**FINAL REPORT**

**of the research project „Molecular and functional characterization of genes controlling resistance to tomato spotted wilt virus and bacterial spot in pepper (*Capsicum annuum* L.)".**

**Grant ID K-111935**

**Task1: Cloning of the pepper *CaTSW* gene controlling resistance to TSW virus**

The identification of the TSW gene conferring resistance against TSWV-P0 was a hot topic in pepper resistance research because TSW is a devastating pathogen of pepper and tomato. The monogenic dominant resistance derived from *Capsicum chinense* is widely used in breeding programs therefore the identification of the gene and developing molecular markers for marker assisted breeding has a great importance.

In order to identify the *Tsw* gene, we initiated a map-based cloning project using a segregating population derived from the cross of TSWV resistant and sensitive parental plants. The genetic analysis of more than 1000 F2 individuals narrowed down the TSW region less than 600 kbp ) containing the resistance locus. The sequence analysis of the candidate genes in the Tsw region identified 18 NBS-LRR-type resistance and other candidate genes, gene models or coding sequences (ORFs, EST, etc.) which can confer resistance to *TSW* virus. The combination of the analysis of pepper transcriptome sequencing projects and amplification of the 18 gene candidates from cDNA templates resulted in the identification of pseudogenes and distinct groups of NBS-LRR genes. The expression pattern of each predicted R-genes in the Tsw region has been proved by amplifying the 10 gene candidates CDS from the TSWV resistant Capsicum chinense PI 159236 accession cDNA. Using the full coding sequence and predicted exon-intron structure we narrowed down the candidate list to four genes, a Virus Induced Apoptotic Factor (VIAF), a truncated NBS-LRR candidate, a non CC NBS-LRR (RGC1) and an R gene containing LRR domains (RGC2) in mock or TSW virus infected leave RNA samples. We focused on the two R-genes (RGC1 and 2) because these genes fitted best into the general model of resistance against TSWV. 63 TSWV sensitive and resistant plants were selected from the *C. chinense* germplasm collection to analyze polymorphisms (SNP and In/Dels) in RGC1 and 2. We found several SNPs in RGC1 between *Capsicum annuum* and sensitive or resistant *C. chinense* transcripts but none of them could be correlated obviously with the resistance. We detected a 17 bp deletion in RGC2 that generates a frame shift resulting in an early translational stop in *C. annuum*. We also identified and analyzed the sequence of RGC2 from three resistant and sensitive plants, respectively from *C. chinense*. We have not found SNPs between the resistant samples but altogether 20 SNPs were detected between the resistant and sensitive sequences. These SNPs resulted in substitutions of 5 residues between sensitive and resistant samples that might be corresponded to the susceptibility to TSWV. The RGC1 and 2 were the best candidates for the TSW resistance gene and we planed transformation experiments with these genes to prove the identity of the TSW gene. We cloned RGC2 (containing an early stop codon due to frame shift in *C. annuum* allele) from *C. chinense* PI 159236 accession, introduced into a binary vector and transformed to TSWV sensitive *Nicotiana tabacum* L. var *Xanthi* and *Capsicum annuum* plants. The presence and the expression of the transformed gene were confirmed by construct and gene specific primers using specific PCR and qRT-PCR. Unfortunately, the transgenic *Nicotiana* and *Capsicum* plants did show resistance to TSWV inoculation, indicating that RGC2 does not correspond to the *Tsw* gene.

In order to speed up the gene identification process, the approach of virus induced gene silencing constructs (VIGS) of 3 NBS-LRR candidate genes was also applied. We generated TRV (tobabco rattle virus) vector-based silencing constructs that were introduced into pepper leaves and we monitored their silencing function altering the hypersensitive responses (HR). One of the VIGS constructs silenced and abolished HR indicating that the gene or similar genes having similarity to the

cloned fragment might be involved in the induction of HR and thus related to the gene responsible for the resistance to TSW (Figure 1). We amplified and cloned the coding sequence (CDS) of this NBS-LRR gene from *C. chinense* PI 159236 accession for further studies.

