



**RELATIONSHIP BETWEEN PH, PECTOLYTIC ENZYMES AND PATHOGENESIS RELATED (PR) PROTEINS ACTIVITY DURING THE WHEAT-FUSARIUM CULMORUM INTERACTION.** M.P. Aleandri, P. Magro and G. Chilosi. *Dipartimento di Protezione delle Piante, Università degli Studi della Tuscia, Via S. Camillo de Lellis, 01100, Viterbo, Italy. E-mail: chilosi@unitus.it*

The variation of ambient pH by *Fusarium culmorum* during growth on mineral medium and in apoplastic fluids from wheat infected seedlings and its influence on the production and activity of pectolytic enzymes and the pathogenesis related (PR) proteins  $\beta$ -1,3-glucanase, chitinase and peroxidase were detected. Fungal development on mineral medium led to a pronounced alkalisation reaching values above 8.0. The increase in ambient pH was accompanied by the enhancement of total pectin lyase activity and number of isoenzymatic forms and was associated with the secretion of ammonia. Similarly, in apoplastic fluids from inoculated seedlings the concomitant ammonia accumulation and rise in pH were recorded. This trend was accompanied by the increase in pectin lyase which therefore could function close to the optimal pH condition. Polygalacturonase was detected as a single isoenzyme only during the early fungal growth. The time course in the 24-48 h interval post infection was characterised by an increase in activity and isoenzymatic differential induction of the selected PRs. Peroxidase was strongly affected by pH. The enzyme activity had the maximum rate at pH 6.0 and thereafter it rapidly declined at higher pH. Maximum peroxidase activity paralleled the appearance of the complete isoenzymatic pattern. Present results suggest that during infection of wheat seedlings by *F. culmorum* the pH modulation favours host colonisation by enhancing the activity of pectin lyase, and simultaneously inhibits the capacity of the host to oppose to the pathogen by interfering with peroxidase, which represents an important component of the defence arsenal.

**INHIBITORY PROPERTIES OF CARNATION (*DIANTHUS CARYOPHYLLUS* L.) FLAVONOIDS TOWARDS *FUSARIUM OXYSPORUM* f. sp. *DIANTHI*.** F. Galeotti<sup>1</sup>, L. Gaggero<sup>1</sup>, M. Dolci<sup>2</sup>, C. Pasini<sup>1</sup> and P. Curir<sup>1</sup>. <sup>1</sup>CRA, Unità di Ricerca per la Floricoltura e le Specie Ornamentali, Corso Inglesi 508, 18038 Sanremo, Italy. <sup>2</sup>Dipartimento di Valorizzazione e Protezione delle Risorse Agroforestali (DiVaPRA), Università degli Studi di Torino, Via L. Da Vinci 44, 10095 Grugliasco, TO, Italy. E-mail: p.curir@istflori.it

Five different flavonoids, among which two new molecules, have been isolated from many carnation (*Dianthus caryophyllus* L.) cultivars, i.e. an apigenin-C-glycoside (CF1), and four kaempferol glycosides, bringing four (CF2), three (CF3) and two (CF4 and CF5) sugar units, respectively. With the aim of elucidating the possible role of these compounds in the pathogenic interactions carnation-*Fusarium oxysporum* f. sp. *dianthi* (*Fod*), the effect on the fungal growth was evaluated under *in vitro* experiments. The biological assays were performed by using the five above mentioned compounds at 35, 350 and 700 micromolar concentrations, respectively: the biological activity was tested against the three most important *Fod* pathotypes (races 2, 4 and 8). The results show that each fungal pathotype displays a different level of tolerance towards a same compound and that the molecules extracted from constitutively *Fod*-resistant carnation cvs (CF2 and CF3) indeed are effective in hastening the *in vitro* fungal growth, particularly when assayed at the highest concentration. This does not imply that a strict correlation between cultivar resistance level and qualitative composition in flavonoids exists, since these molecules need to accumulate in tissues at the

right time and in suitable amounts to efficiently face fungal infection. The biologically active flavonoids, as a part of a multifactorial resistance system, could, however, contribute to trigger plant defensive reactions against pathogenic fungal attacks.

**A SYSTEMS APPROACH TO CHARACTERISING THE *FUSARIUM OXYSPORUM* – *ARABIDOPSIS* INTERACTION.** D.M. Gardiner<sup>1</sup>, L.F. Thatcher<sup>1</sup>, B. Dalrymple<sup>2</sup>, K. Kazan<sup>1</sup>, L.J. Ma<sup>3</sup> and J.M. Manners<sup>1</sup>. *Commonwealth Scientific and Industrial Research Organisation, <sup>1</sup>Plant Industry, <sup>2</sup>Livestock Industries, 306 Carmody Road, Brisbane, 4067, Queensland, Australia. <sup>3</sup>The Broad Institute of MIT and Harvard, 320 Cambridge, Massachusetts 02141, USA. E-mail: donald.gardiner@csiro.au*

*Fusarium oxysporum* causes vascular wilt disease of many crop plants including economically important crops such as cotton, banana and tomato as well as the model plant *Arabidopsis thaliana*. We are using taking a systems approach in both the host and pathogen to dissect mechanisms of resistance, susceptibility, pathogenicity and virulence. The SALK *Arabidopsis* Homozygote T-DNA collection and 10 000 *F. oxysporum* T-DNA mutants are being screened for altered disease phenotypes. As part of this work a genome sequence of an Australian *F. oxysporum* isolate highly pathogenic on *Arabidopsis* will be obtained using Illumina sequencing technology. This combined systems approach to the *F. oxysporum*-*Arabidopsis* interaction will allow the construction of complementary interacting gene networks in host and pathogen, which has the potential to transform our understanding of this organism with a variety of host plants. Data will be presented on the sequencing of our *F. oxysporum* isolate and on progress of the *F. oxysporum* insertional mutagenesis.

**NMR METABOLOMICS FOR MAPPING METABOLITE VARIATION IN EUROPEAN WHEAT.** S.F. Graham<sup>1</sup>, E. Amigues<sup>2</sup>, M. Migaud<sup>2</sup> and R.A. Browne<sup>1</sup>. <sup>1</sup>Institute of Agri-Food and Land Use, School of Biological Sciences, Queens University Belfast, David Kier Building, Stranmillis Road, BT9 5AG, N. Ireland. <sup>2</sup>School of Chemistry and Chemical Engineering, Queen's University Belfast, David Kier Building Stranmillis Road, BT9 5AG, N. Ireland. E-mail: sgraham20@qub.ac.uk

Nuclear magnetic resonance metabolomics is a powerful new tool for investigating passive disease resistance in wheat germplasm. In this study we investigated if there was sufficient variation between European wheat germplasm to justify a mapping approach. The European wheat cultivars Apache, Charger, Claire and Orvantis were analysed at two growth stages, the coleoptile and two week old leaf tissue extracts. There were substantial metabolite differences among wheat European genotypes and between growth stages. Notably the coleoptile extracts had lower abundances of glutamine, glutamate, sucrose and trans-aconitate and more glucose and fructose than in the two week old leaf tissue extracts. These growth stage differences were consistent across all the cultivars (Apache, Charger, Claire and Orvantis) investigated. Comparing the four cultivars, the cultivars Apache and Charger showed the greatest differences in metabolite profiles. Apache had lower concentrations of betaine, the single most influential metabolite in the principal component analysis, in addition to fructose and sucrose. However Apache had higher concentrations of aspartate, choline and glucose than Charger. These findings demonstrate the potential for a biochemical mapping approach using NMR, across European wheat germplasm, for metabolites known to correlate with disease resistance.



