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Novel Approaches for Genetic Resistance to Bacterial Pathogens in Flower Crops

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Bacterial diseases plague many flower crops. Fortunately, for ornamentals cultivated under glasshouse conditions, bacterial diseases are less prevalent than fungal or viral diseases (Dons et al., 1991). Nevertheless, bacterial diseases can be devastating when an outbreak occurs. The most common causal organisms are from five genera of bacteria: *Erwinia*, *Xanthomonas*, *Pseudomonas*, *Clavibacter* (*Corynebacterium*), and *Agrobacterium*. These bacteria cause the familiar symptoms of leaf spots, blights, rots, wilts, cankers, and galls (Powell and Lindquist, 1992) and affect many of the major cut-flower and flowering potted-plant crops (Table 1). Perhaps one of the best known bacterial diseases among ornamentals is xanthomonas bacterial blight/wilt in geranium. Despite disease control advances made since the 1960s, before which the geranium market was virtually nonexistent due to the prevalence of bacterial blight/wilt, the disease continues to be a risk for geranium propagators and growers alike (Whealey, 1994). Forced dumping of geranium stock plants due to an outbreak of *Xanthomonas* even occurred this past year.

Through use of horticultural methods that practice sanitation (Baker, 1957) and pathogen exclusion, bacterial disease problems can be minimized. Sanitation is useless, however, if diseased plants are not first eliminated from the farm. Unfortunately, some growers are still reluctant to do this. Today, culture-indexed cuttings for many cut-flower and potted florist crops can be obtained from specialist propagators. Limited chemical controls, such as antibiotics or copper sprays, also can be used on the farm. However, frequent usage results in pathogen resistance (Cooksey, 1990). Moreover, these controls are not curative, and they do not economically solve extensive bacterial disease problems.

Genetic resistance offers one way to manage disease. When combined with the use of culture-indexed propagules and sanitation, it can be a powerful control. Classical breeding offers some genetic solutions, but little has been published regarding resistance to bacterial diseases of florist crops. This dearth contrasts sharply with the active resistance breeding for other horticultural and agronomic crops and with the progress made in fungal disease resistance in some flowers crops, e.g., *Fusarium* spp. in carnation and tulip, *Puccinia horiana* Henn. (white rust) in chrysanthemum, and *Colletotrichum gloeosporioides* Penz. (anthracnose) in anthurium (Aragaki et al., 1968; Sparnaaij, 1991). Introgression of resistance genes, either through tissue culture manipulations, use of molecular genetics, or by genetic engineering, are supplementary breeding approaches that appear promising. Yet, despite the current and predicted importance of the floriculture industry to U.S. agriculture (1993 wholesale value approached \$3 billion), little use of novel breeding strategies for (bacterial) disease control is evident in either the public or private sector.

In the last several years, numerous papers describing genetic transformation systems for floricultural crops have appeared (Robinson and Firoozabady, 1993). Preceding and during this same period, numerous biological compounds have been identified with potent antimicrobial properties—research driven in part by the biomedical and food science fields. The objective of this paper is to discuss previous and current research that uses novel genetic strategies to control bacterial disease in various cropping systems, with focus on integrating genetic engineering with nonplant resistance genes. A case study for controlling *Xanthomonas* spp., species of which severely limit geranium and anthurium production, is given for anthurium, using genes derived from those of the *Cecropia* moth.

Novel approaches: Cloning resistance genes from plants via molecular genetics

Screening plant extracts for antibacterial activity has identified many potentially useful compounds, but it has seldom led to identification of the associated plant genes. Focusing on plant molecular signals active during early stages of infection has been more successful; many research groups are targeting host genes involved in patho-

Received for publication 20 Oct. 1994. Accepted for publication 16 Nov. 1994. Journal Series no. 4007 of the Hawaii Institute of Tropical Agriculture and Human Resources. Research supported in part by the State of Hawaii Governor's Agricultural Coordinating Committee Grant 89-22 and the Hawaiian Anthurium Industry Association. We thank J. Jaynes, A. Alvarez, D. Norman, and D. Coyne for their contributions. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

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Table 1. Common bacterial diseases of some major cut flowers and flowering potted plants.

