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Classical and Molecular Approaches to Breeding Horticultural Plants for Disease Resistance: Introduction to the Colloquium

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Diseases of horticultural crops have always been a concern of crop producers, processors, merchandisers, and consumers because of reductions in yield and product quality. Excessive chemical application to control diseases of some crops has been a health concern in some countries. Some diseases pose a major constraint, or even a threat, to the production of a crop in a region. Plant breeders, in association with plant pathologists, have had notable successes in breeding disease-resistant varieties, thus ensuring the economic production of a crop. There is now an increased need to breed for disease resistance of horticultural crops because of a loss of chemicals for disease control, environmental and human health concerns, the need to enhance economic competitiveness of horticultural producers and to reduce the genetic vulnerability of crops to pathogens, and an emphasis on using sustainable crop production systems. It is timely to review new concepts and strategies in breeding for disease resistance in horticultural crops in view of the new technologies that have been developed along with the continuing role of classical breeding. This

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colloquium was organized to review recent developments in classical and molecular approaches to breeding diverse horticultural plants for resistance to viruses, bacteria, and fungi. The reviews embody concepts, methods, strategies, and examples of current developments and successes that will interest breeders of all horticultural crops.

A comprehensive classical breeding program for disease resistance involving cooperation between breeders and pathologists encompasses most or all of the following areas: knowledge of the biology, strain, variation and virulence, and epidemiology of the pathogen; development of rapid inoculation methods with repeatable results; use of sets of differential varieties/lines (where available) to provide information on strain variation; easily applied and understood disease rating scales; identification of sources of resistance; knowledge of the genetics of resistance; development of effective selection and breeding schemes for resistance; and widespread evaluation of new lines along with controls in production areas to determine merit for release.

Many graduate students in plant breeding are now heavily involved in using the tools of molecular biology and tissue culture (molecular markers, gene tagging, molecular mapping, transformation methods, and regeneration of specific tissues into intact plants) in the laboratory. They also need to be well versed in the disciplines associated with classical breeding programs. The new tools need to become a part of the total plant breeding program. At this stage, breeders need to combine molecular and classical approaches in enhancing germplasm and developing crop varieties with desirable horticultural traits along with disease resistance. The goal of plant breeding is to enhance the

welfare of a society through genetic improvement of crops, while at the same time improving breeding methods and advancing knowledge in attaining that goal. This perspective needs to be imparted to graduate students, administrators of land-grant institutions, and funding agencies because of the continued erosion of plant breeding programs. It is imperative for the future well-being of humanity that we continue to train plant breeders to meet the stated goal.

In some cases, sufficiently high levels of resistance to some pathogens of many crops have not been secured through classical breeding. In this category are physiological resistance to the fungus *Sclerotinia sclerotiorum* [Lib.] de Bary], some gemini viruses, and bacterial pathogens. Molecular approaches alone, or in combination with classical breeding, may be the most useful approach to attain a desired resistance level. Breeding for virus resistance has proved to be long, difficult, and costly in some crops. Transgenic plants with high levels of resistance to some virus diseases have been developed in recent years through insertion of a gene controlling the virus coat protein into a host genome (see review by R. Grumet). This goal can be achieved in a short time in comparison with traditional breeding procedures. Grumet also discusses other strategies to develop transgenic virus-resistant plants.

Classical breeding procedures have not been successful in introducing high levels of resistance to some bacterial pathogens into acceptable crop lines or varieties. The recent introduction of foreign genes that control specific peptides with antibacterial properties from nonplant sources into desired susceptible horticultural varieties may provide high levels of resistance to a broad spectrum of bacterial pathogens (see review by A.K. Kuehnle). This approach is particularly valuable where it is desired to maintain the original genotype of the variety, except for the introduced foreign gene, and where the classical backcross method is difficult to use or cannot be used to constitute the genotype due to incompatibility, as in apple (*Malus domestica* Borkh.) (see review by S.M. Mehlenbacher).

