Summary of IS-MPMI Meeting, July 1999, Amsterdam

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The 9th International Congress of Molecular Plant-Microbe Interactions was held July 25–30, 1999, in Amsterdam, The Netherlands, with more than 1,000 attendees. Due to the information-packed sessions and the more than 700 posters, attendance at local beer and wine cafes was seriously reduced. Scientific developments reported were substantial, but may be categorized as more evolutionary than revolutionary. Modern genomics approaches were surprisingly not much in appearance, with very little genome sequencing or array data presented. Similarly, the plant-microbe field has not fully employed proteomics or protein structure approaches. However, a clear and welcome trend from discovery by genetics toward biochemical and cell biological approaches was evident. Due to the deluge of information presented in posters and oral talks, we will summarize only some of the outstanding findings presented at the Congress, and cannot be comprehensive. Regrets to colleagues for omissions.

J. Dangl (University of North Carolina) set the tone for the meeting with a summary of our understanding of plant recognition of pathogens and recent results from his own laboratory. He stressed the need for more refined mutant searches with *Arabidopsis* and the need for infusion of biochemical approaches to understand interactions between pathogen avirulence proteins and host plant proteins. Dangl's work extends previous evidence that three different *Pseudomonas* avirulence gene proteins are functional in plant cells when delivered either by the bacterial type III *hrp* secretion system or from T-DNA. He provided evidence suggesting that myristoylation of these three Avr proteins at the N terminus is required for their function as both avirulence and virulence factors. These proteins are localized to the plant plasma membrane. Dangl reported results indicating that it may be possible to use genetics to identify the presumed virulence targets of Avr proteins in plants. He also presented evidence that a host protein can drive excision of a pathogenicity island from the chromosome of *P. syringae* and into a plasmid.

The cloning and characterization of disease resistance genes is continuing rapidly. R. Michelmore (University of California, Davis) suggested that, based on an analysis of plant sequence data bases, the number of leucine rich repeat (LRR) resistance genes in rice may exceed 1,700! J. Jones (Sainsbury Laboratory, UK) discussed the hypervariable LRR domains of disease resistance genes and described experiments suggesting that recognition of the Avr9 avirulence protein from *Cladosporium fulvum* by *Cf9* tomato plants requires at least one additional plant factor. P. Dodds and colleagues (CSIRO Division of Plant Industry, Australia) reported that genes from the P and N flax rust resistance loci have been cloned and sequenced. He also discussed the variable regions of the LRR

domains of the *L* family of allelic resistance genes. B. Staskawicz (University of California, Berkeley) described the cloning by his group of the *Bs2* resistance gene from pepper and showed that it functions in tomato to confer resistance to *Xanthomonas campestris* pv. *vesicatoria*. Notably, however, he observed that the *Bs2* gene does not function in *Arabidopsis*, lettuce, or cotton plants, a finding that may temper enthusiasm for the introduction of cloned resistance genes into a wide array of plants.

The first cloned resistance genes targeting nonspecific elicitors (bacterial flagellin proteins and fungal elicitors) were reported by T. Boller (Friedrich Miescher-Institut, Switzerland) and B. Tyler (University of California, Davis), respectively. G. DeLorenzo (University of Rome, Italy) showed that the polygalacturonase inhibitor proteins of bean, which are associated with the plant cell wall, are LRR proteins and similar in some respects to resistance gene products. Further, she reported that the inhibitor proteins interact with methylated pectin and lipoxygenases and that minor alterations in the sequence of the inhibitor protein can drastically alter the spectrum of polygalacturonases recognized. B. Valent (DuPont Agricultural Enterprise, DE) described exciting recent work with yeast two-hybrid and far western experiments indicating that the AvrPita avirulence protein of *Magnaporthe grisea* and the corresponding Pi-ta disease resistance protein of rice directly interact. Along with immunoprecipitation work by R. Leister and F. Katagiri (University of Maryland and Novartis, CA), these are the first experimental demonstrations that avirulence proteins and LRR resistance proteins may directly interact for initiation of resistance. M. Joosten (Wageningen Agricultural University, The Netherlands) presented data indicating that the *Cladosporium fulvum* Avr4 protein has a specific binding site in *Cf4* tomato plants, but the binding protein has not yet been isolated.