Crop	Disease	Pathogen
Anthurium	Blight	<i>Xanthomonas campestris</i> pv. <i>dieffenbachiae</i>
Begonia	Leaf spot and blight	<i>Xanthomonas campestris</i> pv. <i>begoniae</i> (Takimoto) Dye
Carnation	Bacterial spot and blight	<i>Pseudomonas woodsii</i> (Smith) Stevens
Chrysanthemum	Bacterial wilt	<i>Pseudomonas caryophylli</i> (Burkholder) Starr & Burkholder
	Bacterial blight	<i>Erwinia chrysanthemi</i> Burkholder, McFadden & Dimock
	Bacterial leaf spot	<i>Pseudomonas cichorii</i> (Swingle) Stapp
Freesia	Bacterial scab	<i>Pseudomonas marginata</i> (McCull.) Stapp
Geranium	Leaf spot and blight	<i>Xanthomonas campestris</i> pv. <i>pelargonii</i> (Brown) Dye
	Wilt	<i>Xanthomonas</i> spp.
	Pseudomonas leaf spot	<i>Pseudomonas erodii</i> Lewis and <i>P. cichorii</i>
Gerbera	Bacterial blight	<i>Pseudomonas cichorii</i>
Gladiolus	Bacterial leaf rot	<i>Pseudomonas marginata</i>
	Leaf spot and neck rot	<i>Xanthomonas campestris</i> pv. <i>gummisudans</i> (McCull.) Young, Dye et al.
Lily	Bulb rot	<i>Clavibacter (Corynebacterium) michiganense</i> (Smith) Jensen
Orchids		
Dendrobium	Leaf spot and blight	<i>Erwinia chrysanthemii</i>
	Bacterial soft rot	<i>Pseudomonas gladioli</i> Severini pv. <i>gladioli</i>
	Bacterial canker	<i>Clavibacter (Corynebacterium) spp.</i>
Poinsettia	Bacterial leaf spot	<i>Xanthomonas campestris</i> (Pammel) Dawson
	Soft rot and stem rot	<i>Erwinia</i> spp.
	Bacterial leaf spot	<i>Pseudomonas syringae</i> van Hall
Rose	Crown gall	<i>Agrobacterium tumefaciens</i>

gen recognition/signal transduction or biosynthesis of inducible defense products, such as phytoalexins, pathogenesis-related proteins, and structural proteins (Lamb et al., 1992). Several examples exist for applying this strategy against fungal pathogens, including *Rhizoctonia solani* Kühn (damping-off) (Broglie et al., 1991).

Less progress has been made in combating bacterial diseases; a few examples exist for non-florist crops with host-pathogen systems that show gene $\times$ gene interaction (Flor, 1971). In tomato, restriction fragment length polymorphisms (RFLPs), genetic linkage maps, and F_2 segregating progeny from crosses of near-isogenic lines were used together to identify a tomato chromosome region and a subsequent cDNA clone (CD127) encoding a *Pto* gene for resistance to those races of *Pseudomonas syringae* pv. *tomato* Okabe with the corresponding avirulence gene *avrPto* (Martin et al., 1993). Similar map-based cloning is in progress for isolating the *Arabidopsis* gene *RPM1* for resistance to *P. syringae* pv. *maculicola* McCulloch carrying *avrPmaA1* (T. Debener, H. Liedgens, M. Gerwin, and J. Dangl, unpublished data).

Despite the success of this approach, immediate application of map-based cloning to flower crops appears remote. Genetic linkage and RFLP maps are not yet developed for the major temperate and subtropical ornamentals, and many ornamentals may be unsuitable due to genome size and organization, inbreeding depression, and long generation times. However, a disease resistance gene identified by map-based cloning in one crop could possibly be used to identify a structurally and functionally conserved gene in a target florist crop. For example, the tomato *Pto* gene family clone CD127 shared homology with DNA regions of at least 11 other crop species (Martin et al., 1993).