Figure 1.: Virus Induced Gene Silencing of a NBS LRR TSW candidate. PDS control, Mock control (PI152225), Systemic infection on TSW resistant plant

At this period of the project, a paper was published in New Phytologist (Kim et al 213:886-899, 2016) reporting the genes conferring resistance to Potyvirus and TSW virus infection. The PVR4 and *Tsw* genes were identified by map based cloning. The publication of the sequence of the TSW gene means that we were behind to publish our results at that time. In this paper, the sequences of the *Tsw* gene from TSW virus sensitive *C. chinense* (the *C. chinense tsw* allele) have not been analyzed and published. In order to publish some of our results achieved in the previous years in the TSW resistance projects, we planned to sequence the tsw allele from TSW virus sensitive *C. chinense* plants and analyze the difference between the resistant and sensitive alleles of *Tsw*.

We obtained 63 *C. chinense* accessions from germplasm collections and tested them for sensitivity to TSW virus infection. Ten *C. chinense* accessions showed resistance and 53 were sensitive to virus infection, respectively. Full length CDS of the *CcTsw* and *Cctsw* genes were amplified from cDNA samples, cloned and sequenced from resistant and sensitive accessions. In all the ten TSWV resistant accessions, we identified a 6 kb fragment which was similar in size to the *Tsw* resistant allele identified previously. The amplifications of the *tsw* alleles from cDNA samples of the sensitive genotypes either resulted in smaller PCR products compared to the resistance allele or

failed to generate any PCR products. The sequence analysis revealed that the 4.8 kbp sensitive *Cctsw* allele contained six exons compared to the five exons found in gene variant of the Catsw sensitive allele. The encoded protein contains only four leucine-rich repeats (LRRs) compared to resistant allele of *Tsw* containing eight LRRs. The similarity in the CC-NBS domains between the resistant and sensitive *C. chinense* Tsw alleles was 96% while the sensitive C. chinense and C. annum alleles showed 91% similarity.

Our objective was to develop easy-to-use PCR-based markers for the TSW virus resistance to apply it in marker assisted selection (MAS). We compared the genomic sequences of the resistant *C. chinense* and the sensitive *C. annuum tsw* alleles to detect polymorphism, especially insertions and deletions (In/Del polymorphisms). To develop molecular markers, oligonucleotides were designed for six In/Dels detected in the 25-25 kb region upstream and downstream of the *TSW* gene. We tested different TSW virus resistant and sensitive accessions for the presence/absence of the PCR fragments and we found that one of the six markers located upstream of the *Tsw* gene could be used to detect the *tsw* allele with 95-100% accuracy (Figure 2), while the other markers were less effective. This relatively low accuracy probably due to the large number of genetic changes occurred around the *Tsw* gene since the separation of the *C. annuum* and *C. chinense* species.

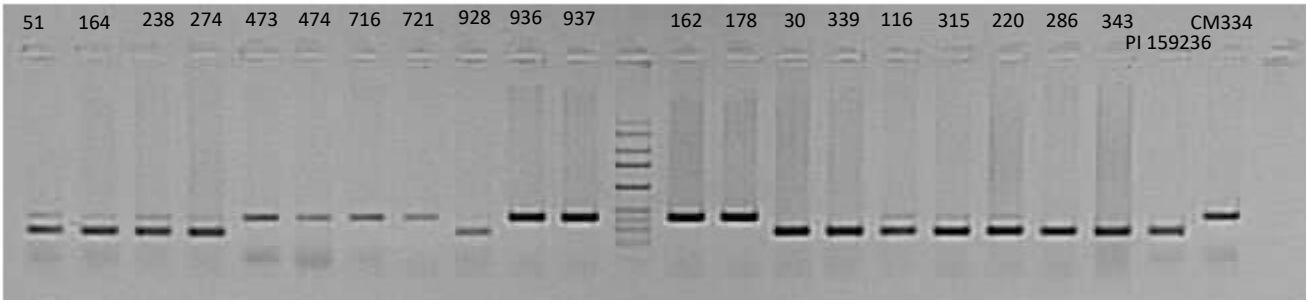


Figure 2.: Detection of resistant and sensitive amplicons of the tightly linked molecular marker to *Tsw* gene, numbers indicate F2 plants followed by resistant and sensitive control samples (PI159235, CM334)

During the screen of 85 *C. chinense* genebank accessions with the developed easy-to-use closely linked codominant PCR-based marker to the *Tsw* gene, we identified a genotype that showed resistance to the TSW virus P0 pathotype in biological test although it carried an allele with the fragment size of the sensitive alleles (plant no. 16 in Figure3). Accordingly, we were unable to amplify the resistant variant of the *Tsw* gene (coding sequence (CDS) of *Tsw* KT751527-PI 159236; 6351 nt, 2117 AA) from this genotype. We analyzed the CDS, promoter and terminator region of the gene which showed length and high sequence similarities besides the few changes in amino acid residues to a previously cloned sensitive tsw allele of a *C. chinense* accession. During the durability tests we concluded that the HR phenotype identified in the unique *C chinense* genotype is probably dependent on the TSW virus isolate and the resistance is partial.

