Session 7 - Biotic Interactions

(P7-1) Structural diversity and evolutionary relationships of Gpa2/Rx1 homologues in potato

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Sequence analysis was carried out on the C-termini of fourteen highly similar members of an R gene family in potato. The fourteen sequences were derived from five different haplotypes, and include the two virus resistance genes Rx1 and Rx2, and the potato cyst nematode resistance gene Gpa2. Apart from these three functional R genes, cDNA was obtained for two other members. Using gDNA and cDNA sequences in a multiple sequence alignment enabled us to identify intron splice sites and putative open reading frames for ten members. Splice site positions appeared to be conserved, while intron sizes were variable. The acidic tail, consisting of two direct sequence repeats is present in Rx1 and Rx2, but absent in Gpa2. For seven members, only one repeat of the acidic tail was observed and in three members the acidic tail was absent. Comparative sequence analysis of all Gpa2/Rx1 homologues revealed that most sequence variation occurs in the LRR
b-strand/b-turn motifs. Ka/Ks ratio analyses indicated that these motifs are also under stronger positive selection pressure than the complete C-terminal part of the genes. Comparing the Gpa2/Rx homologues revealed a patchwork of sequence identities for both paralogues and orthologues suggesting an evolutionary history that involved unequal crossing-over and gene conversion.

(P7-2) The regulation of mycorrhiza-specific P transport in solanaceous species

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Str_Abstract: Arbuscular mycorrhizae represent ancient symbioses thought to have originated more than four hundred million years ago in the roots of plants pioneering the colonization of terrestrial habitats. In these associations, a key process is the transfer of phosphorus as inorganic phosphate to the host plant across the fungus-plant interface. Mycorrhiza-specific phosphate transporter genes, and their regulation are conserved in phylogenetically distant plant species and are selectively activated by fungal species from the phylum Glomeromycota (1). The potato phosphate transporter gene StPT3 (2) is expressed in a temporally defined manner in root cells harbouring various mycorrhizal structures, including thick coiled hyphae (1). The results contrast with the general assumption that arbuscules are the major site of phosphorus transfer and indicate that cell-cell contact between the symbiotic partners is required to induce phosphate transport. To obtain information on the spatial and temporal profile of StPT3 expression, a reporter system based on the Fluorescent Timer, a mutant form of the DsRed fluorescent protein from the coral Discosoma, was used (1). The Fluorescent Timer protein shifts fluorescence color from green to red over time due to slow fluorophore maturation (3). The rate of color conversion has been reported to be independent of protein concentration and therefore can be used to trace time-dependent expression. Overall, the study of phosphate transport mechanisms and respective gene regulation will further our understanding of the intimate interaction between the two symbiotic partners.

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A phosphate transporter expressed in arbuscule-containing cells in potato. Nature 414, 462-470.

(3) Terskikh, A., Fradkov, A., Ermakova, G., Zaraisky, A., Tan, P., Kajava, A. V., Zhao, X.N., Lukyanov, S., Matz, M., Kim, S. *et al.*, 2000.
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(P7-3) QTLs for resistance to the cyst nematode Globodera pallida, deriving from Solanum sparsipilum mapped in resistance gene clusters

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The potato cyst nematode Globodera pallida is one of the most serious potato pests in temperate climates. From economical and environmental points of view, breeding for resistant cultivars is the most desirable method of control. Polygenic resistances to G. pallida were found in potato wild species. We analyzed the resistance originating from S. sparsipilum by a QTL approach. A diploid progeny of 239 clones issued from crosses between S. tuberosum Caspar H3 (susceptible) and S. sparsipilum spl329.18 (resistant) was genotyped with 20 AFLP primer combinations, four CAPS and five SSR markers. Maps of the two parents were constructed according to the double pseudo-test cross design. Resistance assessment was performed by artificial tests on four tubers per clone. Tubers were grown for 4 months in pots filled with soil infested with 10 cysts of G. pallida. Newly formed cysts were visually counted. Two QTLs were detected by Composite Interval Mapping on the chromosomes V (GpaVsspl) and XI (GpaXIsspl) with the resistant alleles originating from spl329.18. GpaVsspl had a major effect (R^2=76.6%) and mapped in the GP21-GP179 resistance gene cluster where major genes or QTLs affecting resistance to viruses, Phytophthora infestans, and potato cyst nematodes have been previously mapped. GpaXIsspl had a minor effect (R^2=12.7%) and mapped on the chromosome XI in the same cluster as the resistance genes R3, R6, R7 against P. infestans and the resistance QTL Gro1.3 from S. spegazzinii against potato cyst nematode G. rostochiensis.