A second strategy, gene tagging, is being applied with rapid progress in several crops, including *Arabidopsis thaliana* L., a model plant with a small genome. In this approach, genes of interest are identified through use of transposable elements (Altmann et al., 1992) or T-DNA insertion (Feldmann and Marks, 1987). Direct application of this approach may be less valuable for polyploid ornamentals, such as roses, chrysanthemums, and orchids, or species with large genomes (genome sizes of the vast majority of ornamental breeding species and hybrids are currently unknown). However, gene sequences identified in *Arabidopsis* could be used to screen genomes of ornamentals in the event that some resistance genes are conserved across genera (Dangl et al., 1992). Alternative strategies that do not rely on RFLP or genetic maps are emerging and are being used to elucidate and manipulate the host-pathogen gene recognition response (Keen et al., 1993).

Novel approaches: Studies using nonplant antibacterial genes

Recent advances have led to the identification, and subsequent gene cloning, of several peptides with antibacterial properties in

arthropods and some mammalian species. These peptides work independently or in concert to kill a range of important Gram-positive and -negative bacteria. Their activities vary, but several appear suitable against plant pathogens (Destéfano-Beltrán et al., 1990; Nordeen et al., 1992; Sinden et al., 1993). We limit our discussion to three broad categories of antimicrobial proteins: insect immune proteins, animal defense proteins, and lysozymes, followed with examples of natural or synthetic antibacterial genes that have been cloned, modified, and introduced for expression in plants.

Insect immune proteins. H. Boman's group in Sweden, using the giant silkworm *Hyalophora cecropia* L., initially isolated and characterized insect antibacterial peptides (Table 2) in some detail during the late 1970s and the 1980s (Boman et al., 1991; Dunn, 1991). These

Table 2. Some antibacterial peptides isolated from arthropods (adapted, in part, from Dunn, 1991).

Species	Life stage/ tissue ^a	Defensive protein	Molecular wt (kDa)
<i>Hyalophora cecropia</i> (giant silkworm)	P	Lysozyme	15
	P	Cecropins	4
	P	Attacins	22
	P	P4	48
<i>Sarcophaga peregrina</i> Meigen (fleshfly)	L	Sarcotoxins I-III	7-26
	CL	Sapacin	4
<i>Phormia terraenovae</i> Robineau Desvoidy (blowfly)	L	Diptericins	9
	L	Defensins	4
	L	Cecropin-like	4
<i>Manduca sexta</i> L. (tobacco hornworm)	L	Lysozyme	14
	L	Cecropin-like	4
	L	Attacin-like	22
	L	P4-like	48
<i>Drosophila melanogaster</i> Meigen L. (fruitfly)	A	Cecropins	4
	GT	Andropin	6
	A	Diptericin	9
<i>Apis mellifera</i> L. (honeybees)	A	Apidacins	2
	A	Abacins	4
<i>Zophobas atratus</i> Blanchard (tenebrionid beetle)	L	Coleopteracin	8
<i>Tachyplesus tridentatus</i> Leach (horseshoe crab)	H	Tachyplesins	2

^aA = adult; CL = cell line; GT = genital tracts; H = hemocytes, L = larva; P = pupa.

peptides are the products of humoral (cell-free) immunity in insects after induction by bacteria. Immune proteins have now been isolated from various insects and other arthropods. This continues to be an active area of research.