**GENETIC AND PHYSIOLOGICAL CHARACTERIZATION OF THE INTERACTION OF *FUSARIUM OXYSPORUM* f. sp. *MELONIS* RACE 1.2 WITH SUSCEPTIBLE AND RESISTANT MELON PLANTS.** R. Herman<sup>1</sup>, Z. Zvirin<sup>1</sup>, I. Kovalski<sup>1</sup>, Y. Brotman<sup>1</sup>, S. Freeman<sup>2</sup>, Y. Denisov<sup>2</sup>, G. Zuri<sup>3</sup>, N. Katzir<sup>3</sup> and R. Perl-Treves<sup>1</sup>. <sup>1</sup>The Mina and Everard Goodman Faculty of Life Sciences, Bar-Ilan University, Ramat-Gan 52900, Israel. <sup>2</sup>Department of Plant Protection, Agricultural Research Organization, Bet Dagan 50250, Israel. <sup>3</sup>Agricultural Research Organization, Neve Yaar Research Center, P.O. Box 1021, Ramat-Yishay 30095, Israel. E-mail: perl@mail.biu.ac.il

An Israeli melon breeding line, BIZ, that is resistant to all four races of *Fusarium oxysporum* f. sp. *melonis* (FOM) has been characterized regarding its response to FOM race 1.2, and the genetic control of this resistance. Resistance is expressed as a recessive trait controlled by two major recessive genes when severe artificial inoculation is applied, but in the field it appeared as a dominant trait. The infection process of a FOM 1.2 strain that expresses the GFP reporter protein was monitored, suggesting the timing and sites in which fungal progression differs between a resistant and a susceptible genotype. An attempt to relate such progression to defense gene expression is under way. A mapping population derived from the cross PI 414723 and BIZ was used to construct a linkage map with ~90 traits and markers, and QTL analysis of FOM1.2 resistance was initiated. A major recessive QTL for FOM1.2 resistance was located in linkage group 2, near a major locus controlling fruit-net development.

**WHEAT TRANSGENIC PLANTS EXPRESSING A BEAN PGIP SUPPORT A ROLE FOR POLYGALACTURONASE ACTIVITY IN THE INITIAL STAGE OF WHEAT INFECTION BY *FUSARIUM GRAMINEARUM*.** M. Janni<sup>1</sup>, S. Benedettelli<sup>2</sup>, A.E. Blechl<sup>3</sup>, F. Favaron<sup>4</sup>, L. Sella<sup>4</sup>, M. Fagioni<sup>5</sup>, L. Zolla<sup>5</sup> and R. D'Ovidio<sup>1</sup>. <sup>1</sup>Dipartimento di Agrobiologia e Agrochimica, Università della Tuscia, Via San Camillo de Lellis, 01100 Viterbo, Italy. <sup>2</sup>Dipartimento di Scienze Agronomiche e Gestione del Territorio Agro-forestale, Università degli Studi, Firenze, Italy. <sup>3</sup>USDA-ARS, Albany, CA, USA. <sup>4</sup>Dipartimento Te.S.A.F. Università degli Studi di Padova, Via dell'Università 16, 35020 Legnaro, PD, Italy. <sup>5</sup>Dipartimento di Scienze Ambientali, Università della Tuscia, Via San Camillo de Lellis, 01100 Viterbo. E-mail: dovidio@unitus.it

*Fusarium graminearum* is one of the predominant causal agents of Fusarium Head Blight (FHB) of wheat worldwide. This fungal pathogen produces trichothecene mycotoxins, including deoxynivalenol (DON). Transgenic wheat plants expressing the *FsTRI101* gene, which encodes a DON acetyltransferase, were partially protected against the spread of *F. graminearum*. *F. graminearum* also secretes an array of enzymes to degrade cell wall polymers. Since some of these enzymes are controlled by apoplastic inhibitor proteins, we are analyzing the feasibility of increasing host resistance by manipulating the activity of these cell wall components. We report the effect of the ectopic expression of a bean polygalacturonase-inhibiting protein (PvPGIP2) in transgenic wheat plants, alone and in combination with the product of the *FsTri101* gene, in protecting wheat plants against *F. graminearum*. We monitor FHB symptom progression in inoculated heads for 23 days. We show that transgenic plants expressing PvPGIP2 show a significant reduction of FHB symptoms from 9 to 23 dpi. A parallel analysis performed on wheat transgenic lines expressing *FsTri101* showed a delayed protective effect starting from 15 to 23 dpi. We also evaluated the effects of combining the *Pvpgip2* and *FsTRI101* transgenes on the spread of the FHB symptoms. The hybrid lines carrying both transgenes show symptom reductions equal to ei-

ther parental line, but an earlier reduction of FHB symptoms (from 11 to 23 dpi) than the parental line expressing only *FsTRI101*. These results demonstrate that PvPGIP2 can slow *Fusarium* infection and support a role for polygalacturonase activity in the initial stage of colonization.

**COLONISATION OF BARLEY ROOTS WITH DSRED EXPRESSING *FUSARIUM AVENACEUM* AND GFP EXPRESSING *FUSARIUM CULMORUM*-COMPETITION AND EFFECT ON TOXIN PRODUCTION.** T. Johansen<sup>1</sup>, M.N. Grell<sup>1</sup>, J. Åhman<sup>1</sup>, S. Olsson<sup>1</sup>, M. Hansen<sup>2</sup> and H. Giese<sup>1</sup>. <sup>1</sup>University of Copenhagen, Faculty of Life Science, Department of Ecology, Section of Genetics and Microbiology, Thorvaldsensvej 40, 1871 Frederiksberg C, Copenhagen, Denmark. <sup>2</sup>University of Copenhagen, Faculty of Life Science, Department of Plant Biology, Plant Physiology and Anatomy Laboratory, Thorvaldsensvej 40, 1871 Frederiksberg C, Copenhagen, Denmark. E-mail: tjo@life.ku.dk