(P7-4) Breeding for resistance to Tobacco Rattle Virus.

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Research at SCRI has clarified the role of weeds and potatoes in the epidemiology of this virus. Potato responses to the virus, including true susceptible genotypes that exhibit fully systemic infections, genotypes with strong hypersensitive-type responses that exhibit classical 'spraing' symptoms in the tuber flesh and a third group of genotypes that appear to exhibit complete resistance to the virus.
The most effective and sustainable approach is the use of heritable plant resistance. Recent research at SCRI examining a tetraploid S. tuberosum population derived from a Record (resistant) x Wilja (susceptible) cross indicates that resistance in the potato cultivar Record appears to be conferred by a single resistance gene. AFLP markers linked to this resistance have been identified. A further population derived from a Pentland Dell (spraing symptoms) x Wilja (susceptible) cross has now been analysed using AFLP markers. The results indicate the role of a separate major gene associated with the incidence of spraing symptoms in the tuber flesh.

(P7-5) A Plant EPF-type Zinc-Finger Protein, CaPIF1, Involves in Defense Against Pathogen Attack

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To better understand the defense responses of plants to pathogen attack, we challenged hot pepper plants with bacterial pathogens and identified transcription factor–encoding genes whose expression patterns were altered during the subsequent hypersensitive response. One of these genes, CaPIF1 (Capsicum annuum Pathogen-Induced Factor 1), was characterized further. This gene encodes a plant-specific EPF-type protein that contains two Cys2/His2 zinc fingers. CaPIF1 expression was rapidly and specifically induced when pepper plants were challenged with bacterial pathogens to which they are resistant. In contrast, challenge with a pathogen to which the plants are susceptible only generated weak CaPIF1 expression. CaPIF1 expression was also strongly induced in pepper leaves by the exogenous application of ethephon, an ethylene-releasing compound, whereas salicylic acid and methyl-jasmonate had only moderate effects. CaPIF1 localized to the nuclei of pepper protoplasts expressing a CaPIF1-smGFP fusion protein. Transgenic tobacco plants overexpressing CaPIF1 driven by the CaMV 35S promoter showed increased resistance to challenge with a tobacco-specific bacterial pathogen. These plants also showed constitutive upregulation of multiple defense-related genes. Moreover, virus-induced silencing of the CaPIF1 ortholog in Nicotiana benthamiana enhanced susceptibility to fire blight pathogen of tobacco. These observations provide the first evidence that an EPF-type Cys2/His2 zinc-finger protein plays a crucial role in the activation of the pathogen defense response in plants.

(P7-6) Molecular analysis of Solanaceae wild species for resistance genes

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In Solanaceae family several resistant genes (R-genes) have been mapped and cloned. Exploring the natural potential of these genes is highly desirable. In fact pathogens are continually becoming resistant to existing resistant genes and pesticides. The wild Solanaceae species represent an important source of resistance genes. Numerous genes for resistance to important pests and diseases have been already identified and characterized in such germplasm. The aim of the present work was to genotype various Lycopersicon and Solanum accessions for molecular diversity in six resistance genes. In particular, 71 accessions belonging to 12 Solanum and 7 Lycopersicon species were tested with specific primers designed using sequences of resistance genes: Pto a serine-threonine protein kinase conferring resistance to isolates of Pseudomonas syringae, Gro1 a TIR/NBS/LRR protein conferring resistance against Globodera rostochiensis and four CC/NBS/LRR proteins conferring resistance against Phytophthora infestans (R1 and RB), the plant vascular disease caused by Fusarium oxysporum f sp. lycoprersici race 2 (I2) the tomato spotted wilt virus (Sw-5) respectively.
Amplified fragments obtained with specific primers differed for number and size. R1primers amplified fragments ranging from 200 bp to 1500 bp, Gro1 primers bands ranging from 390 bp to 700 bp and I2 primers bands ranging from 250 bp to 450 bp. Polymorphic sites potentially differentiating homologues genes were recognised through restriction enzyme analysis of PCR products for Pto and Sw-5. Comparison of orthologous sequences to investigate phylogenetic relationships between Solanaceae resistance genes is in progress.