Three classes of bactericidal proteins have been identified in the giant silkworm: lysozymes, attacins, and cecropins (see review by Boman et al., 1991). The cecropia lysozyme is very similar to chicken egg white lysozyme in enzyme activity. Lysozymes are lytic muramidases that cleave the glycosidic bond between the C-1 of *N*-acetylmuramic acid and the C-4 of *N*-acetylglucosamine in the bacterial peptidoglycan (Jolls and Jolls, 1984). Six forms of attacins, with an approximate molecular weight of 20 kDa, are the largest antibacterial peptides found in the hemolymph of immunized cecropia pupae. Attacin appears to inhibit the synthesis of outer membrane proteins in *E. coli* by interfering with transcription (Carlsson et al., 1991). There are three major forms of cecropins. These cecropins are basic and small, ≈ 4 kDa. They are the most potent group of the cecropia antibacterial factors, with a broad spectrum of activity against Gram-negative and Gram-positive bacteria (Jaynes et al., 1987). Insect, and mammalian, cecropins are considered lytic peptides, with the primary targets being the inner and outer bacterial membrane (Boman, 1991).

Noninsect animal defense proteins. Other antibacterial peptides that kill a wide variety of bacteria and fungi have been isolated from diverse animal tissues (Table 3). Mammalian cells and tissues contain one group of proteins called defensins (Ganz et al., 1990), small basic molecules of 29 to 34 amino acid residues with low molecular weights, ≈ 3 to 5 kDa. Like cecropins, defensins are lytic peptides that form pores (ion channels) in bacterial and artificial membranes (Boman, 1991; Lehrer et al., 1991). Some examples of defensins are from human, rabbit, and rat sources (Table 3). Recently, Selsted et al. (1992) reported a novel bactericidal peptide, indolicidin, with only 13 amino acid residues isolated from the bovine immune system. A Gram-positive and a Gram-negative bacterium were killed in 2 h by the peptide at low concentrations ($2.5\text{--}10\text{ mg}\cdot\text{ml}^{-1}$). Mammals, such as pigs, also produce cecropin-like peptides with molecular weights similar to those found in insects (Table 3). Other defense proteins from mammals include bactericidal/permeability-increasing factor (BPI) proteins (Table 3) (Lehrer and Ganz, 1990) and lactoferrin (Erdei et al., 1994). Additional peptides continue to be discovered.

Amphibian skin contains a high percentage of antimicrobial factors. Magainins are one group of small peptides (23 amino acid residues) isolated from the African clawed frog, *Xenopus laevis* Daudin (Berkowitz et al., 1990; Zasloff, 1987). Magainins and derivatives have broad-spectrum activity on Gram-negative and Gram-positive bacteria, fungi, and parasites (Berkowitz et al., 1990); however, the low potency of native magainins may be inappropriate for plant protection purposes (J. Jaynes, personal communication).

Lysozymes. The most intensively studied lysozymes are those from avian egg white, although mammalian, insect, and invertebrate tissues also contain lysozymes. Differing groups of lysozymes differ in

substrate specificity (Jollès and Jollès, 1984), with molecular weights ranging from 14 to ≈ 25 kDa. Many bacteriophage lysozyme genes and their protein products have been isolated, cloned, and sequenced. Usually two or three lytic proteins are involved in the lytic cycle of bacteriophage. These proteins lyse the bacterial host, which results in the release of new phage particles (Young, 1992). Several genes for phage lysis proteins have been cloned into plant gene expression vectors.

Studies toward genetic engineering for bacterial resistance. Following the suggestions of Jaynes et al. (1987) that genes encoding lytic and other antimicrobial peptides can be engineered to increase bacterial disease resistance in plants, several natural (attacin, chicken lysozyme, T4 phage lysozyme, P22 phage genes 13 and 19, lactoferrin, and cecropin B) or synthetic (SB37, Shiva series) antibacterial genes were used to develop plant-expressible gene constructs (Destéfano-Beitrán et al., 1990; Hippe et al., 1989; Trudel et al., 1992) and then engineered into tobacco and potato. Tobacco transgenic for the modified cecropin Shiva-1 showed resistance to *Pseudomonas solanacearum* Smith in the form of delayed symptoms and decreased plant mortality relative to controls (Jaynes et al., 1993). Potato transgenic for the bacteriophage T4 lysozyme gene with an alpha-amylase export signal sequence showed increased resistance to *Erwinia carotovora* pv. *atroseptica* van Hall with lower tuber maceration and higher sprouting capability compared to controls (Düring et al., 1993). Homogenates of transgenic tobacco tissues expressing a hen egg white lysozyme were active against *Micrococcus* spp. in lysoplate assays (Trudel et al., 1992). Potato tubers expressing a gene for the cecropin derivative SB37 ligated to an alpha-amylase secretory sequence indicated significant, but moderate, differences in soft rot among the tubers from control potato plants and transformants challenged with *Erwinia carotovora* spp. (Hassan et al., 1993). Leaf extract of potato transgenic for SB37 showed activity against *P. solanacearum* race 3 (Montanelli and Nascari, 1991). Tobacco transgenic for human lactoferrin appears resistant to *Pseudomonas* spp. (Z. Zhang, D.P. Coyne, and A. Mitra, unpublished data).