*Fusarium culmorum* and *Fusarium avenaceum* are infamous for causing root rot, seedling and head blight in cereals. The fungi are producers of mycotoxins that are deposited in the grain. Both fungi are soil borne and studies were carried out to register in real time the colonization process of these fungi in barley roots. Two reporter strains: *F. culmorum* expressing the green fluorescent protein (GFP) and *F. avenaceum* expressing the red fluorescent protein (DsRed) were generated. A sterile root system was used for the application of macroconidia, individually or together to determine any competitive effects. Laser Scanning Confocal Microscopic recordings of *F. culmorum* and *F. avenaceum* hyphae were obtained infecting the junctions of epidermal cells and *F. culmorum* growing inside the epidermal cells. Both fungi grow in the intercellular space and can directly enter living plant cells. The fungi do not appear to be antagonistic, but *F. culmorum* appears to have a faster growth rate than *F. avenaceum* and therefore becomes the dominant species under infection. Secondary metabolite profiles for *F. culmorum*, *F. avenaceum* and the combination of the two species under infection of barley roots were obtained by RP-HPLC. The polyketide aurofusarin was produced by *F. culmorum* under barley root infection, while the same peak was missing in the *F. avenaceum* infection assay. The result was confirmed by qualitative RT-PCR on *F. culmorum* cDNA in single and co-infection on the *PKS12*, *PKS6*, *PKS7*, *PKS8*, *NPS2* and *NPS6* were expressed in *F. culmorum* after 7 days of infection of barley roots.

**A *FUSARIUM GRAMINEARUM* MUTANT THAT IS DEFECTIVE IN AUTOPHAGY HAS AN ALTERED INFECTION PATTERN IN CEREALS.** L. Josefsen<sup>1</sup>, J. Svensson<sup>2</sup>, S. Olson<sup>1</sup> and H. Giese<sup>1</sup>. <sup>1</sup>Department of Ecology, University of Copenhagen, Thorvaldsensvej 40, 1871 Frederiksberg C, Denmark. <sup>2</sup>Department of Plant Biology, Thorvaldsensvej 40, 1871 Frederiksberg C, Denmark. E-mail: lojo@life.ku.dk

Recycling of nutrients is a strategy for fungi to survive the stress and starvation they experience prior to plant infection. Autophagy is one of the primary mechanisms for recycling of resources within fungal mycelia. It is dependent on the vesicular enclosetment of portions of the cytosol resulting in the formation of autophagosomes (double layered vesicles). The autophagosomes in turn fuse with vacuoles, which contain digestive enzymes that degrade the contents of the autophagosome and make nutrients available for new production of proteins and organelles. Autophagy has in other plant pathogens been shown to be essential for the infection process. It is thought that autophagy is nec-

essary for turnover of organic matter and optimizing the energy needed for pathogenicity. Using homologous recombination and an *Agrobacterium*-mediated transformation technique we have produced a *Fusarium graminearum* strain where the *Atg8* gene essential for the autophagic process is interrupted. On nutrient rich media the  $\Delta$ *Atg8*-mutant and the WT show identically growth patterns. However, on low nutrient media the mutant performs poorly compared to the WT, indicating that the mutant has lost the ability to recycle internal resources. Barley infection assays show that the  $\Delta$ *Atg8*-mutant is able to infect plant tissue; however, there is a delay in the time of infection and visible symptoms compared to the WT. In wheat infection assays the infection of  $\Delta$ *Atg8*-mutant is limited to the point of infection.

**EXPLORING RESISTANCE IN WHEAT TO FUSARIUM EAR BLIGHT DISEASE.** S. Lee and K.E. Hammond-Kosack. *Centre for Sustainable Pest and Disease Management, Rothamsted Research, Harpenden, Hertfordshire. AL5 2JQ, UK. E-mail: sarah.lee@bbsrc.ac.uk*

*Fusarium* ear blight, caused principally by the fungal pathogens *Fusarium graminearum* and *F. culmorum*, is a devastating disease of wheat. The problems caused by ear infection are two fold: firstly, shrivelling of grain causes a reduction in yield and quality and secondly, the accumulation in the grain of *Fusarium* trichothecene mycotoxins, primarily deoxynivalenol (DON) and its acetylated derivatives 3-ADON and 15-ADON and nivalenol (NIV), results in a reduction in quality and is a concern for food safety. Control of the disease is difficult. The use of resistant cultivars is now considered to be the best control option. In this project, hexaploid wheat genotypes from around the world have been screened in field trials over two years for resistance to FEB. Harvested grain from the trial was analysed using gas chromatography-mass spectrometry to assess the quantity of DON mycotoxin present. Genotypes which showed reduced disease symptoms and/or mycotoxin accumulation are now being analysed further under controlled environment conditions. The infection biology of the more resistant genotypes is being investigated in two ways. Firstly, the measurement of a DON breakdown product DON-3-glucoside will establish whether the mycotoxin is being broken down *in planta*. Secondly, using transgenic isolates of *F. graminearum* producing the reporter protein  $\beta$ -glucuronidase (GUS), the route of infection and DON mycotoxin production is being further explored.

**IDENTIFICATION OF CANDIDATE GENES FOR THE TOMATO I-3 GENE FOR FUSARIUM WILT RESISTANCE.** G.T.T. Lim<sup>1</sup>, G.P. Wang<sup>1,2</sup>, M.N. Hemming<sup>1,3</sup>, D.J. McGrath<sup>4</sup> and D.A. Jones<sup>1</sup>. <sup>1</sup>*Plant Cell Biology, Research School of Biological Sciences, The Australian National University, Canberra ACT 0200, Australia.* <sup>2</sup>*College of Horticulture, South China Agricultural University, Guangzhou 510642, China.* <sup>3</sup>*CSIRO Plant Industry GPO Box 1600, Canberra ACT 2601, Australia.* <sup>4</sup>*Queensland Department of Primary Industries and Fisheries, Bowen QLD 4805, Australia. E-mail: david.jones@anu.edu.au*

*Fusarium* wilt of tomato is caused by the fungus *Fusarium oxysporum* f. sp. *lycopersici* (Fol). Fol race 3 first appeared in Queensland, Australia in the late 1970s and caused huge yield losses by the 1980s. Resistance against Fol race 3 was identified in the wild tomato species *Lycopersicon pennellii* and bred into the cultivated tomato *L. esculentum*. Our goal has been to isolate the I-3 gene for Fol race 3 resistance from the *L. pennellii* accession

LA716 by map-based cloning. A high-resolution genetic map of the I-3 region was generated through the development and mapping of PCR-based markers using recombinants derived from three mapping populations segregating for I-3. A BAC contig covering the I-3 region was then constructed using BACs from an *L. esculentum* cv. Heinz 1706 library identified by an *in silico* bioinformatic approach. Sequencing of an *L. pennellii* BAC clone containing markers flanking and co-segregating with I-3 revealed no resistance gene-like sequences other than a TIR-NBS-LRR pseudogene. Our focus then shifted to a cluster of five S-receptor like kinase (SRLK) genes and one S-locus glycoprotein (SLG) gene in the I-3 region that might be candidates for I-3. Further development of markers within this gene cluster and the subsequent mapping of five recombination breakpoints within this region enabled the candidates for I-3 to be narrowed down to four genes. Gene complementation constructs for the candidate genes have been developed and transformation of these constructs into susceptible tomato plants is currently being carried out.

