

**PL-P09.****MULTIPLE TOSPOVIRUS RESISTANCE BY iRNA IN PLANTS**

*Debat HJ*; Grabele M; Lopez Lambertini P; Ducasse DA  
 Inst. Fitopatol. Fisiol. Veg. IFFIVE-INTA. Cno. 60 Cuadras Km 5 ½  
 (5119) Córdoba, Argentina. E-mail: hdebat@correo.inta.gov.ar

Tospoviruses are among the ten most detrimental plant viruses in the world causing severe economic losses in a wide range of vegetables and ornamental crops. In Argentina, the three main species of this genus are TSWV, GRSV and TCSV, affecting mainly lettuce, tomato, potato, pepper and several ornamental plants.

Here we present a RNA interference resistance strategy based in the generation of double stranded RNA with sequence identity to a highly conserved region of the nucleocapsid gene of TSWV, GRSV and TCSV. In order to induce the host iRNA pathway to target this viral gene a 173bp region was obtained by multiple alignment of the species, amplified, cloned and inserted as an inverted repeat flanking an intron in a plant expression vector.

*Nicotiana benthamiana* plants were agroinoculated with this construction and challenged against each of the Tospovirus species in transient expression experiments. At 35 days post inoculation 100% of TSWV, 70% of GRSV and 60% of TCSV plants displayed a symptomless phenotype and no virus presence was detected by DAS-ELISA.

The approach presented here, based in a hairpin RNA construct targeting a short and highly conserved viral sequence, combines high specificity, a reduced off-target effect, does not relay in viral protein expression, and results in a high frequency broad range virus immunity to three tospoviruses.

**PL-P10.****POSSIBLE ROLE IN LIPID SIGNALLING OF STRAWBERRY CLASS TAU GSTs EXPRESSED IN A BIOTIC INTERACTION**

*Tonello U*; Castagnaro AP; Díaz Ricci JC  
 INSIBIO. Fac. Bioq, Qca y Farm. UNT. Chacabuco 461, 4000 Tucumán. E-mail: ursulatonello@fbqf.unt.edu.ar

In plants, Glutathione S-transferases (GSTs) belong to a family of proteins grouped into four main classes (tau, phi, theta and zeta), playing major roles in protection from biotic and abiotic stresses. We have isolated 17 GSTs class tau sharing high identity with other plant GSTs from strawberry leaves 48 hpi with the fungal strain M23. Proteins presented identities ranging from 29 to 99%, showing conservation in residues involved in GSH binding, and diversification in the H site at the C-terminal domain. Based in total sequence alignments and 3'UTR motifs, five different groups were found in FaGSTs. Comparative phylogenetic analyses among *Arabidopsis* and strawberry tau class GSTs indicated that the proteins expressed in the biotic stress in leaves are orthologues of the AtGSTs induced by salicylic acid (SA) and in response to cyclopentenones. These results agree with the increased values of malondialdehyde assessed as TBARS in leaves extracts 36 hpi, indicating that the generation of lipoperoxide precedes the induction of *FaGSTs*. We have also observed that total SA was increased in phloema exudates in challenged strawberry plants. The expression changes in *FaGSTs* transcripts were confirmed using real time PCR. Different approaches for functional studies of these paralogous genes in strawberry will help to clarify the role of class tau GST members in biotic interactions.

**PL-P11.****EXTRACELLULAR SUBTILISIN-LIKE PROTEIN FROM *Colletotrichum spp.* INDUCES DEFENSE RESPONSES IN PLANTS**

*Chalfoun NR*; Grellet Bournonville CF; Di Peto P; Castagnaro AP; Díaz Ricci JC  
 INSIBIO (CONICET-UNT), Chacabuco 461, 4000, Tucumán, Argentina. E-mail: nadiarchal@yahoo.com.ar

We have earlier reported that the avirulent isolate M23 of *C. fragariae* protects the cv Pájaro of strawberry against anthracnose and that culture supernatant derived from that strain induced HR, autofluorescence, oxidative burst, accumulation of salicylic acid and callose deposition. Analysis of the active fraction indicated that the defense inducing agent consists in a 37 kDa protein. The aim of this work is to characterize the protein secreted by the isolate M23 and to study its activity in other strawberry cultivars and in *Arabidopsis thaliana*. The purified protein was separated by SDS-PAGE and electroblotted in 10 mM CAPS (pH 11). The band excised was subjected to Edman gas-phase microsequencing. Each amino acid sequence obtained was compared to annotated sequences using psi-BLASTP. Partial sequence of the purified protein revealed that it belongs to the family of the subtilisin-like serin-proteases and the mature protein lacks the N-terminal protease inhibiting domains found in homologous proteins. Phytopathological experiments showed that defense eliciting protein exhibited the ability to confer resistance to other strawberry cultivars in different degrees. Experiments also showed that the purified protein could also induced the accumulation of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), superoxide anion (O<sub>2</sub><sup>-</sup>) and callose deposition in *Arabidopsis thaliana*.

**PL-P12.****DIFFERENTIALLY EXPRESSED GENES IN POTATO SPROUTS AFTER POTASSIUM PHOSPHITE APPLICATION**

*Feldman ML*<sup>1</sup>; Machinandiarena MF<sup>1</sup>; Amarilla LD<sup>1</sup>; Guzzo MC<sup>1</sup>; Di Rienzo J<sup>2</sup>; Daleo GR<sup>3</sup>; Andreu AB<sup>1</sup>  
<sup>1</sup>IIB, FCEyN, UNMdP, Mar del Plata, Argentina. <sup>2</sup>FCA, UNC, Córdoba, Argentina. E-mail: mfeldman@mdp.edu.ar

We are studying the role of phosphites in disease control management, yield and potato tuber quality. In brief, our previous results showed a number of promising properties associated with these compounds. When we applied potassium phosphites (KPhi) at 3 litre ha<sup>-1</sup> to seed tubers immediately after cutting, this promoted early emergence, an increased in stem number and diameter, early tuber initiation and an increased in the number of tubers per plant. In addition to these physiological effects, phosphite treatment also resulted in greater resistance in seed tubers to *Phytophthora infestans*, *Fusarium solani* and *Rhizoctonia solani*. In order to understand the mechanisms which regulate these responses, we analyzed the changes in gene expression in tubers seeds at early stage of sprouting after KPhi treatment. Preliminary results of microarray analysis from potato sprouts treated or not with KPhi, showed that 26 genes were upregulated in the treated ones. These genes were classified into 5 groups: plant defense, metabolism, abiotic stress, transcription factors and unknown genes. We performed semiquantitative RT-PCR assays of some of these genes to validate the results. Interestingly one of these genes was *CULLIN 1*, involved in jasmonic acid mediated signaling pathway. This result may support the hypothesis that phosphites could be involved in triggering IR (induced resistant).