(P7-7) A high-resolution map of the H1 locus harbouring resistance to the potato cyst nematode Globodera rostochiensis

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The resistance gene H1 confers resistance to the potato cyst nematode Globodera rostochiensis and is located at the distal end of the long arm of chromosome V of potato. For marker enrichment of the H1 locus a bulked segregant analysis (BSA) was carried out using 704 AFLP primer combinations. A second source of markers tightly linked to H1 is the ultra-high density (UHD) genetic map of the potato cross SH´RH. This map has been produced with 387 AFLP primer combinations and consists of 10,365 AFLP markers in 1,118 BINs (www.dpw.wageningen-ur.nl/uhd/). Comparing these two methods revealed that BSA resulted in one marker/cM and the UHD map in four markers/cM in the H1 interval. Subsequently, a high-resolution genetic map of the H1 locus has been developed using a segregating F1SH´RH population consisting of 1209 genotypes. Two PCR based markers were designed at either side of the H1 gene to screen the 1209 genotypes for recombination events. In the high-resolution genetic map, two of the four co-segregating AFLP markers could be separated from the H1 gene. Marker EM1 is located at a distance of 0,2 cM and marker EM14 is located at a distance of 0,8 cM. The other two co-segregating markers CM1 (in coupling) and EM15 (in repulsion) could not be separated from H1.

(P7-8) Molecular genetic characterisation of the I-3 region of tomato chromosome 7

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Fusarium wilt is a spectacularly damaging vascular wilting disease of tomato caused by the fungus Fusarium oxysporum f. sp. lycopersici (FOL). The disease is both a significant horticultural problem and a useful model system for studying vascular pathogens. FOL race 3 arose in Queensland and Florida in the late 1970s and is now a problem in many tomato-growing areas. Resistance to FOL race 3 was identified in the wild tomato species Lycopersicon pennellii and bred into the cultivated tomato L. esculentum. We are using the extensive genetic resources available for tomato in attempt to isolate the L. pennelli I-3 gene for resistance to FOL race 3 by map-based cloning. Existing molecular markers and known genes mapping to the I-3 region were converted to PCR based Sequence Characterised Amplified Region (SCAR) and Cleaved Amplified Polymorphic Sequence (CAPS) markers, and Radiolabelled Amplified Fragment (RAF) markers mapping to this region were developed de novo. A map of the I-3 region was generated using a set of overlapping L. pennellii introgression lines and an F2 population segregating for FOL race 3. These enabled identification of molecular markers close to I-3 suitable for marker-assisted breeding and fine mapping. A resistance gene analogue (RGA) and a RAF marker were found to cosegregate with resistance. The RGA was cloned and sequence analysis showed that it could not correspond to I-3. Bacterial artificial chromosome (BAC) libraries of tomato genomic DNA were screened with the RGA and RAF markers to construct a BAC contig of the I-3 region. We are further characterising the I-3 region to develop better markers for I-3 and to isolate the gene itself.

(P7-9) Pathogen-Responsive Genes in Hot Pepper Non-Host Resistance Against Soybean Pustule Pathogen

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Modern biotechnologies are expanding our view on plant biology from analyzing individual components of biological system to integrating entire genetic programs and complex mechanisms. Information generated by large -scale genomic sequencing and cDNA micro-array has led to a major revolution in plant biology through revelation of all the genes required to encode major life forms and monitoring of gene expression at the genomic level. Although the entire genomic sequence of Arabidopsis is available, sequencing and analysis of cDNA sequences (ESTs) remains invaluable, especially in other plant species with large genome size. Although hot peppers are getting reputation as an important source of vitamins, medicine and many other areas, consumption and cultivation is being increased in the world, so little attention has been given to the hot pepper plants at the molecular level. Because of the large genome size of hot pepper (2.7 -3.3 X 109 bp), the EST sequencing is one of plausible approach for initial characterization of hot pepper genome structure. Furthermore, the EST clones are being used as a good source of gene expression analysis for isolation of useful genes or understanding of unique biological system. The selected ESTs are microarray deposited onto a glass slide followed by scoring hybridization signal with RNA from target and reference sample. This method is a powerful tool to measure the differential expression of massive genes, simultaneously. Here we present practical application of EST sequencing and cDNA micro-array technology for isolation of defense-related useful genes and understanding of plant defense mechanism(s).

