# SEMINAR

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TEMASEK LIFESCIENCES LABORATORY

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**The Auditorium (Level 1)**
**Hosted by: Dr YIN Zhongchao**

# Homology-independent Breakdown of Transgenic Resistance by More Virulent Virus Strain and the Solution

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Underlying the mechanism of post-transcriptional gene silencing (PTGS), coat protein (CP)-transgenic papaya generated for control of Papaya ringspot virus (PRSV) in Hawaii represents the first successful application of genetically modified fruit crop. Accordingly, we have also generated coat protein (CP)-transgenic papaya lines with broad-spectrum resistance to different geographic strains of PRSV. During the field tests approved by government, an unrelated potyvirus Papaya leaf-distortion mosaic virus (PLDMV) was detected. Transgenic papaya lines carrying a chimeric untranslatable-CP construct to confer double resistance to both PRSV and PLDMV were further obtained. However, during repeated field tests of the transgenic lines in the past decade, more virulent strains of PRSV able to overcome the single-virus or double-virus resistance were noticed. Analysis by virus recombination indicated that a recombinant virus containing the silencing suppressor HC-Pro from a more virulent strain 519 can break down the transgenic resistance in a transgene-homology independent manner. Analysis by agroinfiltration confirmed that 5-19 HC-Pro, with variations in five aa positions, has a stronger PTGS suppression capability. Consequently, we further generated new transgenic papaya lines carrying an untranslatable HC-Pro construct of the supper PRSV strain 5-19 to disarm the viral weapon for suppressing PTGS. Ninety of the lines showed high degrees of resistance to both common strain YK and more virulent strain 5-19. Our results indicate that silencing suppressor-transgenic papaya lines can solve the problem resulting from homology-independent resistance breakdown. Currently, hybrid papaya varieties with the chimeric-CP resistance and HC-Pro transgenic resistance have been generated. They confer broad-spectrum resistance to different geographic PRSV strains and PLDMV and have a great potential for global application.

**Key words:** Papaya ringspot virus, transgenic resistance, virulent virus strain, post-translational gene silencing, silencing suppressor

Shyi-Dong Yeh received a Bachelor degree in Plant Pathology in 1974 and a Master Degree in Plant Pathology in 1979 from National Chung Hsing University (NCHU). He was then supported by a governmental scholarship to join Cornell University and obtained his Ph.D. degree in Plant Pathology in 1983. After one year of postdoctoral study at Cornell University, he came back to the Department of Plant Pathology as an associate professor at NCHU in 1984. He was promoted to a full professor in 1989, appointed as the Director of Biotechnology Center in 2001, and promoted as the Vice President of the university in 2004. His major responsibility was the coordination of teaching and research programs on biotechnology at NCHU. After he stepped down from the postion of VP, he was promoted as the Chair Professor at NCHU up to now. In 2012, he was appointed by the Ministry of Science and Technology as the Director of International Plant and Food Biotechnoogy Center at NCHU. In conjuction with UCDavis, USA, he has carried out several agri-biotech programs for Vietnam and Thailand, and set as a model to introduce and deploy modern agricultural technology in developing countries. During his Ph.D. study at Cornell University, he created several nitrous-acid induced mild strains of Papaya ringspot virus (PRSV), a noxious virus in tropics and subtropics, and then they were successfully applied in large-scale for control of PRSV by cross protection after he came back to Taiwan. This approach successfully maintained the production of papaya during 1984-1993 before the current netting method. In late 1980s, his research was shifted to the transgenic approach for control of PRSV. He created a new method for transformation of papaya by Agrobacterium and generated several transgenic papaya lines that carry the CP gene of a Taiwan strain of PRSV and confer a wide-spectrum resistance to different geographic strains of PRSV. However, an unrelated virus Papaya leaf-distortion mosaic virus (PLDMV) and several super strains of PRSV were found able to breakdown the transgenic resistance during intensive field tests. Consequently, he sucessfully developed a new transgenic system to provide broad-spectrum resistance against both PRSV and PLDMV, and also to the super strains of PRSV. Due to his excellent performance in PRSV research, Dr. Yeh received three times Outstanding Award for Research from National Council of Science of Taiwan, R.O.C. during 1994-98, and the national secientific award from the Ministry of Education in 2005. Also, he was elected as the fellow of American Phytopathology Soceity in 2008, and the fellow of American Association for the Advancement of Science in 2008. Professor Shyi-Dong Yeh is an internationally renowned leader in the areas of genetic organization of insect-borne plant viruses, molecular mechanism of plant-virus interactions, and development of novel approaches for control of noxious important plant viruses by transgenic resistance and cross protection. He has obtained many domestic and international patents which have great contributions to agricultural biotechnology industries for world markets.

**Recent Publications:**

1. Peng. J.-C, Chen. T.-C., Raja. J. A. J., Yang. C.-F., Chien. W.-C., Lin C.-H., Liu. F.-L., Wu. H.-W., and **Yeh. S.-D**. (2014) Broad-spectrum transgenic resistance against distinct tospovirus species at the genus level. PLoS One 9(5):e96073.
2. Yang. C.-F., Chen. K.-C., Cheng. Y.-H., Raja. J. A. J., Huang. Y.-L., Chien. W.-C., and **Yeh. S.-D**. (2014) Generation of marker-free transgenic plants concurrently resistant to a DNA geminivirus and a RNA Tospovirus. Scientific Reports 4:5717, 1-11.
3. Kung, Y.-J., You, B.-J., Raja. J. A. J., Chen, K.-C., Huang, C.-H., Bau, H.-J., Yang, C.-F., Huang, C.-H., Chang, C.-P., and **Yeh, S.-D.** (2015) Nucleotide sequence-homology-independent breakdown of transgenic resistance by more virulent virus strains and a potential solution. Scientific Reports. 5:9804, 1-10.