The groups of V. Williamson (University of California, Davis) and I. Kaloshian (University of California, Riverside) showed that the *Mi* disease resistance gene in tomato confers resistance not only to root knot nematodes but also to an aphid. In addition, the Kaloshian group has used mutagenesis experiments to identify a second tomato gene that is required for *Mi* function. B. Baker (USDA and University of California, Berkeley) showed that the P50 fragment of tobacco mosaic virus (TMV) replicase is the elicitor of *N* gene-mediated defense responses. She also reported results of a powerful genetic screen for *N* gene transgenic tomato plants and the isolation of mutants that did not exhibit TMV resistance.

Considerable progress in intracellular signaling during plant defense was reported at the Congress. X. Dong (Duke University, NC) isolated a suppressor of NPR signaling in *Arabidop-*

sis and showed that it encodes a novel protein with weak homology to a retinoblastoma, presumably a transcription suppressor. In conjunction with the group of P. Ronald (University of California, Davis), the Dong group also showed that rice plants carry an NPR homolog and reported that it interacts in the yeast two-hybrid system with two other rice proteins. Several groups reported on the role of MAP (mitogen-activated protein) kinases. D. Scheel (Institute of Plant Biochemistry, Halle, Germany) identified three parsley MAP kinases that were activated by phosphorylation and the group of J. Jones (Sainsbury Laboratory, UK) showed that a calcium-dependent kinase in tomato may be important, as determined by viral-induced gene silencing. D. Klessig (Rutgers University, NJ) reported that NO signals plant defense by activating G proteins and opening calcium channels. Furthermore, as in animals, aconitase in tobacco is affected by NO.

Programmed cell death (PCD) was an important part of the meeting. The groups of D. Gilchrist (University of California, Davis) and E. Lam (Rutgers University, NJ) reported that expression of the baculovirus P35 gene, an inhibitor of PCD in animals, functions in transgenic plants and reduces the severity of some but not all plant diseases. Most notably, the P35 gene did not confer resistance to powdery mildew fungus, perhaps to be expected since it is a biotrophic pathogen. C. Lacomme (Scottish Crop Research Institute, UK) further showed that expression of the BAX gene, which induces PCD in animals, also induces localized cell death in tobacco. All of these results imply that PCD is similar in animals and plants and may be harnessed for disease control.

Phytoalexins have long been thought to be an important part of active defense in plants against pathogens. At this meeting, W. Broekaert (Katholieke Universiteit Leuven, Belgium) reported that the *pad3* mutant in Arabidopsis, shown earlier to block phytoalexin synthesis (J. Glazebrook, Novartis, CA), does not affect resistance to *Botrytis* spp. but drastically reduces resistance to *Alternaria* spp. These results increase the legitimacy of phytoalexins as a major part of the plant defense arsenal. Preformed plant toxic compounds have likewise been seen as important for resistance to pathogens. A. Osbourn (Sainsbury Laboratory, UK) summarized her elegant work demonstrating that only fungal strains that produce a specific detoxifying enzyme can attack oat plants containing the saponin avenacin. The Osbourn group is also currently making progress in identifying enzymes important in the biosynthesis of this preformed compound.

D. Baulcombe (Sainsbury Laboratory, UK) presented promising results from the use of viral-induced gene silencing (VIGS) to reduce expression of targeted plant genes. This rapid technique is anticipated to have major applicability in assessing the role of particular plant genes in defense. Several papers addressed mechanisms involved in the local and systemic movement of plant viruses in plants. Y. Okinaka and co-workers (Kyoto University, Japan) identified plant proteins that interact in the yeast two-hybrid system with the brome mosaic virus coat protein and demonstrated that the central region of coat protein is required for long-distance viral movement. R. Beachy (Danforth Center, MO) reported details of virus movement involving the movement protein of TMV. This work was particularly impressive because cell biological techniques were used to study movement protein and virus localization.

