricultural Improvement Station has distributed these seedlings to farmers, enabling them to grow high-quality bamboo shoots. We believe that only a firm commitment to basic research will allow us to continue to develop such innovative biotech applications.

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