

A PROPOSED NEW STANDARD PROTOCOL FOR DIAGNOSIS OF *XYLELLA FASTIDIOSA*

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The Interim Commission on Phytosanitary Measures of the International Plant Protection Convention (IPPC) adopted recommendations on the publication of International Standards for Phytosanitary Measures (ISPM). This guideline produces standardized documents describing procedures and methods for the detection and identification of pests of quarantine significance. The documents are reviewed by a panel of experts which also includes members from the regional plant protection organizations (i.e. NAPPO, EPPO, COSAVE, etc). These protocols describe procedures and methods for detection and identification of pests that are regulated by contracting parties and relevant for international trade. These are addressed to diagnosticians/diagnostic laboratories performing official tests as part of phytosanitary measures and provide reliable diagnostic protocol(s) for relevant pests. There is a need to develop the protocol for detection of *Xylella fastidiosa* (Xf) in several hosts. We drafted such a document for Xf detection in 2005. Here we propose to update that protocol in the light of recently developed Xf diagnostic procedures and genomics data. The proposed protocol also includes the recently developed bioassay for Xf in the model plant *Nicotiana tabacum* cv. SR-1. This highly sensitive host is an excellent indicator plant to test the pathogenicity of Pierce's disease and almond leaf scorch disease strains of Xf. The procedure includes the use of *in vitro*-propagated tobacco plants grown in controlled environment (i.e., light and temperature) room. The SR-1 plants are grown in small pots to reduce space requirements, and symptoms appear in only 6-8 weeks. Xf strains from different plant hosts induce distinct symptoms in SR-1 tobacco. The protocol is applicable for disease surveys, and for quarantine and certification programs.

Section 4: Pathogen and Disease Management



ENABLING TECHNOLOGIES FOR GRAPE TRANSFORMATION

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Reporting Period: The results reported here are from work conducted June 2006 to September 2006. Work discussed here reflects a project funded for the period of June 1, 2006 to May 31, 2008.

ABSTRACT

Patenting of agricultural biotechnologies has expanded dramatically over the last 25 years and can represent a significant barrier to new crop development. Thus, navigating the intellectual property (IP) rights of commonly used research tools is essential to prevent downstream legal or regulatory obstacles for deployment of promising new technologies. The research proposed here seeks to develop and test a grape-specific transformation system for developing genetically engineered *Vitis* that addresses legal IP issues, meets high technical standards and is designed with attention to the emerging regulatory framework. The proposed plant transformation system can serve as a platform tool for the practical deployment of transgenic Pierce's disease (PD) control strategies.

INTRODUCTION

PIPRA, the Public Intellectual Property Resource for Agriculture, is a public sector multi-institutional program designed to provide the framework to manage IP and develop tools that will facilitate humanitarian or commercial development of promising agricultural innovations. In research to control PD, several transgenic strategies have been tested and show long-term promise. However, the gene transfer tools utilized for the research are, in general, proprietary and do not provide features that are likely to be compatible with evolving regulatory frameworks. As a consequence, promising research conducted today may need to be replicated with different tools and technologies if transgenic plants are ever to be deployed for commercial field production. The objective of the research proposed here is to design and test a plant transformation system that addresses IP and regulatory issues and that could be used for research and commercial deployment of transgenic PD control strategies in grapes.

OBJECTIVES

1. Design, develop, and validate a grape-specific transformation system that addresses legal IP, technical and regulatory considerations.
2. Develop alternatives to *Agrobacterium*-mediated transformation for California wine grapes and/or cultivars suitable for generating root stocks.
3. Develop strategies to disseminate biological resources under appropriate licensing agreements for the PD community.
4. Explore collaborative opportunities with researchers developing PD control strategies to link the developed transformation technologies with specific PD resistance technologies.

RESULTS

PIPRA has proposed to identify a suite of complimentary technologies that are scientifically functional and legally deployable for public research and potential commercial uses. Described below are technologies believed to meet these needs.

Plant Transformation

Of a limited number of high efficiency plant transformation methods, the method of choice for essentially all researchers is *Agrobacterium tumefaciens*-mediated transformation. In this process, genes are delivered to plant cells via contact with *Agrobacterium* that harbor plant transformation vectors containing a DNA cassette flanked by *Agrobacterium* T-DNA borders. The T-DNA sequences facilitate transfer and integration of the desired transgene into the plant genome. Patent coverage for *Agrobacterium*-mediated transformation in the U.S. is uncertain because of a long interference which has delayed issuance of the primary patent for over 20 years. By comparison to its European counterpart we can reasonably conclude that when the US patent issues, it will contain methods claims to the use of *Agrobacterium* and T-DNA border