**MOLECULAR MECHANISMS DETERMINING FUSARIUM CROWN ROT DISEASE OF WHEAT.** J.M. Manners<sup>1</sup>, A. Stephens<sup>1,2</sup>, D.M. Gardiner<sup>1</sup>, O. Desmond<sup>1,2</sup>, P. Schenk<sup>2</sup>, A. Munn<sup>2</sup> and K. Kazan<sup>1</sup>. <sup>1</sup>*Commonwealth Scientific and Industrial Research Organisation, Division of Plant Industry, Queensland Bioscience Precinct, Brisbane, 4067.* <sup>2</sup>*The University of Queensland, Brisbane 4072, Australia. E-mail: john.manners@csiro.au*

*Fusarium* pathogens cause crown rot disease in wheat which is the major *Fusarium*-incited disease of wheat in Australia and in many other arid regions. Infection of the stem base by *F. graminearum* appears to have three phases comprising, spore germination and the development of a superficial mycelium on the leaf surface, followed by invasion of the outer leaf whorl and migration to the crown, where subsequently extensive necrotrophic colonisation of the crown and inner stem parenchyma tissues occurs. Wheat defence genes are induced in the stem at all stages during infection by *Fusarium* pathogens and defence activation by jasmonate application prior to inoculation reduces disease development. Trichothecene mycotoxins are produced in the crown and can also induce wheat defence gene transcripts, active oxygen production and apoptosis. However, these toxins are probably only functionally important to disease development at late stages of infection because fungal *Tri5* mutants cause similar necrotic crown rot symptoms to wild type, but appear to be impaired in their ability to colonise the crown and stem parenchyma. The reduced growth of the *Tri5* mutants in infected plants is associated with enhanced wheat defence gene induction at this late stage of infection compared to that induced by the wt strain. We are interested in genes in the host and pathogen that function at the early establishment stages of infection and have undertaken gene expression profiling using the wheat and *F. graminearum* Affymetrix chips and we will report on genes that are currently undergoing functional analysis.

**INSIGHT INTO THE MOLECULAR REQUIREMENTS FOR PATHOGENICITY OF FUSARIUM OXYSPORUM f. sp. LYCOPERSICI THROUGH LARGE-SCALE INSERTIONAL MUTAGENESIS.** C.B. Michielse, R. van Wijk, L. Reijnen, B.J.C. Cornelissen and M. Rep. *Plant Pathology, Swammerdam Institute for Life Sciences, University of Amsterdam, The Netherlands. E-mail: michiels@science.uva.nl*

*Fusarium oxysporum* f. sp. *lycopersici* is a soil-borne fungus

that causes vascular wilt disease in tomato by penetrating the plant roots and colonizing the plant xylem vessels. In order to identify genes involved in pathogenicity 10,209 random insertion mutants were generated using T-DNA of *Agrobacterium tumefaciens* as an insertional mutagen. All transformants were screened for loss of pathogenicity on tomato. This led to the identification of 20 non-pathogenic and 86 reduced-in-virulence mutants. The genomic regions flanking the T-DNA were isolated by TAIL-PCR. In total 129 potential pathogenicity genes were identified, several of which were known pathogenicity genes, such as class V chitin synthase, Zn(II)2Cys6 transcription factor FOW2, carbon catabolite derepressing protein kinase SNF1 and mannose-6-phosphate isomerase. Based on the putative function of the proteins identified, several general and specific processes seem to play a role in pathogenicity, e.g. certain metabolic pathways, peroxisome biogenesis and protein mannosylation. Complementation of three insertion mutants confirmed a role for peroxisomes and a probable cell wall mannosidase in pathogenicity.

**AGROBACTERIUM TUMEFACIENS-MEDIATED TRANSFORMATION OF NONPATHOGENIC AND ANTAGONISTIC FUSARIUM OXYSPORUM WITH THE JELLYFISH GENE GFP.** V. Mondello<sup>1</sup>, J.G. Macia Vicente<sup>2</sup>, L. Torta<sup>1</sup> and S. Burrano<sup>1</sup>. <sup>1</sup>Dipartimento SENFIMIZO – Sez. Patologia vegetale e Microbiologia agraria, Università degli Studi, Viale delle Scienze 2 90128 Palermo, Italy. <sup>2</sup>Departamento de Ciencias del Mar y Biología Aplicada, Universidad de Alicante Ap. Correos 99, 03080 Alicante, Spain. E-mail: santella@unipa.it

To study the evolution of the fungal endophytes colonization in host plant tissues, three strains of *Fusarium oxysporum*, isolated from roots of asymptomatic mango trees (*Mangifera indica* L.) with established antagonistic activity towards some soil-borne pathogens, were transformed, via *Agrobacterium tumefaciens*-mediated transformation, with the genes encoding the green fluorescent protein GFP, using the vector pCAMBgfp with an hygromycin resistance cassette. The transformation was carried out according to Bundock *et al.* (1995). Hygromycin-resistant mutants, expressing the GFP, were obtained for all the *F. oxysporum* strains, with an average of 27 mutants for each, within 10 days after transformation. To identify the strains with a stable antibiotic-resistance and GFP expression, all the GFP-mutants were cultivated in PDA+ hygromycin for 30 days. After this selection, the stable strains were further screened by comparison for growth rate and sporulation level with the respective wild types. Only 7 mutant strains with a high level of GFP expression were found to be very similar to the wild ones. To evaluate the colonization ability of the *F. oxysporum* GFP-mutants, inoculation assays are being carried out with barley seedlings.

**FUSARIUM VS. SUGAR BEET: CHARACTERIZATION OF PATHOGENICITY AND COLONIZATION PATTERNS OF DIFFERENT FUSARIUM SPECIES.** E. Nitschke and M. Varrelmann. Institute of Sugar Beet Research, Holtenser Landstr. 77, 37079 Göttingen, Germany. E-mail: varrelmann@ifz-goettingen.de

*Fusarium* infections of sugar beet are well-known in USA but there are only a few reports from European countries thus raising questions towards an understanding of the host-pathogen interaction. Our investigations aimed at determining to what extent sugar beet could serve as a host for colonisation by different *Fusarium* species (*F. culmorum* and *F. graminearum*) known to be pathogenic for cereals and to compare their potential with a

species (*F. oxysporum* from different geographical origin) known to be pathogenic to sugar beet as well as a species (*F. sambucinum*) characterized by its high saprophytic behaviour. Pathogenicity tests towards a *Fusarium*-susceptible sugar beet line were conducted by inoculation with spore suspensions ( $10^5$  spores/ml) of 9-week-old plants in the greenhouse. Clear differences in the aggressiveness of the investigated *F. oxysporum* isolates were observed. North American isolates were most aggressive and resulted in plant death during the cultivation period. French isolates induced typical vascular discoloration, while German isolates did not interfere visibly with the plant development. Sugar beet infection with *F. culmorum*, *F. graminearum* and *F. sambucinum* did not result in distinctive symptom development. Detailed microscopical studies on colonization patterns of the different isolates revealed systemic infections for all analysed pathogenic species, but not for the saprophytic one. The results show the potential of *Fusarium* infections for sugar beet, whereas the implicated risk for mycotoxin accumulation in sugar beet needs further research and is aim of ongoing studies.

