Soares Filho W., S. Dos, Z.J.M. Cordeiro, K. Shepherd, J.L.L. Dantas, S. de Oliveira e Silva & M.A.P. da Cunha. 1992. The banana genetic improvement programme at CNPMF/EMBRAPA, Brazil. Pp. 339-346 *in* Breeding bananas and plantains : proceedings of an International Symposium on Genetic Improvement of Bananas for their Resistance to Diseases and Pests (J. Ganry, ed.). CIRAD-FLHOR, Montpellier, France.

Stover R.H. & I.W. Buddenhagen. 1986. Banana breeding: polyploidy, disease resistance and productivity. Fruits 41:175-191.

Swennen R. & D. Vuylsteke. 1990. Aspects of plantain breeding at IITA. Pp. 252-266 *in* Sigatoka leaf spot disease: Proceedings of an international workshop (R.A. Fullerton & R.H. Stover, eds). San José, Costa Rica.

Tomekpé K., P. Rowe, H. Tezenas du Montcel & D. Vuylsteke. 1995. Plantain and Popoulou/Maia Maoli Breeding: current approaches and future opportunities. Workshop INIBAP/MARDI, Serdang, Malaysia.

Vakili N.G. 1967. The experimental formation of polyploidy and its effect in the genus *Musa*. Amer. J. Bot. 54: 24-36.

Can model plants help banana improvement through biotechnology?

Martin B. Dickman

Bananas must cope with numerous environmental challenges, particularly with fungal and bacterial pathogens as well as pests and abiotic stresses. This situation is exacerbated by the limited diversity of cultivars. Moreover, traditional breeding strategies are problematic due to a low female fertility, sterility, ploidy and poor seed set. As a result, classical genetics is difficult and limited, as well as being extremely time consuming. Taken together, the difficulty in conventional beeeding, limited genetic diversity and poorly controlled diseases all point to the necessity of developing alternative strategies for banana improvement. Biotechnological approaches are particularly appropriate for this crop. This review will focus on two distinct, but overlapping issues: (i) the role of model plants in providing avenues leading to approaches for banana improvement through biotechnology and (ii) conceptual approaches for generating bananas with enhanced resistance to disease and other environmental stresses.

The attraction of genetic engineering

While the "track record" regarding successful applications of recombinant DNA approaches in generating transgenic plants with enhanced agronomic traits (especially involving disease resistance) is limited at best, it is also fair to say that this technology has considerable experimental power. It is now evident that many important techniques (e.g. genetic transformation, gene silencing) are now possible in bananas (James Dale, personal communication) and are mainly limited by the choice of gene(s). In other words, what do we insert? Moreover, while the technology for gene manipulation has been available for a number of years for many plants, success under field conditions has been hampered by our overall lack of understanding of the essential determinants and pathways mediating stress/disease. However, thus more effective genes and strategies are likely to ensue given the availability of genome sequences.

Model plants

This section will discuss two model plants; *Arabidopsis* and rice. *Arabidopsis* has served as an invaluable model plant in numerous aspects of plant biology, including pathology and stress physiology, with many insights viewed to be directly applicable to crop plants. In addition, *Arabidopsis* has a number of key experimental features: the genome is sequenced, microarray chips are commercially available and a considerable number of mutants have been characterized. In addition, reverse genetics will continue as a powerful tool to examine gene function in *Arabidopsis*.

However, *Arabidopsis* is a dicot and is not a crop plant. On the other hand, rice is both a monocot (and thus may be more closely related to banana) and a crop plant, but is not so well genetically characterized, although the complete sequence of the rice genome will soon be available. Moreover,

many of the experimental features available in *Arabidopsis* are being developed for rice; some of which are already in use (e.g. rice T-DNA knockout lines). The rice genome is relatively small; about 3-4 times larger than *Arabidopsis* (Resink and Buell 2004). Moreover, a number of predicted genes found in rice have homologs in *Arabidopsis* (Rice Chromosome 10 Sequencing Consortium 2003). Another important consideration is the fact that rice, along with other closely related plants, exhibit a relatively high degree of synteny (Gale and Devos 1998).

Since banana genomics is in its infancy with limited sequence information, it is premature to draw conclusions as to a singular comparative strategy and to what degree synteny will be conserved. However, initial studies have been done (Aert *et al.* 2004). Interestingly, comparison of the banana genome structure and organization, derived from preliminary studies of BAC end sequencing, with the ones of rice and *Arabidopsis* suggested that banana may actually be closer to *Arabidopsis* than to rice (Chris Town, personal communication). If this preliminary observation holds up, then banana is positioned in a rather unique place, a monocot with more affinity to dicots than other monocots.