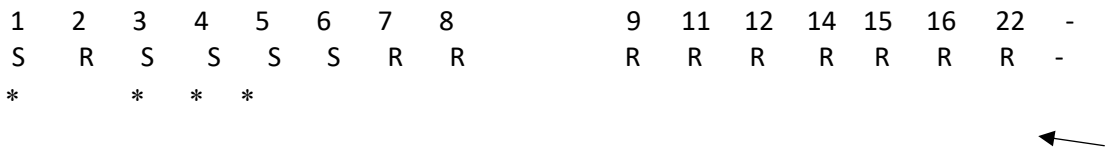


Figure 3.: Ampification of full length CDS of resistant (arrow) and sensitive (asterix) TSW alleles. Phenotypes are indicated under the sample numbers. Note the plant no. 16 that carries a fragment of the size of the sensitive allele but it showed resistance to TSW virus.

In order to further analyze the mechanism of the Tsw resistance mechanism and analyze the expression changes regulated by the *Tsw* gene, we generated *Nicotiana benthamiana* plants carrying the *Tsw* gene. The resistant *Tsw* allele was cloned into binary vector and clones were tested by transient expression in *N. benthamiana* leaves (Figure 4a and 4b). Positive clones were selected based on the presence of necrotic lesions following TSW virus inoculation and used to generate stable transformant *N. benthaminana* plants using *Agrobacterium tumefaciens*-mediated transformation of tobacco leaf discs (Figure 5). Twelve independent regenerated plants (T0) were tested by amplifying partial genomic segments and the whole transcript of *Tsw*. T0 seeds of the transformed plants carrying *Tsw* transgene have been collected. Leaf samples were collected from mock and TSW virus infected transgenic tobacco plants and total RNA was purified. The total transcriptome (RNAseq) of mock and infected transgenic *N. benthaminana* have been sequenced using Illumina platform. The analysis of the transcriptional activity of HR phenotype related genes is still in progress.

Figure 4a.: Transient expression of the *Tsw* allele in TSW virus infected *N. benthamiana*

Figure 4b.: Symptoms of TSW virus infection on *N. benthamiana* leaves transiently expressing the resistant allele of *Tsw*.

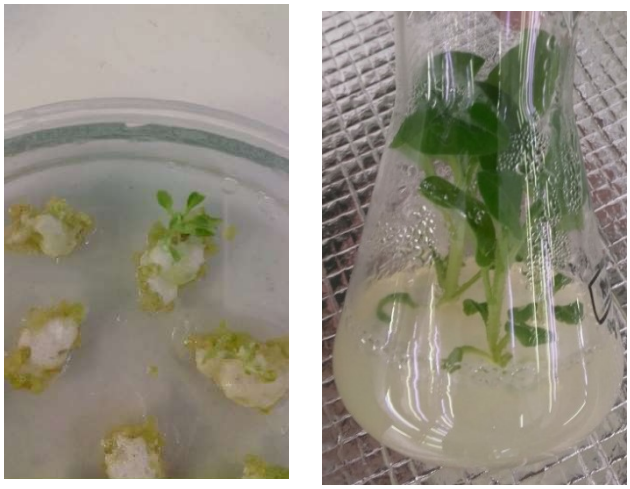

Figure 5.: Regenerated *N. benthamiana* plantletscarrying the pepper *Tsw* resistance gene.

