

GUEST EDITORIAL



Martin Bruce Dickman, born 1953. Charles Bessey Prof. of Plant Pathology, U. of Nebraska, Lincoln, NE (UN-L). B.Sc. in Tropical Crops (1979), M.Sc. (1982) and Ph.D. (1986) in Biochemistry and Plant Pathology, all at the U. of Hawaii, Hilo, HI. 1986-87, Post-doctoral Research Fellow, Inst. of Biological Chemistry, Washington State U., Pullman, WA. 1987-93, Asst. Prof., Dept. of Plant Pathology, UN-L. 1993-97, Assoc. Prof., Dept. of Plant Pathology, UN-L. 1997 – present, Professor, Dept. of Plant Pathology, UN-L. 1988 – present, Consultant, ISCO Corp., Lincoln, NE. 1999 – present, Consultant, IDUN Pharmaceuticals, San Diego, CA. *Awards and honors:* 1991, U. of Nebraska, Inst. of Agriculture and Natural Resources, Junior Faculty Recognition for Excellence in Research

Award; 2002, U. of Hawaii, Hilo, Distinguished Alumni Award; 2003, Charles Bessey Professor, UN-L; 2003, Fellow, Am. Phytopathol. Soc. *Other activities:* Co-taught the first course on Internet 2 (with Kansas State and Oregon State Universities): Molecular Plant – Microbe Interaction. Jan. 1991–June 2002, Assoc. Ed., *Applied and Environmental Microbiology*; July 1996 – June 2000, Assoc. Ed., *Mycologia*; July 1996 – June 1999, Senior Ed., *Archives of Microbiology*; June 1997 – present, Senior Ed., *Physiological and Molecular Plant Pathology*; Aug. 2001 – present, Senior Ed., American Phytopathological Society Press; 1990 – 2001, Chairman and Founder, Genetic Basis for Pathogenicity in the Genus *Colletotrichum*, NCR 173; 1991–92, Chairman, Am. Phytopathological Soc. Biochemistry, Physiology, and Molecular Biology Committee. *Research interests:* Plant – pathogen interactions; mechanisms of fungal pathogenicity and disease development; signal transduction; plant apoptosis; comparative pathobiology.

Comparative Pathobiology Approaches to Generating Transgenic Crop Plants with Enhanced Resistance to Fungal Pathogens

Modern agriculture provides abundant productivity for consumers but now faces increasingly severe challenges. Crop protection against pathogenic microorganisms is a major constraint in production agriculture. Fungal diseases historically are the major microbial pathogens of cultivated plants (1). Increased levels of international trade, although providing economic benefit for producers, present new risks from importation of pests or disease agents which can threaten the survival of agricultural enterprises. Increased international travel by the general public has also created opportunities for disease agents to gain quick and easy access to countries. Traditional control measures including breeding, chemical sprays and management practices (*e.g.* crop rotation) have continually and successfully delivered disease resistant/ tolerant cultivars and alleviated disease pressure. However, plant breeding can be time consuming and in some cases has led to the evolution of new virulent fungal races, in large part a result of monocultural agricultural practices. In addition, plant breeding regimes are not always successful measures against complex

multigene diseases or diseases which lack natural resistance sources.

While biotechnology approaches have offered an alternative strategy for plant disease, most attempts to utilize these approaches have failed. Moreover, we cannot ignore the possible environmental risks that must be considered with genetically modified organisms (GMOs) because, for the most part, the results of careful studies and risk assessment are unknown. This issue is also a matter of political and social concern and remains controversial but will not be discussed further (but see 4,16,17). Genetically modified crops presently involve four crops containing a few transgenes grown in four countries (24). Biotechnological approaches have yet to achieve success in controlling fungal diseases. Although powerful tools are available, detrimental effects on crop yield, plant growth and development have resulted in limited effectiveness. Furthermore, the choice of a given transgene and our understanding of the essential compatibility/incompatibility determinants have limited measurable success.