Gene transfer across species

The *Arabidopsis* NPRI gene is a well-characterized central player in regulating systemic acquired resistance (SAR). When overexpressed in *Arabidopsis*, enhanced disease resistance occurs. To evaluate the role of NPRI in monocot plants, the *Arabidopsis* gene was overexpressed in rice and transgenic plants were challenged with the rice bacterial blight pathogen *Xanthromonas oryzae* pv. *oryzae*. Transgenic plants exhibited enhanced levels of resistance, although not as pronounced as the resistant cultivar (Chern *et al.* 2001). These studies indicated that a dicot gene can be expressed and confer a useful phenotype in a monocot and suggests that monocot and dicot plants share a conserved pathway that mediates resistance.

The identification and characterization of major resistance genes (R genes) is an important, active field of investigation, and banana is no exception (McDowell and Woffenden 2003, James Dale personal communication). Monocot R genes appear to be in the CC (coiled coil), NBS (nucleotide binding site) and LRR (leucine rich-repeat) structure class as opposed to the TIR (toll-like inverted repeat) NBS LRR that predominates in dicots, including *Arabidopsis* (for a review of R genes, see Martin *et al.* 2003).

Hulbert and colleagues recently described an interesting approach for identifying novel sources of resistance (Zhao *et al.* 2004). This group inoculated a number of maize lines with the bacterium that causes leaf streak of rice. Lines that induced a hypersensitive response (HR) when challenged were identified. This phenotype suggests that these maize plants were able to recognize the rice pathogen. Following crosses, genetic control of the HR segregated as a single dominant locus, suggesting the possibility of a single R gene. The responsible maize gene was map-based cloned and, when inserted into rice, conferred resistance to the bacterial streak disease pathogen (S. Hulbert, personal communication). This is more than just a demonstration, as this disease is very severe in areas (e.g. China) where hybrid cultivars are extensively utilized and are very susceptible to leaf streak. Moreover, these studies suggest that the same genes may be involved in non-host resistance and specific resistance.

Programmed cell death

Multicellular organisms eliminate unwanted, damaged or used cells by a gene-directed programmed cell death (PCD) process. PCD is genetically controlled cellular suicide and plays a critical role 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, autoimmune diseases) 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.

By far, the most common of the cell suicide responses in animal species is “apoptosis”. Apoptosis refers to a constellation of characteristic morphological changes that animal cells typically undergo when dying by activation of the endogenous cell suicide program. The execution of this program is often associated with cell shrinkage, membrane bleeding, nuclear and cytoplasmic condensation, and DNA fragmentation. These DNA fragments coalesce to form membrane-bound apoptotic bodies that, in animals are rapidly phagocytosed and digested by macrophages. In this way, the dead cells are rapidly and cleanly removed and cellular leakage of noxious and possible dangerous contents in avoided.

In contrast, necrosis results from cellular injury, cells swell and lyse, releasing cytoplasmic material, which in animals often triggers an inflammatory response. While the distinction between these two forms of death is not always clear, in necroses, the cell is not an active participant in its demise. Apoptosis is usually also associated with the activation of nucleases that degrade chromosomal DNA into small oligonucleosomal fragments, which when electrophoresed, result in a characteristic “DNA ladder”.

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 (e.g. Bcl-2, Bcl-xl, CED-9, IAP) or pro-apoptotic proteins (e.g. Bax, Bid, caspases), which do battle with each other in making life-death decisions for the cell. Ectopic overexpression of certain types of anti-apoptotic genes can render animal cells markedly resistant to a wide range of cell death stimuli, including nutrient deprivation, irradiation, cytotoxic chemicals, hypoxia and disease (Navarre and Wolpert 1999).

Programmed cell death in plants

In plants, programmed cell death plays a normal physiological role in a variety of processes, including (a) deletion of cells with temporary functions, such as the aleurone cells in seeds and the suspensor cells in embryos; (b) removal of unwanted cells, such as the root cap cells found in the tips of elongating plant roots and the stamen primoridia cells in unisexual flowers; (c) deletion of cells during sculpting of the plant body and formation of leaf lobes and perforations; (d) death of cells during plant specialization, such as the death of tracheary element (TE) cells; and (e) leaf senescence (Dickman and Reed 2003). Regulation of cell death pathways also occurs in response to abiotic stimuli (Jones and Dangl 1996). In some cases, cell suicide programmes are also activated during pathogen attack in both resistant and susceptible plant-pathogen interactions (Beers 1997, Mitsuhara *et al.* 1999).