From these reports, it appears that expression of antibacterial genes in plants may confer potentially effective bacterial disease resistance. Current studies are expanding the targets for insect immune genes and their derivatives to include several horticultural crops (Jaynes, 1993). This strategy could be similarly applied to florist crops when successful gene transfer and tissue culture methods are developed.

Genetic engineering of floricultural crops

Gene transfer, such as is possible through genetic engineering, is particularly attractive for ornamentals with a lengthy juvenile phase, such as roses, orchids, bulb crops, and tropical exotics. Goals of ornamental genetic engineering appear thus far to have focused on altering phenotype, such as flower color, rather than on decreasing production costs, such as by incorporating disease resistance. Methods for engineering all the major florist crops exist (Table 4), although regeneration difficulties and cultivar differences still limit routine application of these methods (Hutchinson et al., 1992). Development of genotype-independent tissue culture and gene-transfer protocols are a focus of several research programs.

Control of bacterial blight in *Anthurium*: Current research

Anthurium andraeanum Linden ex André and its hybrids, *A. andraeanum* Hort., are economically important ornamental monocots for subtropical and tropical regions. Anthurium is used as a cut flower, flowering potted plant, and more recently as a landscape plant. Hawaii currently supplies about one-fifth of the global production. Anthurium is also widely cultivated in temperate climates in glasshouses, ranking 13th among cut flowers sold in the Dutch auctions.

New cultivars of this outbreeding, clonally propagated crop are developed through sexual hybridization, progeny evaluation, and selection. There are good opportunities for interspecific hybridization within the genus (*Anthurium* consists of ≈ 1000 species). The Univ. of Hawaii has an active breeding program, with more than two dozen cultivars released in the past 30 years (Kamemoto and Kuehnle, 1995).

Table 3. Some antibacterial peptides isolated from mammals and amphibians.

Species	Defensive protein	Molecular wt (kDa)
Human	Defensin	5
	Bactericidal/permeability increasing (BPI)	55–60
Rabbit	Defensins	4
	Neutrophil peptides	4
Pig	Cecropin P1	3.3
	Tracheal antimicrobial peptide	3.4–4.0
	BPI	25
	Indolicidin	1.4
Rat	Defensins	3.2–3.8
Mouse	Cryptidins	4
Guinea pig	Neutrophil cationic peptides	2.6
<i>Xenopus laevis</i> (African clawed frog)	Magainins	2–3
	PGQ	1.4–2.0
<i>Bombina variegata</i> L. (frog)	Bombinin-like	3

Table 4. Genetic engineering feasibility for some florist crops.

Crop	Method	Selected references
Anthurium	Agrobacterium	Kuehnle and Sugii, 1991a
Carnation	Agrobacterium	Lu et al., 1991
Chrysanthemum	Agrobacterium	Courtney-Gutterson et al., 1993
Gerbera	Agrobacterium	Elomaa et al., 1993
Gladiolus	Agrobacterium	Graves and Goldman, 1987
Lily	Agrobacterium	Cohen and Meredith, 1992
	Particle gun	Peñafiel and Kamo, 1994
Orchid	Particle gun	Kuehnle and Sugii, 1992
Rose	Agrobacterium	Firoozabady et al., 1994
Tulip	Particle gun/Agro	Wilmink et al., 1992

Genetic engineering of anthurium recently became feasible after it was shown that some cultivars can be infected by *Agrobacterium tumefaciens* Smith & Townsend (Kuehnle and Sugii, 1991a), a well-established vector for plant genetic transformation. Tissue culture protocols were also available for some Hawaiian cultivars (Kuehnle and Sugii, 1991b). However, as a perennial crop with a long juvenile stage (2 to 3 years) and slow seed maturation (6 months), the impact of classical breeding efforts and transgenic germplasm takes many years to be realized.