Advances were also reported in the development of transgenic plants for disease control. S. Beer (Cornell University, NY) and colleagues showed that expression of the *Erwinia amylovora hrpN* gene in plants shows promise for improved resistance. Y. Nishizawa and colleagues (National Institute of Agrobiological Resources, Japan) showed that rice plants transgenic for chitinase or glucanase genes showed enhanced resistance to rice blast. All of these results indicate that the promise of transgenic plants for disease control may be close to realization.

Progress is rapidly being made on characterizing fungal plant pathogens. K. Mendgen (Universität Konstanz, Germany) described the identification of specific amino acid and hexose transporters in haustoria of the bean rust fungus. We are beginning to understand the basis of these specialized fungal structures for nutrient uptake from host cells. J. Kronstad (University of British Columbia, Canada) reported the isolation of elements in the cyclic AMP (cAMP) signaling pathway for activation of pathogenicity genes in *Ustilago maydis*. R. Kahmann (University of München, Germany) reported that, in addition to cAMP signaling, an independent pheromone signaling pathway involving MAP kinases is also required in *U. maydis*. The two signaling cascades activate Prf1, which in turn transcriptionally activates a large number of fungal genes involved with pathogenic development.

J. Taylor (NRC Plant Biotechnology Institute, Canada) and several other groups reported the characterization of ABC transporters in fungal pathogens. These transmembrane proteins have different specificities for metabolites transported out of the cell in an ATP-dependent manner. Some of the transporters work to detoxify plant preformed compounds or phytoalexins, and as such may be important virulence factors. Characterization of host-selective toxins produced by fungal pathogens also has progressed. T. Tsurushima and S. Mayama (Kobe University, Japan) presented evidence indicating that production of a toxin by crabgrass-specific strains of *Magnaporthe grisea* permitted them to attack that host plant. T. Gaffney (Novartis, NC) introduced a model fungal pathogen, *Ashbya gossypii*, with a genome of only about 8.8 Mb, and two innovative approaches being used by his group to identify targets for fungicide therapy.

Recent progress has occurred in the study of nematode pathogens. For example, the group of J. Bakker (Wageningen Agricultural University, The Netherlands) reported that *Globodera rostochiensis* produces a pectate lyase and, of great potential interest, the nematode also secretes a mitogenic factor (perhaps a peptide) that may be involved in the galling phenotype. Several groups also reported on advances in the use of biological control bacteria in applied settings. These included L. Thomashow (USDA and Washington State University) on further characterization of antifungal metabolite production by various *Pseudomonas* strains. F. O'Gara (University of Ireland, Cork) reported that homocysteine lactone-mediated quorum sensing by *Pseudomonas fluorescens* strains is required for the production of antifungal compounds.

Progress continues to be rapid with bacterial plant pathogens. A. Collmer (Cornell University, NY) reported on additional characterization of the type III *hrp* gene secretion system in *Pseudomonas syringae*. Evidence is now strong that this system introduces certain bacterial avirulence proteins, via *hrp* pili, directly into plant cells. Collmer also presented

data indicating that the signature for *hrp* secretion may be the structure of the 5' terminus of the mRNA, as recently observed in *Salmonella* spp. Several groups, including the Collmer group and those of I. Barash (Tel Aviv University, Israel), J. Murillo (Universidad Pública de Navarra, Spain), and J. Mansfield (Wye College, UK), characterized pathogenicity islands in bacterial pathogens analogous to those in mammalian pathogens. B. Kunkel (Washington University, MO) reported that the *avrRpt2* gene of *Pseudomonas syringae* may also have a virulence function through suppressing plant defense. In addition, the group reported the isolation of more than 40 new and unknown genes that are expressed specifically in the plant host, through the use of in vivo expression technology. The group of M. Van Montagu (University of Gent, Belgium) reported on recent work with the bacterial gall pathogen *Rhodococcus fascians*. These results indicate that the pathogen makes a previously uncharacterized cytokinin as well as a mitogenic factor that may be involved in the galling and stunting phenotypes observed in infected plants. Finally, the group reported results suggesting that the bacteria may directly invade plant cells.