Though the biochemical mechanisms responsible for cell suicide in plants are largely unknown, a variety of reports suggest similarities to the programmed cell death that occurs in animal species. For example PCD in plants typically requires new gene expression, and thus can be suppressed by cycloheximide and similar inhibitors of protein or RNA synthesis (Dickman and Reed 2003). The morphological characteristics of plant cells undergoing PCD also bear some striking similarities to apoptosis in animals, though the presence of a cell wall around plant cells imposes certain differences. Akin to animal cells, PCD in plants is associated with internucleosomal DNA fragmentation (DNA ladders) and the activation of proteases (Ryerson and Heath, 1996, Solomon *et al.* 1999).

In addition to its role in developmental processes in plants, cell suicide plays an important role in interactions of plants with a variety of pathogens, including bacteria, fungi and viruses (Mittler and Lam 1996). One of the best studied of these plant responses to pathogens is the hypersensitive response (HR). Upon exposure to certain pathogens, plant cells in the immediately affected area undergo a rapid cell suicide response that is theoretically intended to kill the cells near the site of infection, thereby limiting spread of pathogens. The HR is associated with the expression of a variety of plant defense genes and the induction of programmed cell death. The HR is usually preceded by rapid and transient responses, including ion fluxes, alterations

in protein phosphorylation patterns, pH changes, changes in membrane potential, release of reactive oxygen species (ROS), and oxidative cross-linking of plant cell wall proteins (Richberg *et al.* 1998).

Although plant cell suicide (HR) may be effective in limiting the spread of certain viruses, bacteria, and fungi (in particular, those with a biotrophic lifestyle), it is counterproductive for limiting necrotizing pathogens that utilize the decaying cell corpse as a food base in the case of certain bacteria and fungi. For example, hallmark features of apoptosis have been observed in plants (during compatible interactions) that are sensitive to toxin-producing necrotrophic fungi, including *Fusarium moniliforme* (fumonisin), *Alternaria alternata* (AAL toxin), and *Cochliobolus victoriae* (victorin) (Navarre and Wolpert 1999, Piedras *et al.* 1998). Thus, plant programmed cell death can accompany both susceptible and resistant reactions, suggesting common biochemical pathways during both interactions.

Engineering resistance to pathogens

Proof-of-concept experiments indicate that it is possible to genetically engineer plants for pathogen resistance without interfering with normal programmed cell death responses needed for plant development. For example, Mitsuhara *et al.* demonstrated that the expression in tobacco of cytoprotective Bcl-2 family of proteins from humans (Bcl-X_L) and nematodes (CED-9) resulted in increased cellular resistance to UV irradiation and paraquat. Work in my lab has provided further evidence indicating that Bcl-2 proteins function in plants. Transgenic tobaccos were generated harboring various anti-apoptotic proteins including human Bcl-2, chicken Bcl-X_L, nematode CED-9 and baculovirus Op-IAP (Dickman *et al.* 2001). When the necrotrophic fungal pathogen *Sclerotinia sclerotiorum*, which has an extremely broad host range (more than 400 species), was inoculated onto tobacco plants harboring these transgenes, the usually susceptible plants became highly tolerant and in most cases, completely resistant. Eventually, the fungus stops growing, presumably after having depleted its nutritional source, and, importantly, the fungus fails to colonize and infect transgenic plant tissue even with extended incubation. Similar results occurred with other necrotrophic fungi including *Botrytis cinerea* and *Cercospora nicotianae*.

A unifying aspect of these results with the fungi tested is that all three fungal pathogens are necrotrophs; thus, these fungi require host plant cell death to grow, colonize and reproduce in the host milieu. In the case of the broad host-range pathogen *S. sclerotiorum*, it has been assumed that this aggressive, indiscriminate pathogen with an impressive arsenal of destructive enzymes and toxins simply overwhelms plants. Our data suggest that this interaction is more sophisticated; the pathogen specifically interacts with the plant by triggering host cell death pathways. Inhibition of this pathway, presumably by anti-apoptotic gene products, prevents fungal infection even though the fungus has its full complement of virulence factors. Thus, necrotrophic pathogens may co-opt plant host cell death pathways for successful colonization and disease development. Redirection of plant cell death pathways by necrotrophic pathogens may be essential for disease development to occur.