(P7-10) Domain swapping to identify sequences required for induction of a hypersensitive response by the disease resistance genes encoded HRT and RPP8 proteins in Arabidopsis

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HRT encodes a protein with coiled-coil regions (CC), a nucleotide binding site (NBS), and leucine-rich repeats (LRR) that confers hypersensitive response (HR) against Turnip crinkle virus (TCV). The NBS and LRR domains in HRT are present in many disease resistance genes in plants and in regulators of apoptosis in animals. To investigate structure-function relationships of HRT, 11 chimeras have been made by swapping of domains between the HRT and its paralog, RPP8, which confer resistance to Peronospora parasitica Emco5. Outside of the LRR region, HRT and RPP8 proteins share 98% amino acid identity while their LRR regions are less conserved (87% identity). The ability of these chimeric proteins to induce an Avr-dependent HR to TCV was then assessed using an Agrobacterium-mediated transient expression assay. Co-expression of the HRT and its elicitor, the TCV coat protein (CP), results in rapid cell death. Following infiltration into N. bentahamiana leaves, three constructs elicited an HR (HRds1, HRds3, and HRds10). The chimeric construct HRds10, in which LRRs 8-14 of RPP8 were exchanged with the corresponding sequences from HRT, induces cell death in the absence of viral coat protein. We also generated transgenic Col-0 plants expressing the 11 chimeric constructs under control of CaMV 35S promoter or HRT promoter. Further analyses with Arabidopsis transgenic plants are in progress.

(P7-11) Identification and Characterization of Putative Transcription Factors Involved in Nonhost Resistance in Hot Pepper Plants

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To investigate the molecular mechanisms of non-host resistance in pepper, hot pepper plants (Capsicum annuum cv. Bukang) were inoculated with non-host pathogen (Xanthomonas axonopodis pv. glycines 8ra: Xag8ra) and genes that are specifically induced or down-regulated during hypersensitive response (HR) were isolated by cDNA microarray analysis. cDNA microarrays containing 4,685 unigene clones were analyzed by hybridization using the labeled cDNA probes derived from nonhost pathogen infected and uninfected pepper leaves. This cDNA microarray approach allowed identification of 88 putative transcription factors, of which were isolated as being up- and down-regulated more than two fold ratio upon Xag8ra infection. Thirty five clones, which were selected from rapid reverse northern analysis, were carried out Northern blot analysis. This resulted in the isolation of 2 clones whose corresponding mRNA levels in leaves was significantly up-regulated upon Xag8ra inoculation. Nucleotide sequence analysis showed that these clones had homology with scarecrow and AT-hook transcription factors. Expression analysis of these genes during various pathogen and abiotic stresses treatments was performed. Overexpression and VIGS experiment will be conducted in tomato microtom plant and Nicotiana.benthamiana respectively.

(P7-12) Identification of potato cyst nematode Globodera rostochiensis factors involved in the H1-dependent incompatible plant-nematode interaction

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The H1 locus of potato confers resistance to the potato cyst nematode G. rostochiensis and is the only nematode resistance gene with a proof for a gene-for-gene interaction. The H1 resistance response triggers a localized hypersensitive reaction (HR) within the cells surrounding the feeding site shortly after its initiation. It is postulated that this response occurs due to the H1-dependent recognition of a nematode elicitor, most likely secreted by either the dorsal or subventral glands or by the amphids. Two G. rostochiensis lines (line 19 and line 22) have been previously described to differ in their (a)virulence; line 19 is avirulent on potato genotypes harboring the H1 locus, while line 22 is virulent. In order to identify nematode interacting components, cDNA libraries are being produced from avirulent line 19 J2 infective stage ‘heads’ and ‘tails’. To ensure a good library quality in spite of the low-efficient procedure of material collection, the T7 polymerase mRNA amplification system is used. Using a suppressive subtractive hybridization method, common cDNA clones will be removed from the ‘head’ cDNA library by subtracting the ‘tail’ cDNA library. Random cDNA clones of the resulting ‘head’ cDNA library will be then cloned into a binary vector and expressed in leaves of the resistant and susceptible potato clones SH and RH, respectively, using Agrobacterium tumefaciens transient transformation assay (ATTA). Observation of an H1-dependent HR can be the first step to the isolation and further analysis of putative nematode avirulence factors.