**Task 2: Cloning of *Cabs6* gene controlling resistance to *Xanthomonas campestris* pv. *vesicatoria* (Xcv)**

In order to identify pepper lines carrying the *Cabs6* resistance gene, we started to grow plants of PI271322 USDA and PI271322 FL gene bank accessions supposed to carry *Cabs6*. Plants were started to grow in the second half of 2014 and following self-pollination, the progeny were inoculated with the *Xcv* strains carrying different avirulence factors avrbs1, avrbs2, avrbs3 and avrbs4 and a strain lacking any avrbs proteins. Following testing the phenotype of more than 400 plants originating from PI271322 USDA, five plants carrying *Cabs6* were selected for crosses to establish segregating populations. Out of the 70 progeny of PI271322 FL, we selected four plants to use them as parental lines following inoculation tests. The selected 9 parental individuals were crossed with the maternal pepper Fehérözön 5 plants. The hybrid nature of the 90 F1 plants were tested with microsatellite (SSR) markers and hybrid F1 plants were self-pollinated to develop F2 mapping populations. We tested the polymorphism of 98 SSR markers distributed along the arms of the 12 chromosomes of pepper and found that 65 SSR markers showed polymorphism between the parental lines (PI271322 and Fehérözön 5) in agarose gels or following SSCP (single-strand conformation polymorphism) analysis. We have infected 92 F2 plants and determined their resistance or susceptibility to *Xcv*. F2 individuals from F1 hybrid plants were grown and leaf samples were collected for DNA analysis. We determined their genotypes for the 65 SSR markers showing

polymorphisms between the parental lines. These 65 SSR markers were more or less equally distributed along the 12 chromosomes of pepper. The linkage analysis between the resistance phenotype to *Xcv* and the molecular markers revealed a genomic region on chromosome 6 containing the locus of resistance to *Xcv* (*bs6* locus). Parallel to our studies, a research group at University of Florida published a project summary presenting the genetic mapping of the of the Xcv (*Cabs6*) resistance. Based on their results, the locus of *Cabs6* was positioned into a 27 Mbp region on chromosome 6 confirming our mapping results of *Cabs6*.

In order to define the position of the *bs6* locus more precisely, we mapped additional 30 SSR markers that determined a genomic region of 1.5 Mb wherein the *Cabs6* gene is located. The sequence of the region is known from pepper line CM334 and landrace Zunla-1 genetic background except four large gaps. These gaps were sequenced using BAC clones covering the region. The genomic fragments used to construct the BAC (Bacterial Artificial Chromosome) library were derived from a susceptible *C. annuum* plant and thus the library did not contain the resistant allele of the *bs6* gene.

In order to confirm the phenotype of the F2 plants to *Xcv* infection and determine the homozygosity or heterozygosity of sensitive F2 plants for the *bs6* locus, progeny analysis of selected F2 plants was carried out. We have selected 20 resistant and 20 susceptible F2 plants of mapping population and their 20-20 offspring, respectively were inoculated with *Xcv* and scored for the susceptibility to *Xcv* infection. Surprisingly, we found in several cases (about 20% of the F2 plants) that the phenotypes of the F3 plants did not confirm the predicted genotype of the sensitive F2 plants. We considered that a modifying locus in the genetic background might affect the resistance phenotype. We have analyzed the segregation ratio of resistant phenotype of F2 progenies and it showed the expected 3:1 Mendelian segregation ratio of the susceptible and resistant phenotypes. Based on this, we believe that it is unlikely that a locus in the background affected the resistant phenotype in the F3 offspring but we rather assume that the discrepancy is due to the different physiological parameters of plants at the time of infection or the bacterial culture used for inoculation. Therefore parameters of the infection were meticulously fine-tuned to optimize the infection protocol:

- plants were grown in growth chambers under controlled light and temperature conditions

- pepper plants developed until the fourth leaf stage were used for bacterial infection

- we have monitored the growing of the bacterial population in inoculated leaves of susceptible and resistant lines from the inoculation time (0 DAI) to determine the optimal initial Colony Forming Unit (CFU) of the bacterial culture for infection. Based on these data we determined concentration of 2 x $10^8$ cfu/ml suspension was optimal for inoculation to score the phenotype

- in addition to beside carrying out the bacterial infection at the 4-6 leaf stage, we repeated the *Xcv* infection twice at later developmental stages to confirm the susceptibility/resistance to *Xcv* infection

Applying the optimized infection method, we scored the plants of the mapping population of the previous cross between Fehérözön 5 (Fö5) and PI271322 plants and based on the determined resistance phenotypes we continued the mapping of *Cabs6* resistance gene. Using the genetic markers, the rough map position of the resistant locus was determined between two SSR genetic markers spanning approximately 2 cM genetic distance of the pepper genome. In order to define the position of the *Cabs6* gene more precisely, additional ~2000 F2 plants were genotyped and analyzed for their resistance to *Xcv* infection. The fine mapping narrowed down the *Cabs6* region into a

genomic region of about 1 Mbp between two codominant microsatellite (SSR) markers. The sequence of this genomic region was obtained from the sequence database of the cultivated pepper Zunla-1 and CM334 and used to analyze the gene content and generate additional markers for further genetic mapping. Within the region flanked by the newly developed markers, the genome sequence of Zunla-1 and CM334 have been analyzed by using the online software FGENESH. The analysis identified 25 genes including housekeeping genes and other ones predicted to function in general biological processes (cell signaling, metabolism, etc.).