Still, these results do not prove that plants and animals share common features of apoptosis. However, when *S. sclerotiorum*

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The model plant Arabidopsis *whose genome has been sequenced*

was inoculated onto wild type tobacco, DNA fragmentation was observed in the form of characteristic “ladder”, a common feature of apoptotic responses. Further, fungal induced DNA fragmentation was detected by terminal deoxynucleotide transferase-mediated dUTP end labeling (TUNEL) of DNA 3'-OH groups which also indicated the presence of apoptotic bodies. Importantly, when transgenic plants were inoculated with *S. sclerotiorum*, not only were the plants resistant, but there was no laddering nor were there any TUNEL positive cells. In addition, experiments with tobacco mosaic virus shows that in N gene mediated resistance, the resulting HR exhibits TUNEL reacting tobacco cells and that in transgenic tobacco containing anti-apoptotic genes, cell death (HR) is suppressed. Thus, we have evidence for apoptotic pathways being involved in both susceptible and resistant plant responses. Induction of these pathways is dependent on the genetics of the host/pathogen and the life style of the pathogen. Moreover, we have recently demonstrated that these same transgenic plants are tolerant to heat, cold, salt and drought (Li and Dickman 2004). Importantly, these data clearly suggest that homologous pathways are operative in plants and animals.

We are currently in the process of generating transgenic bananas harboring these anti-apoptotic genes. The two major fungal diseases of bananas (black leaf streak and Fusarium wilt) both fit the conceptual framework for disease control; in other words, there are necrotrophic fungi. Thus we are cautiously optimistic that the transgenic bananas will exhibit tolerance/ resistance. We will also evaluate such lines for tolerance to abiotic stresses (heat, cold, salt, drought).

Conceivably, enormous opportunities exist for using animal models of programmed cell death to dissect cell death pathways in plants. Such information can lead to a mechanistic understanding of the regulation of plant cell death, an area that is not well understood and is of fundamental importance for plant biology. Thus understanding and eventual exploitation of cell life/death pathways in plants can be used for protection of banana against pathogens and environmental stresses.

References

Aert R., L. Sagi. & G. Volckaert. 2004. Gene content and density in banana (*Musa acuminata*) as revealed by genomic sequencing of BAC clones. Theor. Appl. Genet. 109:129-139.

Beers G.P. 1997. Programmed cell death during plant growth and development. Cell Death and Differentiation 4:649-661.

Chern M.-S., H.A. Fitzgerald, R.C. Yadov, P.E. Canalas, X. Dong & P.C. Ronald. 2001. Evidence for a disease-resistance pathway in rice similar to the NPR1 - mediated signaling pathway in *Arabidopsis*. Plant J. 27:101-113.

Dickman M.B. & J.C. Reed. 2003. Paradigms for Programmed Cell Death in Animals and Plants. Pp. 26-43 *in* Programmed Cell Death in Plants (J. Gray, ed). Blackwell Publishing, UK.

Dickman M.B., Y.K. Park, T. Oltersdorf, W. Li, T. Clemente & R. French. 2001. Abrogation of disease development in plants expressing animal anti-apoptotic genes. Proc. Nat’l. Acad. Sci. 98:6957-6962.

Gale M.D., & K.M. Devos. 1998. Comparative genetics in the grasses. Proc. Nat’l. Acad. Sci. 95:1971-1974.

Jones A.M. & J.L. Dangl. 1996. Logjam at the Styx: programmed cell death in plants. Trends in Plant Science 1:114-1109.

Li W. & M.B. Dickman. 2004. Abiotic stress induces apoptotic-like features in tobacco that is inhibited by expression of human Bcl-2. Biotech. Letters 26:87-95.

Martin G.B., A.J. Bogdanove & G. Sessa. 2003. Understanding the functions of plant disease resistance proteins. Annu. Rev. Plant Biol. 54:23-61.

McDowell J.M. & B.J. Woffenden. 2003. Plant disease resistance genes: recent insights and potential applications. Trends in Biotechnology 21:178-183.

Mitsuhara I., K.A. Malik, M. Miura & Y.Ohashi. 1997. Animal cell-death suppressors Bcl-x(L) and Ced-9 inhibit cell death in tobacco plants. Curr. Biol. 9: 775-778.

Mittler R. & E. Lam. 1996. Sacrifice in the face of foes: pathogen-induced programmed cell death in plants. Trends in Microbiol 4:10-15.

Navarre D.A. & T.J. Wolpert. 1999. Victorin induction of an apoptotic/senescence-like response in oats. Plant Cell 11:237-249.