**A TRANSPOSON-BASED GENE TAGGING APPROACH IN FUSARIUM CULMORUM.** G. Ortu<sup>1,2</sup>, M. Dufresne<sup>2</sup>, V. Balmas<sup>1</sup>, M.-J. Daboussi<sup>2</sup> and Q. Migheli<sup>1</sup>. <sup>1</sup>Dipartimento di Protezione delle Piante, Unità di Ricerca Istituto Nazionale Biostrutture e Biosistemi, Università degli Studi, Via E. De Nicola 9, 07100 Sassari, Italy. <sup>2</sup>Institut de Génétique et Microbiologie, Université Paris-Sud, F-91405 Orsay Cedex, France. E-mail: qmigheli@uniss.it

*Fusarium culmorum* (W.G. Smith) Sacc. is one of the most common incitants of crown and foot rot of wheat worldwide and is able to produce type B trichothecenes. Aiming at elucidating the mechanisms involved in pathogenicity and mycotoxin production of *F. culmorum*, we have recently started a transposon-based mutagenesis approach to identify genes governing these characters without *a priori* knowledge on their function. We used the *impala* element, which was already shown to transpose in a wide range of Ascomycete species. A collection of 300 revertant strains has been generated and tested for pathogenicity on wheat under glasshouse condition. Following two rounds of wheat assays, 7 mutants, more or less altered in pathogenicity have been identified among 175 strains exhibiting a reinsertion event. To date, one non-pathogenic mutant has been characterised in detail. Loss of pathogenicity results from the insertion of *impala* in a region close to the 5' end of a gene encoding a putative HMG Co-A reductase, a function already described as involved in *F. graminearum* pathogenicity (Seong *et al.*, *Fungal. Genet. Biol.* 43: 34-41, 2006). Complementation of this mutant with the *HMR1* gene of *F. graminearum* PH1 allowed complete restoration of its pathogenicity, thus confirming that this gene in *F. culmorum* is not essential for growth and development, but it is essential for pathogenicity.

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**INTERACTION BETWEEN THE PATHOGENICITY MAPK CASCADE AND THE CATABOLITE REPRESSOR PATHWAY IN THE CONTROL OF CELL-WALL DEGRADING ENZYME ACTIVITY IN FUSARIUM OXYSPORUM.** N. Rispaill and A. Di Pietro. Departamento de Genética, Universidad de Córdoba, Campus de Rabanales, Edificio Gregor Mendel (C5),

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Mitogen-activated protein kinase (MAPK) cascades are conserved eukaryotic signalling pathways that play important roles in cellular processes. The orthologue of the yeast Fus3/Kss1 MAPKs is essential for virulence in plant pathogenic fungi. Thus, mutants of the soilborne fungus *Fusarium oxysporum* lacking the Pathogenicity MAPK Fmk1 are non-pathogenic on tomato plants. The mechanisms underlying MAPK activation and its exact role in plant infection remain unknown. We found that  $\Delta fmk1$  mutants grown on apple slices show a dramatic growth inhibition and lack of fruit tissue maceration compared to wild-type strain, due to a decrease in cell-wall degrading enzymes (CWDE) activity. Plate assays for a number of CWDE revealed a significant reduction in the activity of pectinolytic enzymes in the  $\Delta fmk1$  strain. Since these enzymes are under strict control by glucose repression, we investigated the crosstalk between the MAPK cascade and the catabolite repressor (CreA) pathway. Adding as little as 0.2% (w/v) glucose to the medium completely abolished the ability of the  $\Delta fmk1$  mutant to degrade polygalacturonic acid (PGA). By contrast, the wild-type strain retains its ability to degrade PGA in presence of up to 5% glucose. These results suggest that (i) absence of the Fmk1 MAPK leads to an increased effect of the CreA pathway; (ii) the Fmk1 MAPK cascade competes with the CreA pathway in activation of pectinolytic genes. Currently, we are generating knock-out mutants of components of the pathogenicity MAPK cascade to elucidate the interaction between Fmk1 MAPK cascade and the CreA pathway in the control of CWDE and virulence.

**INTERACTION OF A ROOT-KNOT NEMATODE WITH FUSARIUM WILT RACES 1 AND 4 ON RESISTANT COTTON.** P.A. Roberts and T.R. Mullens. Department of Nematology, University of California, Riverside, CA 92521, USA. E-mail: philip.roberts@ucr.edu

Root-knot nematode (*Meloidogyne incognita*) interacts with *Fusarium wilt* (*Fusarium oxysporum* f. sp. *vasinfectum*) as a disease complex on cotton. In California, mild races of *Fusarium wilt* (races 1 and 2) have predominated and result in wilt symptoms only in cotton fields infested with *M. incognita*. Recently, a virulent strain of wilt (race 4) was identified in fields without apparent nematode infestation. In greenhouse pathogenicity tests, three cotton cultivars (*Gossypium hirsutum* cvs. Acala SJ-2 and Acala NemX, and *G. barbadense* cv. Pima S-7) were challenged with *Fusarium wilt* races 1 and 4, with or without co-inoculation of *M. incognita*. In co-inoculations, race 1 caused the greatest wilt damage on the wilt and nematode susceptible Acala SJ-2, intermediate damage on wilt resistant and nematode susceptible Pima S-7, and least damage on wilt susceptible and nematode resistant Acala NemX. These results indicated nematode resistance was more effective than wilt resistance in suppressing race 1 wilt. In F<sub>1</sub> hybrids with both nematode and wilt resistance, wilt symptoms were suppressed strongly. Race 4 caused severe wilt symptoms on Pima S-7 with or without nematodes, and caused milder wilt symptoms on Acala SJ-2 although the symptoms were greater in the presence of *M. incognita*. On nematode resistant Acala NemX, race 4 caused moderate wilt symptoms without nematodes, and wilt symptoms were even less in the presence of *M. incognita*. Thus *M. incognita* can increase wilt induced by race 4 on cotton, but the effect is cultivar-specific and may be reduced by activation of nematode resistance.