Bacterial blight and its current control. Due to the outbreak of a bacterial blight in Hawaii in the early 1980s (Nishijima and Fujiyama, 1985), Hawaii's anthurium production steadily declined between 1986 and 1993 from a farmgate value of \$10 million to about \$6 million. Blight of anthurium is caused by the bacterium *Xanthomonas campestris* pv. *dieffenbachiae* McCull. & Pirone (Xcd). Most commercial cultivars are susceptible to Xcd; the bacterium infects at least 11 other ornamental aroids in addition to anthurium and can cause foliar and systemic symptoms in anthurium. Foliar symptoms include water-soaked and necrotic spots, usually starting at the leaf margin due to entry at the hydathodes. Systemic infections lead to a general yellowing of entire leaf blades. Plants with systemic infection in the stem, petioles, and spathes have high internal bacterial populations and usually die (Nishijima and Fujiyama, 1985). The disease can be spread by various means, such as splashing rain or irrigation, or the use of contaminated cutting tools during cultural operations, such as pruning and handling. Most Hawaiian anthurium production is in shadehouses, which are exposed to rain and overhead irrigation.

Current control measures include strict sanitation (Nishijima, 1988). Although the antibiotic combination of streptomycin and oxytetracycline has been used in some cases, the bacteria developed streptomycin resistance in a short time. Therefore, antibiotics are not recommended for routine control (Alvarez et al., 1989; Nishijima, 1988). Studies using several chemicals, such as Physan (Maril Products, Tustin, California) and Aliette 80 WP (Rhone-Poulenc Agrochimie, Lyon, France), have led to some abatement of bacterial infection (Alvarez et al., 1990, 1991). A clean stocks program is currently being developed with Hawaii state resources. Despite implementation of controls by many growers, annual anthurium production has not increased.

Conventional breeding. Using classical hybridization and selection in our breeding program, we have attempted to transfer the apparent systemic resistance from *Anthurium antioquiense* Engler to the cultivated *A. andraeanum* Hort. F₁ hybrids had a high degree of resistance to the bacteria. However, due to the small flower of *A. antioquiense*, the F₁ hybrids have to be backcrossed to the cultivated varieties to obtain resistant plants with horticulturally desirable characteristics (Kamemoto et al., 1990). This objective could take a decade, or longer, to achieve. More importantly, the genetics of the available resistance are not understood, and the quality of the final resistance may not be as high as desirable. Using other tolerant germplasm, we recently released two cultivars with improved resistance, 'Kalapana' and 'Tropic Ice' (Kamemoto and Kuehnle, 1995). After several years, both cultivars eventually succumbed to blight (anthuriums remain in production from 5 to 9 years). The mode of inheritance of the available resistance in the breeding germplasm remains to be determined.

Genetic engineering. The overall goal of our research is to introduce various antibacterial genes and synthetic derivatives from

Hyalophora cecropia and bacteriophages, in suitable vectors, into anthurium cultivars followed by plant characterization and evaluation for bacterial resistance. We used two cultivars in our study, 'Rudolph' and 'UH1060'. Several lines of putatively transformed plants were obtained for the antibacterial genes *Att*, *P13*, and *T4* from constructs Ca2Att, Ca2P13, and Ca2T4, respectively, as shown by polymerase chain reaction and Western analysis (Chen, 1993).