Pennell R.I. & C. Lamb. 1997. Programmed cell death in plants. Plant Cell 9:1157-1168.

Piedras P., K.E. Hammond-Kosack, K. Harrison & J.D.G. Jones. 1998. Rapid Cf9 and Avr-dependent production of active oxygen species in tobacco suspension cultures. Mol. Plant Micro. Interact 11:1155-1166.

Rensink W.A. & C.R. Buell. 2004. *Arabidopsis* to rice. Applying knowledge from a weed to enhance our understanding of a crop species. Plant Physiol. 135: 622-629.

Rice Chromosome 10 Sequencing Consortium. 2003. In depth view of structure. Activity and evolution of rice chromosome 10. Science 300:1566-1569.

Richberg M.H., D.H. Aviv & J.L. Dangl. 1998. Dead cells do tell tales. Curr. Opin. Plant Biol. 1:480-488.

Ryerson D.E. & M.C. Heath. 1996. Cleavage of nuclear DNA into oligonucleosomal fragments during cell death induced by fungal infection or by abiotic treatments. Plant Cell 8:393-402.

Solomon M., B. Belenshi, M. Delledonne, E. Menachem & A. Levine. 1999. The involvement of cysteine proteases and protease inhibitor genes in the regulation of programmed cell death in plants. The Plant Cell 11:431-444.

Zhao B.Y., E. Ardales, E. Bresset, L.E. Claflin, J.E. Leach & S.H. Hulbert. 2004. The Rxo1/Rba1 locus of maize controls resistance reactions to pathogenic and non-host bacteria. Theor. Appl. Genet. 109:71-79.

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Diseases and pests: A review of their importance and management

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Diseases and pests are increasingly limiting factors in smallholder and export production, and can cause catastrophic losses (Jones 2000a). Diseases are the reason breeding programs were established in Trinidad, Jamaica, Honduras and Nigeria, and have been cited as a primary reason for the creation of INIBAP (Buddenhagen 1993). It is most appropriate that a session of this meeting is devoted to these production constraints.

Musa diseases and pests are significant problems worldwide. Diseases affect all portions of the plants, are caused by fungi, bacteria and viruses, and have been the subjects of entire books (Jones 2000a, Stover 1972, Wardlaw 1961). Pests, although of an overall lower importance, are nonetheless serious production factors in their own right (Gold *et al*. 2001, Gold *et al*. 2002, Gowen and Quénéhervé 1990). This short review lists the most important of these problems and concludes with a discussion of some current issues.

The major diseases

Fungal diseases

Diseases that are caused by fungi are most common and destructive (Jones 2000). Leaf spot diseases caused by species of *Mycosphaerella* result in moderate to severe damage wherever significant rainfall occurs (Jacome *et al*. 2003). Black leaf streak disease, better known as black Sigatoka and caused by *Mycosphaerella fijiensis*, is most important. It occurs throughout the humid, lowland tropics and has a wide host range that includes the Cavendish subgroup (AAA) and plantains (AAB). In some areas, eumusae leaf spot, caused by *Mycosphaerella eumusae*, Sigatoka, caused by *Mycosphaerella musicola*, and speckle, caused by *Mycosphaerella musae*, are equally or more important. Fusarium wilt, caused by *Fusarium oxysporum* f. sp. *cubense* (Foc), is a lethal and widespread problem on this crop (Ploetz and Pegg 2000). It devastated the export trade that depended on 'Gros Michel' AAA until ca. 1960. A recently recognized variant, tropical race 4 (TR4), affects Cavendish cultivars and threatens export and smallholder production of it and many other cultivars outside its endemic, Southeast Asian range. Of serious but lesser concern are: the leaf spot diseases cladosporium speckle, caused by *Cladosporium musae*, and freckle, caused by *Guignardia musae*; the post-harvest problems anthracnose and crown rot, caused primarily by *Glomerella musae*; and root rots caused by *Cylindrocladium*/*Calonectria* spp. (Jones 2000b, Jones 2000c, Muirhead and Jones 2000, Ploetz *et al*. 2003a).

Bacterial diseases

Bacteria cause several types of diseases, the most significant of which are vascular wilts (Thwaites *et al*. 2000). With the exception of the Philippines, Moko, caused by race 2 of *Ralstonia solanacearum*, is restricted to the Western Hemisphere. It has eliminated the highly susceptible 'Bluggoe' (ABB) in many production areas in the west. In contrast, blood disease, caused by a *Ralstonia* sp.