**ROLE OF *FUSARIUM OXYSPORUM* WHITE COLLAR 1 PHOTORECEPTOR ON CAROTENOGENESIS, UV RESISTANCE, HYDROPHOBICITY AND VIRULENCE ON MAMMALIAN HOSTS.** M.C. Ruiz-Roldán<sup>1</sup>, V. Garre<sup>2</sup>, J. Guarro<sup>3</sup>, M. Marine<sup>3</sup> and M.I.G. Roncero<sup>1</sup>. <sup>1</sup>Departamento de Genética, Universidad de Córdoba, Edificio Gregor Mendel, Campus de Rabanales, 14071 Córdoba, Spain. <sup>2</sup>Departamento de Genética y Microbiología, Facultad de Biología, Universidad de Murcia, 30071 Murcia, Spain. <sup>3</sup>Unitat de Microbiologia, Facultat de Medicina y Ciències de la Salut, Universitat Rovira y Virgili, 43201 Reus, Tarragona, Spain. E-mail: ge2rurom@uco.es

Light regulates developmental and physiological processes in a wide range of organisms, including filamentous fungi. *Fusarium oxysporum*, the causal agent of the vascular wilt disease in a wide variety of crops and an emerging human pathogen, contains a putative photoreceptor Wc1, the orthologue to WC-1 of *Neurospora crassa*. Isolation and characterisation of the *wc1* gene revealed that the predicted protein contained all the characteristic domains present in other fungal photoreceptors, including a conserved LOV domain, two PAS dimerization domains, a nuclear localization sequence and a Zn-finger DNA binding domain. *Wc1* is expressed constitutively at very low levels and does not regulate expression of *wc2* gene. Targeted disrupted mutants showed that this gene is involved in formation of aerial hyphae when grown on solid medium under white light. Defects in aerial hyphae development could be related to altered hydrophobicity, since  $\Delta wc1$  mutants showed altered colony surface hydrophobicity under white light, as well as differential expression pattern of the hydrophobin gene *hyd1*.  $\Delta wc1$  mutants were also affected in induction of carotenogenesis by light, indicating that this gene is involved in this process. Additionally, *Wc1* contributes to photoreactivation after UV treatment and controls light induced up-regulation of the photolyase gene *phr1*. Pathotypic behaviour of  $\Delta wc1$  mutants on tomato plants was unaltered, indicating that this gene is dispensable for pathogenicity on host plants. By contrast, mutation of *wc1* impairs virulence on immunodepressed mice. These results demonstrate that light perception in *F. oxysporum* plays important roles in the development and behaviour of this ascomycete fungus.

**INTERPRETATION OF THE REGULATORY MOTIFS OF THE *FUSARIUM OXYSPORUM* f. sp. *RADICIS-LYCOPERSICI* ENDOPOLY GALACTURONASE PG2 GENE PROMOTER IN *SACCHAROMYCES CEREVISIAE*.** M. Salgado<sup>1</sup>, C. Callejas<sup>1</sup>, E. Espeso<sup>2</sup>, A. de las Heras<sup>1</sup>, A. Villalvilla<sup>1</sup>, C. Vázquez<sup>2</sup> and M.T. González-Jaén<sup>1</sup>. <sup>1</sup>Department of Genetics, <sup>3</sup>Department of Microbiology III, University Complutense Madrid, Jose Antonio Novais 2, 28040 Madrid, Spain. <sup>2</sup>Centro de Investigaciones Biológicas, Consejo Superior de Investigaciones Científicas (CSIC), Ramiro de Maetzu 9, 28040 Madrid, Spain. E-mail: tegonja@bio.ucm.es

*Fusarium oxysporum* f. sp. *radicis-lycopersici* (FORL) causes an important disease in tomato characterized by cell wall degradation. The characterization of cell wall-degrading polygalacturonase enzymes and their transcriptional regulation is crucial to gain knowledge on the role of these enzymes in pathogenesis. The objectives of this work were to perform both *in silico* and *in vitro* analyses of the upstream region of the endopolygalacturonase encoding gene *pg2* (GenBank Accession number AF078156) in *Saccharomyces cerevisiae*. This gene is transcriptionally regulated in FORL in response to carbon source and ambient pH. Two CREA and two PaC motifs were present in the 724 bp upstream region of *pg2* gene. This upstream region and shorter fragments were fused with the  $\beta$ -galactosidase reporter gene in a *Saccharomyces cerevisiae* vec-

tor. The induction of the reporter gene was analyzed in response to different carbon sources and the results compared to the induction pattern in *in vitro* cultures of FORL.

**DIALLEL ANALYSIS OF GIBBERELLA STALK ROT (*FUSARIUM GRAMINEARUM*) RESISTANCE IN MAIZE. R. Santiago<sup>1</sup>, L.M. Reid<sup>2</sup>, X. Zhu<sup>2</sup>, A. Cao<sup>1</sup> and R.A. Malvar<sup>1</sup>.** <sup>1</sup>Misión Biológica de Galicia. Spanish Council for Scientific Research (CSIC), Apartado 28, E-36080, Pontevedra, Spain. <sup>2</sup>Eastern Cereal and Oilseed Research Centre, Agriculture and Agri-Food Canada, Central Experimental Farm, Building 99, Ottawa, Ontario, Canada, K1A0C6. E-mail: rsantiago@mbg.cesga.es

Stalk rot of maize caused by *Fusarium* species leads to substantial yield losses every growing season around the world. *Fusarium graminearum* Schwabe is only one of many species responsible for stalk rot, but is considered to possess the highest pathogenicity (the ability to cause disease) and aggressiveness (the amount of disease caused). A diallel cross of 5 inbreds selected from previous evaluations for resistance to *F. graminearum* via the stalk was evaluated. Artificial inoculation was accomplished with a spore suspension injected into the first internode above the uppermost aerial root node. Disease severity ratings, yield, and lodging in the parents, crosses and reciprocals were evaluated in two environments. Analyses of variance and general (GCA) and specific combining ability (SGA) analyses were performed. There were significant differences between inbred lines and hybrids for disease severity ratings. Inbreds CM174 and CO325 were susceptible, whereas inbreds 73405 and 73353 were resistant. It is remarkable the good GCA of the inbred lines 73353 and 73405. The interaction genotype  $\times$  year was significant for yield and lodging, consequently significant differences between hybrids in each year were checked. For lodging is remarkable the negative effect in hybrid combination of the inbred CM174. In summary, GCA and SCA effects were significant for all traits at least in one year. Selection of additive effects based exclusively on inbred lines is not sufficient to confer good resistance and yield to hybrids, additional selection should be carried out among the hybrids to look for favorable dominance effects.