Resistance of regenerated anthurium was tested by challenge-inoculation with the highly virulent Xcd strain D150. Kanamycin-resistant plantlets with Ca2Att, Ca2P13, and Ca2T4 showed a noticeable delay in disease symptom development compared with nontransformed controls (Chen, 1993). Counts from bacterial reisolation of inoculated Ca2Att plantlets correlated well with the degree of disease symptoms. Greenhouse spray inoculation and natural infection studies of mature Ca2Att and Ca2T4 plants indicated some tolerance among individuals to Xcd strain D150. However, due to symptomless infections of nontransformed controls, proper statistical analysis of our data becomes essential. Our results with these genes are supported by other studies with delayed symptom expression or resistance in apple transgenic for attacin (Norelli et al., 1993) and potato transgenic for T4 (Düring et al., 1993). Thus, the use of cecropia and bacteriophage genes may confer some bacterial disease tolerance into anthurium. However, tests for effectiveness need to include more samples and more field tests to identify lines with suitable levels of resistance.

Prospects

Results from the limited body of work using lytic peptides against bacterial pathogens remains encouraging. Other developing strategies should also be considered. For example, stimulation in the plant of systemic acquired resistance (SAR) (Ryals et al., 1994) is one area that deserves attention by researchers for potential use in florist crop protection. Apparently, this type of resistance is effective against bacterial pathogens in the field for several weeks (Ku'c, 1982) and can be induced by a variety of biotic and abiotic agents. By itself, this gene-induction strategy could be particularly useful for fast-cycling crops such as chrysanthemum (10- to 12-week production time). For genetic improvement purposes, it may be possible to select and hybridize plants with strong SAR. Akin to this is high phytoalexin production in *Fusarium*-resistant, but not susceptible, carnation (Baayen et al., 1991). A second strategy is extension of the parasite-derived resistance concept (Sanford and Johnston, 1985) to those bacterial diseases whose symptoms in ornamentals may be associated with production of a bacterial toxin (Walton and Panaccione, 1993). The value of this approach is evident in examples of reduced toxin sensitivity, or none, in transgenic tobacco expressing bacteria-derived genes (Anzai et al., 1989; de la Fuente-Martinez et al., 1992). Finally, since protoplast and tissue culture manipulations have improved greatly for ornamentals in recent years, selection of resistance among somaclones (van den Bulk, 1991) or introgression of resistance genes from wild relatives merit further attention by breeders.

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Use of Random Amplified Polymorphic DNA Markers in Breeding for Major Gene Resistance to Plant Pathogens

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Markers are of interest to plant breeders as a source of genetic information on crops and for use in indirect selection of traits linked with the markers. Selection based on the marker would be more efficient provided there was tight linkage between the marker and the trait of interest [<5 centimorgans (cM)] and assuming selection for the marker was more convenient (faster, cheaper, reproducible, expressed earlier). Markers for disease resistance offer the additional advantage of permitting selection for resistance in the absence of the pathogen or a variant of the pathogen. This feature is of particular interest to a plant breeder who may be reluctant either to work directly with or to introduce a pathogen where quarantine restrictions prevent its introduction. Markers linked to various resistance genes would greatly facilitate breeding for multiple disease resistance, since selection based on markers could be readily incorporated into a breeding program. This approach would be of particular interest with pathogens that exhibit inconsistent expression due to environmental or other factors. A single technology that would indirectly permit selection for diverse disease resistance genes would be highly desirable and extremely useful in breeding for disease resistance. Indirect selection as an efficient and practical means of developing disease-resistant cultivars will depend on the identification of markers that are easy to score and tightly linked to different resistance genes.

TYPE OF MARKER

Until recently, indirect selection has not been practical due to the lack of suitable markers, or the undesirable pleiotropic effects of many morphological markers. In recent years, marker-assisted selection (MAS) has received attention as a useful method for improving major gene disease resistance in horticultural crops. The concept of MAS, when applied to monogenic disease resistance, dictates that selection for one or more resistance genes is conducted by selecting a marker (or two flanking markers) tightly linked to the gene of interest (Melchinger, 1990). The use of this approach with morphological markers has been limited primarily due to the few such markers available in most crop

species, their major effects on plant phenotype (often deleterious mutants), and the inability to score multiple morphological mutant traits in a single segregating population (Paterson et al., 1991). The development of markers based on allelic variants of specific enzymes (isozymes; Tanksley and Orton, 1983) and, most notably, of those based on variation in the length of DNA fragments obtained by digestion with restriction endonucleases (RFLPs; Botstein et al., 1980) has overcome these limitations.