In most cases attempts to generate fungal disease-resistant plants have employed plant 'defense' genes, such as PR proteins (23). Infection of many plant species with various pathogens induces a number of genes and proteins (*e.g.* PR protein, phytoalexins, structural proteins and wall appositions, reactive oxygen species). The key question for all of these responses is whether they are the *cause* or *consequence* of resistance. First posed by Heath more than 30 years ago (14), the question remains if these measurable responses are specifically addressing disease challenge as a primary resistance response or whether they are secondary general stress plant responses. This question is not just academic (an excellent topic for classroom discussion), but is extremely important to biotech companies and researchers who devote considerable resources to generating transgenic crop plants with enhanced agronomic traits. If the choice of a candidate gene (which is critical) is merely part of a general stress response and not specific for resistance, then it is unlikely that a plant harboring this gene will effectively be resistant/tolerant to a given disease in the field. Indeed, there is an impressive list of laboratory/greenhouse successes using the defense gene approach (*e.g.* 2,5,12). However, there is a similar 'list' using like strategies where resistance was not observed (*e.g.* 8,19,21). Why? There are probably several reasons. Many of the genes used in these types of studies have been *correlated* with defense responses but generally have not been proven to be causal for resistance, especially under field conditions. For example, chitinase is known to be antifungal. The fact that plants have chitinase but no chitin, and fungal cell walls do, prompts the question "why would plants have an enzyme for which there is no substrate?" Thus, plant chitinases are assumed to mediate plant defense. Chitin is a common fungal constituent of cell walls and by itself elicits plant defense reactions. It has been reasonably postulated that plant chitinases degrade fungal chitin, and that degradation products induce further defense responses. Chitinase also possesses lysozyme activity and may also protect against invading bacteria. Although chitinase induction is fairly well understood, the alternative functions of plant chitinases have received little attention. Nevertheless, a plant endochitinase has recently been shown to mediate early somatic embryo development of carrot (9). Thus, chitinases may be a plant signal which regulates development. Despite intensive research on the numerous plant chitinases, and despite all of our knowledge regarding properties of the enzyme, the actual role of the enzyme in defense remains unclear. Transgenic plants with elevated chitinase levels have been described (*e.g.* 7 and references therein). In one report, tobacco plants transformed with a basic tobacco chitinase, driven by a constitutive

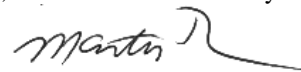
promoter, accumulated up to 120-fold more active chitinase than nontransformed plants. However, transformed plants proved as susceptible to the fungus *Cercospora nicotianae* as controls. Recently, field and greenhouse tests were conducted with transgenic wheat constitutively expressing chitinase, β -1,3-glucanase (another common PR protein), or combinations of them, to evaluate scab (*Fusarium graminearum*) resistance. Although some lines appeared promising in greenhouse studies, none of the transgenic lines had scab resistance under field conditions (3).

It is not the intent of this article to suggest that defense genes are without merit. Much has been learned from these studies, especially with respect to the complexity of plant defense signaling and gene function in plants. PR proteins are also excellent 'biomarkers' for defense responses. Thus, the question arises "where should emphasis be placed?" There is now a shifting emphasis towards marker-assisted breeding and more precise control of gene regulation. For example, pathogen-inducible promoters are being developed (*e.g.* 20) which, in theory, will minimize metabolic expense when compared with the commonly used 35S constitutive promoter. Such promoters will limit expression to sites of pathogen challenge and may also alleviate some of the detrimental effects on plant growth. Alternatively, strategies can be developed from a better understanding of the disease process itself. While easier said than done, such understanding promises to identify targets upon which interference may yield durable resistance. Why? From the pathogen standpoint, genes that block what a fungus requires for pathogenicity or virulence, make it difficult for the pathogen to mutate and overcome this type of resistance, which would require generating new pathogenicity/virulence determinants. Thus, at least in theory, durable resistance could be achieved. Similarly, in gene-for-gene systems, cloning and initial characterization of numerous resistance (R) genes (15) also promises not only to increase the understanding of defense pathways, but also to identify strategies and rules for experimental design. For example, it is now apparent that R genes are effective only in closely related plant species, precluding deployment over larger taxonomic distances (13).