Recent developments in *Agrobacterium* research were highlighted in a series of four presentations. E. Nester (University of Washington) reviewed the role of *Agrobacterium* chromosomal genes in the transformation process. Among the chromosomal virulence genes discussed were several, including *chvB*, *chvG*, and *chvI*, that have homologues in the animal pathogen *Brucella abortus*. Mutation of these genes rendered both pathogens nonpathogenic. Other *Agrobacterium* talks stressed the role of the plant in crown gall tumorigenesis. S. Gelvin (Purdue University, IN) discussed the involvement of a plant phosphatase in regulating, by phosphorylation/dephosphorylation, the nuclear import of VirD2 protein and, hence, the VirD2/T-strand complex. He also described Arabidopsis T-DNA insertion mutants that were resistant to *Agrobacterium* transformation (*rat* mutants). His laboratory has identified and cloned numerous plant genes involved in bacterial attachment (including an arabinogalactan protein gene) and T-DNA integration (including histone H2A and *myb*-like transcription factor genes).

P. Hooykaas (Leiden University, The Netherlands) described experiments that shed light on the role of the VirF protein in plant transformation. Using a yeast two-hybrid system, he showed interaction of VirF with Arabidopsis Skp1 proteins. Because Skp1 proteins are part of complexes involved in targeted proteolysis and entry of the cell into S phase, he suggested that VirF might help stimulate plant cells to divide and become more susceptible to transformation. The development of in vitro nuclear import and T-DNA integration systems was discussed by A. Ziemienowicz and B. Hohn (Friedrich Miescher Institut, Switzerland). Research with HeLa and tobacco BY2 cells indicated that both VirD2 and VirE2 proteins are important for nuclear targeting of the T-strand.

The new results on rhizobial and mycorrhizal symbioses combine molecular, cellular, and genetic studies, and have broadened from the previous focus on Nod factors. Genetic studies in model plant species have paid off with the first molecular identification of a plant gene required for nodulation: *nin1*, a transposon-tagged gene in *Lotus japonicus*, cloned and identified as a putative transcription factor that may have a

membrane spanning domain (J. Stougaard, University of Aarhus, Denmark). H. Franssen and T. Bisseling (Wageningen Agricultural University, The Netherlands) and D. Cook (Texas A&M University) and colleagues have used the synteny of *Medicago* spp. and *Pisum* spp. to focus on a genetic region likely to harbor the sym-2 locus of *Pisum* spp. that controls recognition of particular Nod factor structures. High density maps of *M. truncatula* have been constructed by taking advantage of extensive natural polymorphisms (T. Huguet, INRA-CNRS, Toulouse, France).

Phenotypes of plant mutants are helping dissect early stages of response in *M. truncatula* and *L. japonicus* (C. Gough, J. Denarie, et al., INRA-CNRS, Toulouse, France; K. Szyglowski and F. DeBruijn, Michigan State University; V. Penmetsa and D. Cook, Texas A&M University). Promoter trap screens have revealed both nodule-specific and developmentally interesting *L. japonicus* genes (H. Spaik, Leiden University, The Netherlands). The early cellular responses of root hairs to Nod factors include ionic fluxes in *M. sativa*, which are spatially defined for specific segments of the cell (H. Felle, University of Giessen, Germany; A. Kondorosi, CNRS Gif-sur-Yvette, France). Calcium spiking is present in at least five species of wild-type plants, and is specifically blocked in certain non-nodulating plant mutants (R. Wais and S. Long, Stanford University, CA). At least some of these mutants are also blocked in an oxidative burst occurring in the first minutes to hours (S. Ramu and D. Cook, Texas A&M University). Early plant gene expression can be followed by reporter fusions, and pharmacological interventions suggest likely secondary signals downstream of Nod factor perception (D. Barker, INRA-CNRS, Toulouse, France).