If an introduced foreign gene does not provide a high enough level of disease resistance, it may be useful to determine if enhanced levels of resistance can be obtained by combining the foreign gene with genes for moderate resistance that accumulated in lines through classical breeding procedures. The stability of expression of the foreign gene and its expression in the desired plant parts also must be determined.

Gene pyramiding to combine resistance to various races of a

pathogen to enhance durability of resistance and to reduce genetic vulnerability to the pathogen has been difficult using classical procedures. The difficulty resides in the repeated testing needed with strains to identify the required genes and in the problem of epistasis of genes, which requires test crosses to detect the hypostatic gene. The use of RAPD molecular markers to "tag" genes for resistance to various strains of rust and bean common mosaic virus is now being used by J.D. Kelly (see review paper) to pyramid genes for resistance to strains of those pathogens in common beans (*Phaseolus vulgaris* L.). Selection for resistance to the above pathogens using molecular markers in the absence of the disease likely also will be practicable.

Breeding for resistance to a pathogen whose inheritance is complex and of low heritability may be difficult using classical breeding procedures. Molecular-marker-assisted selection (MAS) may be more efficient than phenotypic selection under certain conditions. This procedure probably will require detection of quantitative trait loci (QTLs) that would explain a major portion of the variability for the trait. If the heritability of the trait can be increased through improved screening procedures and if appropriate population sizes are used, then QTL detection will be improved.

Mehlenbacher provides a broad overview of current developments in breeding small fruit and tree fruit using classical methods, molecular mapping, MAS, and transformation procedures. He also discusses the role of classical and molecular approaches in breeding crop plants and the current status of plant breeding in public institutions in the United States.

The colloquium concluded with a presentation by Eskridge (see review paper) dealing with the use of nonparametric statistical methods, because data obtained from experiments dealing with disease reactions do not adhere to the standard assumptions underlying the classical analysis of variance. Examples are provided of the value of using nonparametric procedures vs. parametric methods. Detecting more disease-resistant lines to various pathogens in diverse situations is possible using nonparametric methods (Eskridge).

This colloquium is broad in scope and contains subject matter of interest to all breeders of horticultural plants. The information should be especially useful to those involved in teaching graduate courses in plant breeding and for graduate students entering the profession, because it contains "state of the art" information on breeding for disease resistance.

Genetic Engineering for Crop Virus Resistance

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The advances of the past decade in plant transformation and the genetic engineering of virus resistance have provided some of the most dramatic recent developments in our ability to increase genetic resistance to plant diseases. In fact, engineered virus resistance was one of the first successful demonstrations of the introduction of any agriculturally useful trait into plants (Gasser and Fraley, 1989). Engineered plant virus resistance remains a highly active area of research and development by public institutions and industry.

One of the major contributing factors to the early, rapid, and widespread development of genetically engineered virus resistance is the source of genes that are used. As will be discussed further, with the exception of some recent experiments with animal-based systems (antibodies, Tavladoraki et al., 1993; interferon-related proteins, Truve et al., 1993), in every case the genes that were used came from the

genome of the virus (Table 1). To my knowledge, there are no examples at this time where engineered virus resistance was achieved using a gene from the host. The practical significance of our ability to use viral genes is that they generally are easy to clone. The use of such genes, however, is only possible with the concurrent development of appropriate plant transformation technologies.

In the past 8 years, there have been more than 70 reports of genetically engineered virus resistance. The number of plant viruses for which engineered resistance has been achieved, the number of plant species into which these genes have been introduced, and the types of genes that have been used are increasing rapidly. This review focuses on the progress that has been made toward the development of virus-resistant crops and will emphasize applied aspects of the work rather than discussions of mechanism. I will use various examples, including those from my laboratory group, to illustrate different aspects of the engineered resistance. Recent reviews related to this topic include those by Beachy et al. (1990), Fitch and Beachy (1993), Gandani et al. (1990), Grumet (1990, 1994), Hull and Davies (1992), Nelson et al. (1990), Scholtz et al. (1993), and Wilson (1993).

To develop an engineered crop with an agriculturally useful trait,

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