RFLP markers offer significant advantages over isozymes. They demonstrate more detectable loci and alleles, are phenotypically neutral, and can be scored at any stage of plant development. RFLP markers have been used extensively to "tag" useful genes in common bean (*Phaseolus vulgaris* L., Degremont and Vallejos, 1994; Nodari et al., 1993), lettuce (*Lactuca sativa* L., Landry et al., 1987; Paran et al., 1991), pepper (*Capsicum annuum* L., Tanksley et al., 1988), potato (*Solanum tuberosum* L., Barone et al., 1990; Ritter et al., 1991), and tomato (*Lycopersicon esculentum* Mill., Klein-Lankhorts et al., 1991a; Nienhuis et al., 1987; Osborn et al., 1987; Sarfatti et al., 1991; Young et al., 1988). However, development of RFLP markers involves a tedious, expensive, multistep process that requires considerable investment in personnel, equipment, chemicals, and safety concerns if radioactive probes are used. The technology is not compatible with the needs of those breeders involved in resistance breeding and working with large populations and limited budgets.

Recently, a molecular marker based on the polymerase chain reaction (PCR) has been developed that alleviates these potential limitations. PCR was modified to develop a new form of molecular marker: the random amplified polymorphic DNA (RAPD) marker (Welsh and McClelland, 1990; Williams et al., 1990). Genetic mapping and gene tagging using RAPD markers has several advantages over other methods (Kesseli et al., 1992; Williams et al., 1990): 1) a universal set of primers can be used and screened in a short period, 2) no isolation of cloned DNA probes or preparation of hybridization filters is required, and 3) only small quantities of DNA are needed, allowing the use of simple and rapid methods for genomic isolation (Afanador et al., 1993; Edwards et al., 1991; Wang et al., 1993).

Since their inception, RAPDs have been used to tag major resistance genes in barley (*Hordeum vulgare* L., Barua et al., 1993), common bean (Haley et al., 1993, 1994a, 1994b, 1994c; Johnson and Gepts, 1994; Johnson et al., 1994; Jung et al., 1994; Miklas et al., 1993), lettuce (Kesseli et al., 1992; Michelmore et al., 1991; Paran et al., 1991), oat (*Avena sativa* L., Penner et al., 1993), rice (*Oryza sativa* L., Mohan et al., 1994), tomato (Klein-Lankhorts et al., 1991b; Martin et al., 1991; Williamson et al., 1994), and wheat (*Triticum aestivum* L., Schachermayr et al., 1994), and to assist in developing comprehensive genetic maps and within-species relationships in several plant species (alfalfa, *Medicago sativa* L., Echt et al., 1992; apple, *Malus domestica* Borkh., Koller et al., 1993; blueberry, *Vaccinium darrowi*, Rowland and Levi, 1994; broccoli and cauliflower, *Brassica oleracea* L., Hu

Received for publication 20 Oct. 1994. Accepted for publication 16 Nov. 1994. Research supported in part by grant DAN 1310-G-SS-6008-00 from the USAID Bean/Cowpea Collaborative Research Support Program, the Michigan Agricultural Experiment Station, and the U.S. Dept. of Agriculture (USDA)—Agricultural Research Service. Mention of a trademark or a proprietary product does not constitute a guarantee or warranty of the product by the USDA and does not imply its approval to the exclusion of other products that may also be suitable. I acknowledge the assistance of L. Afanador, S.D. Haley, P.N. Miklas, J.R. Staveland, and R.A. Young in the development of RAPD markers in common bean and for their critical review of this manuscript. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

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