While there is not yet identification of a plant receptor that transduces a signal in response to Nod factors, studies have advanced on definition of Nod factor binding proteins. G. Stacey (University of Tennessee) reported characterization of a chitin binding site in soybean, and cloning and expression studies of a *M. truncatula* apyrase type protein. A. Niebel and J. Cullimore (INRA-CNRS, Toulouse, France), reported the apyrases are a multi-gene family in *M. truncatula*; they have cloned and tagged these for assay of Nod factor binding. A particulate fraction with Nod factor binding activity shows specificity for the reducing end structure (J. Bono and J. Cullimore, CNRS, Toulouse, France). Host chitinases are clearly diverse; these may play a role in early stages of response and/or may tie the symbiosis response to developmental and pathogenesis strategies (M. Schultze, University of York, UK; M. Holsters, University of Gent, Belgium).

Bacterial signals continue to be revealed by genetic and biochemical approaches. On the Nod factor front, I. Lopez-Lara and O. Geiger (Technical University of Berlin, Germany) showed that the function of NodG is likely to be as an ACP/keto-acyl-reductase. Quorum sensing is likely to be as important in *Rhizobium* spp. populations as for other prokaryotic organisms. There appear numerous genetic systems for production and response, and several distinct species of quorum sensing molecules, in *R. leguminosarum* bv. *viciae* and in *R. etli* (F. Wisniewski-Dyé and J. A. Downie, John Innes Centre, UK; V. Rosekeyer and J. Vanderleyden, K. U. Leuven, Belgium). Plants themselves may produce compounds with activity on quorum sensing systems (W. D. Bauer, Ohio State University), although with distinct chemistry.

Bacterial genetics continues to reveal loci with physiological responses that suggest environmental and/or symbiotic roles. Numerous genes responding to microaerobic conditions have been characterized in *S. meliloti* (J. Trzebiatowski and F. de Bruijn, Michigan State University). Genomics should be a rich future field, as shown by the regulation studies already made possible by the sequence of the NGR234 Sym plasmid (C. Marie, X. Perret, and W. Broughton, University of Geneva, Switzerland). The complete genome of *Sinorhizobium meliloti* is being determined by a multi-national group (INRA, Toulouse, France, on-line site: currently <<http://sequence.toulouse.inra.fr/meliloti.html>>) and a random 1-X pass sequence data base has been released (Stanford University, on-line site: currently <<http://cmgm.stanford.edu>>). Other projects are also beginning.

While a substantial portion of early nodulation can be accounted for through Nod factor effects, it is equally clear that much of nodule development never occurs in the absence of bacterial invasion. For example, it appears that the plant commitment to establish a nodule requires a certain degree of infection, which can be revealed by timed rescue of conditional auxotrophic infection mutants (K. D. Noel, Marquette University, WI). How invasion occurs in symbiosis, and what plant and bacterial components are required, is a fast-growing research area.

The bacterial surface is known to be important for infection, although the function is not known. An active fragment of a specific size can be defined for the *Sinorhizobium meliloti* EPS-I molecule (B. Pollock, H. Cheng, and G. Walker, Massachusetts Institute of Technology). A number of posters from several groups likewise point to an important role for lipopolysaccharides (LPS), possibly at more than one stage in development. Given that both plant and bacterial carbohydrates are important for symbiosis, it is intriguing that bacteria secrete cellulose-digesting glycanases that are active only when in contact with something on the bacterial surface (J. A. Downie, John Innes Centre, UK). Other bacterial genes are probably also involved: an in vitro expression search (IVET) revealed genes that appear to be expressed in either infection or early differentiation (V. Oke and S. Long, Stanford University, CA). A critical gene for bacterial differentiation is *bacA*, which has been shown to be conserved and required for pathogenesis in *Brucella* spp. (K. LeVier and G. Walker, Massachusetts Institute of Technology). This finding may point to some fundamental signaling process between prokaryotes and eukaryotic hosts.