(P7-13) The Rpi-blb2 gene from Solanum bulbocastanum is allelic to the Mi-1 gene from tomato and confers broad spectrum resistance to Phytophthora infestans both in cultivated potato and tomato

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The necessity to develop potato and tomato crops that possess durable resistance against the oomycete pathogen Phytophthora infestans is increasing as more virulent, crop-specialized and pesticide resistant strains of the pathogen are rapidly emerging. Isolation of genes that code for resistance from wild Solanum species and their subsequent introduction as cisgenes into existing varieties could be a means of exploiting potentially durable late blight resistance present in the Solanum gene pool. Here we describe the positional cloning of the Solanum bulbocastanum derived Rpi-blb2 gene, which confers resistance to complex isolates of P. infestans. The Rpi-blb2 locus was initially mapped in several tetraploid back-cross populations, derived from highly resistant complex interspecific hybrids designated ABPT, to the same region on chromosome 6 as the Mi-1 gene from tomato. Due to suppression of recombination in the tetraploid material, further fine mapping was carried out in a diploid intraspecific S. bulbucastanum F1 population. Bacterial artificial chromosome (BAC) libraries, generated from a di-haploid ABPT derived clone and from the resistant S. bulbocastanum parent clone, were screened with markers linked to resistance in order to generate a physical map of the Rpi-blb2 locus. Molecular analyses of both ABPT and S. bulbocastanum derived BAC clones spanning the Rpi-blb2 locus showed it to harbour 14 Mi-1 gene analogues (MiGA). Of these, 5 were genetically determined to be candidates for Rpi-blb2. Complementation analyses showed that one ABPT- and one S. bulbocastanum-specific MiGA were able to complement the susceptible phenotype in both S. tuberosum and tomato backgrounds. Sequence analyses of both genes showed them to be identical. The Rpi-blb2 protein shares 82% sequence identity to the Mi-1 protein, which in tomato confers resistance to root knot nematodes (Meloidogyne spp), aphids (Macrosiphum euphorbiae) and whiteflies (Bremisia tabaci).

(P7-14) A transgenic petunia system to screen for regulatory mutants in mycorrhiza-specific phosphate transport

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Phosphorus (P) is a major limiting macronutrient for plant growth. Although P may be abundant in soil, high sorbing capacity for P, P mineralization and fixation of P in organic soil matter result in low availability of the soluble orthophosphate (Pi), which is preferred by plants. Petunia belongs to one of more than 80% of vascular plant species that form a symbiosis with arbuscular-mycorrhizal (AM) fungi enabling the plant to access Pi far beyond the root-soil interface, while the fungus receives plant carbohydrates.
We have identified a plant Pi transporter, StPT3, which is involved in Pi transfer across the fungus-root interface in the AM symbiosis. Recent results showed that the StPT3 gene is inducible by fungi from the phylum Glomeromycota, and that signal perception and transduction mechanisms leading to mycorrhiza-specific Pi transporter gene expression are conserved in evolutionary distant plant species. In order to screen for mutants in the signaling cascade of mycorrhiza-dependent Pi transport, petunia W115 plants were transformed with a bi-directional promoter construct with both the GUS and the luciferase reporter gene under the control of the StPT3 promoter. These are presently being used to generate a mutant population by crossing with petunia line W138 containing the non-autonomous transposable element dTph1. Mycorrhized transgenic plants lacking GUS activity will be putative mutants of receptors or positive regulators in mycorrhiza-specific Pi transport. Non-mycorrhized plants expressing constitutive luminescence are likely to harbor mutations in repressors of StPT3 promoter activity. An analysis of the transgenic W115 petunia system will be presented.