Another useful approach exploits the idea of 'comparative pathobiology', that is, common elements of disease resistance and susceptibility between kingdoms (*e.g.* type III secretion systems in animal/plant pathogenic bacteria). Cellular communication has several highly conserved aspects, whether we are looking at yeast, filamentous fungi, plants or animals (see 6,22). Besides bacterial secretion, innate resistance in plants and animals has intriguing similarities (R genes in plants and their homologs in animals). This has become an active and clear area of research and will likely contribute to our understanding of molecular events that define host-pathogen interactions. We have been studying the apoptosis response in plants following pathogen challenge. Apoptosis, also known as programmed cell death (PCD), is the fastest growing field of biomedical research today and is becoming an increasingly popular research area in plant biology. PCD plays critical roles in a wide variety of normal physiological processes. In humans and other animals, dysregulation of this natural cell death pathway contributes greatly to diseases characterized by either excessive cell accumulation (cancer, restenosis, autoimmunity) or inappropriate cell death (stroke, myocardial infarction, inflammation, AIDS, Alzheimer's and other neurodegenerative diseases). In addition, most viruses and intracellular bacteria control the cell death pathway in the host cells they infect, thus linking apoptosis to infectious diseases. As in animals, a programmed type of cell death occurs in plants as part of normal growth and development, including reproduction, seed germination, aerenchyma

formation, tracheary element differentiation, sieve element differentiation, and senescence (11). Moreover, cell suicide programs are activated, at least in some cases, during pathogen attack in both resistant and susceptible interactions (11).

The genes that control programmed cell death are conserved across wide evolutionary distances, defining a core set of biochemical reactions that are regulated in diverse ways by inputs from myriad upstream pathways. These genes encode either anti-apoptotic or pro-apoptotic proteins, which do battle with each other in making cell life–death decisions. One question is whether plants display analogous features to mammalian apoptosis during defense against pathogen attack. We generated a number of transgenic crop plants that express animal anti-apoptotic genes. These genes (human *bcl-2*, chicken *bcl-xl*, *C. elegans ced-9*, and insect *sf-tap*) all suppress apoptotic death in animal cells. Recently, we have shown that expression of these genes in tobacco, abrogates disease development in plants infected with necrotrophic fungi, including *Sclerotinia sclerotiorum*, *Botrytis cinerea*, and *C. nicotianae*, suggesting that disease development requires host cell death pathways (10). Plants with inactivating mutations in these transgenes did not protect against pathogens (10). In addition, characteristic apoptotic features (chromatin condensation, DNA fragmentation) appeared in susceptible plants during infection, but not in transgenic resistant plants. Thus, plant cell death can have two very different outcomes: susceptibility (as described above for necrotrophic pathogens) and resistance (as is well documented by the hypersensitive response in gene-for-gene interactions). These transgenic plants also displayed tolerance/resistance to abiotic stresses (heat, cold, salt, drought), suggesting either converging pathways for biotic and abiotic stress responses, and/or that common signals arise from both types of stress (Dickman, unpublished). Similar results, in terms of disease resistance, were obtained by Gilchrist and colleagues using the p35 anti-apoptotic gene from baculovirus which, when expressed in tomato, conferred protection against necrotrophic fungi and a bacterium (18). Conceivably, enormous opportunities exist for using animal models of programmed cell death to dissect cell death pathways in plants, leading to a mechanistic understanding of the regulation of plant cell death. In addition, exploitation of cell life/death pathways in plants can be used for protection of crops against fungal plant pathogens of economically important crops, which we are currently field testing.



Martin Dickman
Dept. of Plant Pathology
University of Nebraska
Lincoln, NE 68583, USA

[Fax: +1-402-472-2853; e-mail: mdickman@unlnotes.unl.edu]

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