**GENE DISRUPTION APPROACH TO INVESTIGATE THE ROLE OF *FUSARIUM GRAMINEARUM* AND *FUSARIUM VERTICILLIOIDES* POLYGALACTURONASES DURING PLANT INFECTION. L. Sella<sup>1</sup>, A. Tomassini<sup>1</sup>, F. Giacomello<sup>1</sup>, A. Raiola<sup>1</sup>, R. D'Ovidio<sup>2</sup>, W. Schäfer<sup>3</sup> and F. Favaron<sup>1</sup>.** <sup>1</sup>Dipartimento Territorio e Sistemi Agro-Forestali - Gruppo di Ricerca in Patologia Vegetale, Università di Padova, Via dell'Università 16, 35020 Legnaro, Italy. <sup>2</sup>Dipartimento di Agrobiologia e Agrochimica, Università della Tuscia, Via San Camillo de Lellis, 01100 Viterbo, Italy. <sup>3</sup>Department of Molecular Phytopathology and Genetics, University of Hamburg, Biocenter Klein Flottbek, Hamburg, Germany. E-mail: luca.sella@unipd.it

*Fusarium graminearum* and *F. verticillioides* are two important pathogens of cereal species, causing yield and quality losses. These fungi are known to produce polygalacturonase (PG) activity during liquid culture, but the role played by these enzymes during plant infection has not been ascertained yet. In particular, *F. graminearum* secretes two endo-PG isoforms, encoded by the two putative *endo-pg* genes contained in its genome, and *F. verticillioides* secretes an isoform which has been previously characterized together with its encoding gene. In order to establish the role of these PGs in pathogenesis, we have obtained by targeted

homologous recombination the transformation-mediated disruption of their *pg* encoding genes, and the virulence of each knock-out mutant has been therefore evaluated by infecting host plants. Two different strains of *F. graminearum* have been transformed: preliminary infection experiments of wheat plants seem to indicate that both *pg* knock-out mutants maintain the capability to infect wheat, although colonization of spikes appears delayed compared to the wild-type strain. Infection experiments with the *F. verticillioides* *pg* knock-out mutant have been performed on maize seedlings and corn husks: the mutant maintains the capability to infect both tissues, but it shows a clearly evident delay in the progression of symptoms. The demonstration of the importance of *F. graminearum* and *F. verticillioides* PGs in pathogenesis might contribute to develop strategies aimed to increase the resistance of host plants to infection by these pathogens.

**TESTING THE EFFICACY OF AN RNA INTERFERING CONSTRUCT TARGETED AT THE TRICHOHECENE BIOSYNTHESIS GENE *TRI6* IN *FUSARIUM CULMORUM*. B. Scherm<sup>1</sup>, M. Orrù<sup>1</sup>, V. Balmas<sup>1</sup>, E. Azara<sup>2</sup>, T.M. Hammond<sup>3</sup>, N.P. Keller<sup>3</sup> and Q. Migheli<sup>1</sup>.** <sup>1</sup>Dipartimento di Protezione delle Piante, Unità di ricerca Istituto Nazionale Biostrutture e Biosistemi, Università degli Studi, Via E. De Nicola 9, 07100 Sassari, Italy. <sup>2</sup>Istituto di Chimica Biomolecolare del CNR, Sezione di Sassari, Traversa La Crucca 3, Località Balduca, Li Punti, 07040 Sassari, Italy. <sup>3</sup>Department of Plant Pathology, University of Wisconsin, Madison, WI, USA. E-mail: qmigheli@uniss.it

Post-transcriptional regulation of eukaryotic genes through interception and degradation of mRNA is known as RNA silencing. Silencing is initiated when dsRNA is processed into small RNAs, 21-26 nucleotides (nt) in length, by the RNaseIII enzyme Dicer. These small RNAs are then incorporated into silencing-effector complexes, which they guide to complementary nucleic-acid targets. Aim of this study was to test the efficacy of an RNA silencing construct to suppress mycotoxin production in the plant pathogen *F. culmorum* (W.G. Smith) Sacc., incitant of crown and foot rot on wheat. Transformation of a highly virulent strain of *F. culmorum* with IRT containing sequences corresponding to the trichothecene biosynthesis gene *tri6* was achieved by using the hygromycin B resistance gene *hph* as selectable marker in PEG-mediated co-transformation of fungal protoplasts. The pattern of integration indicates that most transformants underwent homologous recombination events with partial deletion of the endogenous *tri6* gene. The *tri6*-specific IRT did not alter physiological characteristics, such as spore production, pigmentation, and growth rate on solid media. Pathogenicity assays, carried out to evaluate whether impairment in deoxynivalenol (DON) production in the *tri6*-IRT strains correlates with a loss of virulence, showed decreased disease indices (20-50%) for 13 of the 22 tested strains. Gene expression profiles of *tri6* and *tri5* after 7 days in culture will be screened by real-time RT-PCR analysis, and production of deoxynivalenol *in vitro* detected by HPLC after extraction of the culture filtrates.

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**GENETIC MAPPING OF THE LOCUS CONFERRING BOTH PATHOGENICITY AND PERITHECIUM DEVELOPMENT BY USING A NATURAL MUTANT OF *FUSARIUM GRAMINEARUM*.**

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*Fusarium graminearum* species complex is the primary pathogen causing Fusarium head blight of wheat and barley in the world. A natural pathogenicity mutant (*F. asiaticum* 0225022) was found in our study on Japanese *F. graminearum* species complex. The symptom of this mutant could not be spread beyond the inoculated wheat spike. Strain 0225022 also could not develop perithecia on carrot agar medium by the knock down method. We obtained progenies from the crossing between a wild type strain (*F. graminearum* s. str. 0407011) and the mutant strain. Pathogenicity and perithecium development of the 40 individual progenies were examined. These traits were completely linked and segregated with a segregation of 1:1 ( $P=0.21$ ,  $\chi^2$ -test), although segregation distortion in some genomic regions was previously reported in *F. graminearum* s. str. x *F. asiaticum* cross. We analyzed the genome of 20 progenies with sequence-tagged markers. Two markers in the chromosome 2 showed close linkage (10 cM) to pathogenicity and perithecium development. Gene survey on the *F. graminearum* genome sequence database indicated that mating type ideomorph (MAT) is present near (ca. 360 or 620 kb-distance) these markers. A PCR-RFLP marker (HS296/ClaI) was developed in MAT1-2-1(FG08893.1) and it showed complete linkage to pathogenicity and perithecium development based on the results of 40 progenies. This suggested that pathogenicity and perithecium development could be lost by natural mutation in the MAT or region close to the MAT.