An enduring mystery on the plant side is what the plant contributes to infection thread structure and growth. N. Brewin and colleagues (John Innes Centre, UK) characterized a 110-kDa plant glycoprotein found in infection threads, MGP, that corresponds to a gene product in the extensin category of cell wall proteins. MGP can be insolubilized via oxidative cross linking; physiological and biochemical evidence suggests diamine oxidase and/or oxalate oxidase as plausible pathways for generating peroxide in the right place and time. The growth of infection threads is highly dynamic: P. Van Spronsen and J. Kijne (Leiden University, The Netherlands) reported a correlation of the differentiation state of cells with the width and morphology of the infection threads that pene-

trate them. Several laboratories are hot on the track of cytoskeleton rearrangements that may correlate with formation of successful infection events within plant cells (K. Vanden-Bosch, Texas A&M University; A. Emons, Wageningen Agricultural University, The Netherlands; L. Cárdenas, UNAM, Cuernavaca, Mexico; H. Spaink, Leiden University, The Netherlands).

Overall nodule development likely exploits the plant's own programs for development and for cell division and function. E. Kondorosi and colleagues (CNRS, Gif-sur-Yvette, France) discovered ccs52, a *Medicago* nodule-specific homologue of WD-repeat cell division inhibitors, that may mediate the transition from meristematic activity to differentiation in the plant. Other putative transcription factors include a likely homeobox domain protein in *Lotus* nodules (M. Grönlund and E. Østergaard, Aarhus University, Denmark). M. Djordjevic (Australian National University, Australia) used diverse markers of cell activity to show that nodules forming in the mature region of the root appear to do so by recruiting cells activated by being adjacent to emerging lateral roots, possibly revealing an ancestral pathway for nodule development. It is likely that plant programs involve manipulation of synthesis, transport, or sensitivity to known plant hormones such as auxin (H. Spaink, Leiden University, The Netherlands; C. Grosjean, University of Warwick, UK) and possibly gibberellin (M. Holsters, University of Gent, Belgium), as well as a possibly morphogenic plant gene product, ENOD40 (M. Crespi, CNRS, Gif-sur-Yvette, France; H. Imaizumi-Anraku and M. Kawaguchi, University of Tokyo, Japan; H. Franssen, Wageningen Agricultural University, The Netherlands). Finally, plants control their nodulation via systems that prevent formation of excess nodules. The endogenous plant signals that down-regulate nodules can be provoked without a full nodulation signal (T. Van Brussel, Leiden University, The Netherlands), and act at an early stage prior to responses such as ENOD11 expression (E. Journet and D. Barker, INRA-CNRS, Toulouse, France).

The mycorrhizal symbioses, with different morphology and broader host range, are being analyzed via plant as well as microbial genetics and genomics. Ectomycorrhizal EST array analysis has shown symbiosis-regulated sequences (F. Martin, INRA, Nancy, France). Clones encoding putative phosphate transporters have been obtained both from the endomycorrhiza *Glomus* and its host *Medicago truncatula*; expression studies suggest that some are involved in constitutive host functions (M. Harrison, Noble Foundation, OK). Mutant studies of *M. truncatula* and of *L. japonicus* hosts have confirmed that some common host functions are required for *Rhizobium* spp. and mycorrhizal symbioses (M. Harrison, Noble Foundation, OK; M. Parniske, Sainsbury Laboratory, UK); the nature of the mycorrhizal infection process is being dissected by analysis of *Lotus* mutants, some of which show defects at specific morphological stages of infection (M. Parniske, Sainsbury Laboratory, UK; P. Bonfante, CNR, Torino, Italy). Natural diversity has provided another set of tools in the form of near-isogenic lines of *Trifolium* with distinct mycorrhizal behaviors (J. Webb, Institute of Grassland and Environmental Research, UK). Direct genetic study, combined with the molecular advances made possible by new cloning initiatives, should be highly informative about the details of the cellular interaction.