**THE APPLICATION OF MOLECULAR MARKERS FOR PARTIAL RESISTANCE AGAINST FUSARIUM CROWN ROT IN HEXAPLOID AND TETRAPLOID WHEATS.** M.W. Sutherland<sup>1</sup>, W.D. Bovill<sup>1</sup>, M. Horne<sup>1</sup>, A. Lehmensiek<sup>1</sup>, F. Eberhard<sup>1</sup>, C. Percy<sup>1</sup>, G.B. Wildermuth<sup>2</sup>, S. Simpfendorfer<sup>3</sup> and R. Hare<sup>3</sup>. <sup>1</sup>Centre for Systems Biology, Faculty of Sciences, University of Southern Queensland, Toowoomba QLD 4350, Australia. <sup>2</sup>Queensland Department of Primary Industries and Fisheries, Leslie Research Centre, Toowoomba QLD 4350, Australia. <sup>3</sup>New South Wales Department of Primary Industries, Tamworth NSW 2340, Australia. E-mail: markstuth@usq.edu.au

In most cereal-growing regions of Australia, the principal cause of crown rot in winter cereals is *Fusarium pseudograminearum*, although *F. culmorum* is a significant cause in cooler Southern regions. Analysis of Australian populations to date indicates that while there is variation in aggressiveness, there is no evidence for pathogenic race structure. Using wheat doubled haploid mapping populations incorporating a range of resistance sources (2-49, CPI133814, IRN497, Sunco and W21MMT70), quantitative trait loci (QTL) conditioning partial resistance to crown rot have been identified in seedling and field trials. Different sources of seedling resistance have unique but overlapping combinations of contributing loci, while seedling and field resistance QTL may differ within single host genotypes. Resistance to crown rot in these sources appears to be independent from resistance to head blight, caused by *F. graminearum*. The degree of phenotypic variation contributed by particular loci varies across genetic backgrounds and trial environments, consistent with the epistatic interactions that are detected

by the analysis tools Epistat and QTLNetwork 2.0. Nevertheless, attempts to pyramid resistances from several sources have met with some success. Tetraploid durum wheats are highly susceptible to crown rot under favourable field conditions and the disease is a major constraint on durum production in Australia. While partial resistance has not yet been identified in any durum breeding lines, recent attempts to cross improved resistance from hexaploid sources into tetraploid wheats are encouraging. Currently resistance QTL from hexaploid sources are being analysed for their effectiveness in subsequent generations derived from hexaploid x durum crosses.

**ANALYSIS OF THE INFECTIVE PROCESS OF FUSARIUM OXYSPORUM f. sp. MELONIS IN MELON BY CDNA-AFLP.** K. Szafranska<sup>1</sup>, F. Fusari<sup>2</sup>, L. Luongo<sup>3</sup>, A. Ferrarini<sup>1</sup>, A. Polverari<sup>4</sup>, M. Delledonne<sup>1</sup>, N. Ficcadenti<sup>2</sup> and A. Belisario<sup>3</sup>. <sup>1</sup>Dipartimento Scientifico e Tecnologico, Strada Le Grazie 15, 37134 Verona, Italy. <sup>2</sup>CRA, Unità di Ricerca per l'Orticoltura (CRA-ORA), Via Salaria 1, 63030 Monsampolo del Tronto (AP), Italy. <sup>3</sup>CRA, Centro di Ricerca per la Patologia Vegetale (CRA-PAV), Via C.G. Bertero 22, 00156 Roma, Italy. <sup>4</sup>Dipartimento di Scienze, Tecnologie e Mercati della Vite e del Vino, Villa Ottolini-Lebrecht, 37029 San Floriano di Valpolicella (VR) Italy. E-mail: alessandra.belisario@entecra.it

Several species of *Fusarium* cause wilting on cucurbits, Fusarium wilt of melon is caused by *Fusarium oxysporum* f. sp. *melonis* (Fom) which is one of the most economically important disease worldwide. The most effective control measure to prevent Fusarium wilt is through host resistance. Four races of Fom are presently known (0, 1, 2, and 1,2), one of which, race 1,2, is able to overcome the resistance of commonly cultivated varieties. No genes have been identified in melon that confer high levels of resistance to race 1,2. A transcriptomic approach was undertaken by cDNA-AFLP on melon plants cv. Charentais *Fom*-2 infected with race 1 (avirulent) and race 1,2 (virulent), at 2, 4, 8 and 21 days after inoculation. RNA from fungal colonies of the two races was also included into the analysis, to improve the identification of possible fungal transcripts expressed in the host during infection. A combination of 128 primers was used. Cluster analysis brought to differentially expressed bands which were selected for the following expression profiles: (i) genes modulated in the incompatible interaction or (ii) in the compatible interaction only; (iii) genes modulated in both interactions with different profiles; (iv) genes expressed in plant, but showing a band of similar size also in the fungal samples, which might be of fungal origin. cDNA fragments were eluted from the gels, sequenced, and searched for homology in databases. Few differences in gene expression have been detected between virulent and avirulent race grown in culture, which represent the basis for races 1 and 1,2 characterization.

**THE EFFECT OF FUSARIUM SOLANI METABOLITES ON DEFENSE REACTIONS OF POTATO CELL CULTURE AND THEIR BIOLOGICAL ACTIVITY.** A.S. Utarbayeva<sup>1</sup>, O.A. Sapko<sup>1</sup>, R.M. Kunaeva<sup>1</sup>, K.D. Rahimov<sup>2</sup> and D.B. Zhabaeva<sup>1</sup>. <sup>1</sup>M.A. Aitkhozhin Institute of Molecular Biology and Biochemistry. <sup>2</sup>Central Laboratory of Biocontrol, Certification and Preclinical Investigations, Almaty, Kazakhstan. E-mail: a.utar@mail.ru

This study aims at examining the effect of culture filtrate (CF) and mycelium metabolites of *Fusarium solani* on the growth and viability of potato (*Solanum tuberosum*) cell culture, characterized by high resistance to the fungus, and on the accumulation of phy-

toalexins (FA) and activity of peroxidase (POD, EC 1.11.1.7). The CF was characterized by low phytotoxicity and growth regulated activity. The CF influenced the FA accumulation, which had directly proportional dependence on CF concentration and initial resistance of cells. Change of activity of cytoplasmic POD depended on resistance of cells and infection loading. Cells of tolerant cultivar were characterized by the higher increase (170-250%) activity of enzyme in comparison with sensitive ones (20-70%). The induction of cell-wall bound forms of POD depended only on CF concentration. The mycelium metabolites showed high phytotoxicity of water-soluble thermostable isolates and moderate cytostatic activity of lipophilic components. Ethanol and acetone

fractions of mycelium had elicitor activity. The FA accumulation in suspension cells induced by metabolites was characterized by inverse dependence on initial tolerance of cells: sensitive cells accumulated FA 1.2-1.7 times more than cells of tolerant cultivar. The water-soluble fraction caused rapid (within an hour) inhibition of the activity of cytoplasmic POD and induction of cell-wall bound POD activity. Ethanol isolates stimulated activities of both forms of POD, but more significant increase (by 8 times) was observed in cell-wall bound forms. The biological activity of fungal extracellular metabolites was investigated. The findings indicated antiallergic, antispasmodic and antibacterial (antistaphylococcal) activities of CF metabolites.