In order to continue the fine mapping of the *bs6* gene, additional plants were genotyped and analyzed for their resistance to *Xcv* infection. Based on the genotypes determined for the border markers, altogether 35 individuals showing recombinations were selected for further genetic analysis. We identified 19 recombinant plants with maternal homozygous (sensitive allele)/heterozygous genotypes and 16 recombinant plants with heterozygous/paternal homozygous (resistant allele) genotypes (Fig. 6). As a next step, the genotypes of the 35 individuals showing recombinations between the border markers (151 and 120 on Fig. 6) were determined for internal genetic markers (markers highlighted by grey background in the first column).

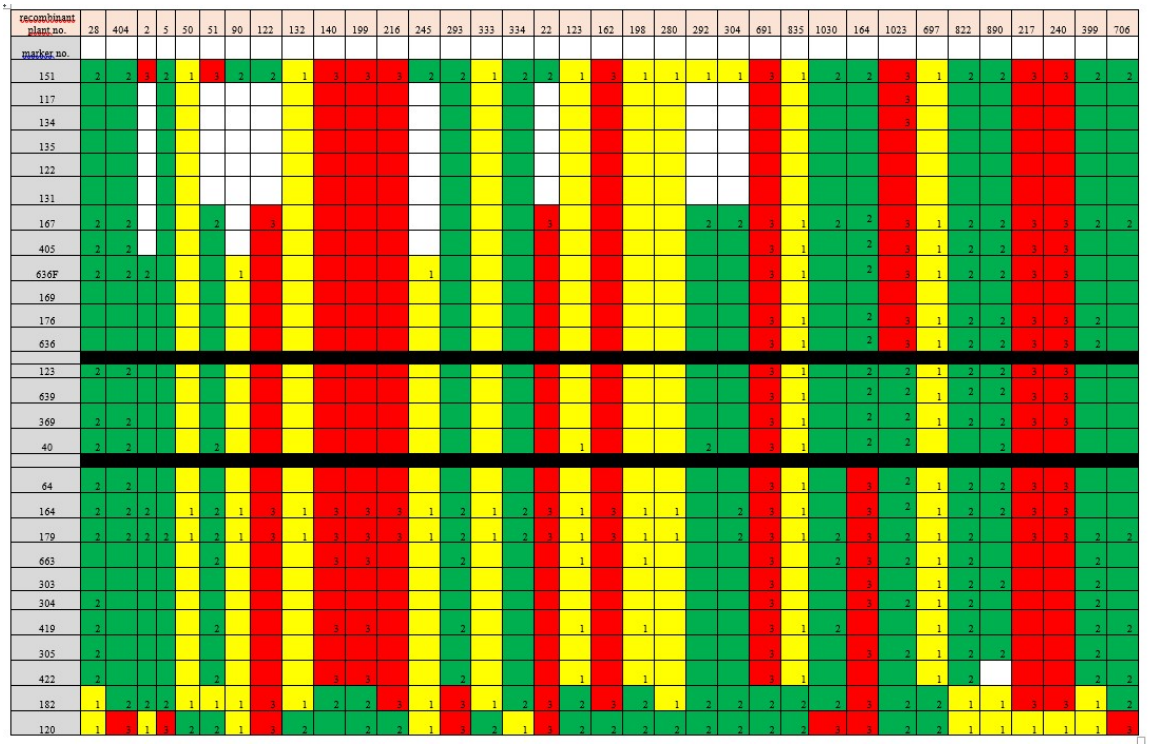


Figure 6.: The color-coded genotypes of the 36 plants in the *bs6* region. Codes for the genotypes: yellow – maternal allele; red – paternal allele; dark green – heterozygous genotype.

The novel genetic markers 123 and 40 were used to delimit the region of the *bs6* locus into a ~40 Kbp region (Fig. 6.). To validate our results we have selected two recombinant (plant no. 1023 and 164) F2 plants of mapping population and their 40-40 offspring were inoculated independently three times with *Xcv* and scored for the susceptibility to infection. The phenotypes of the F3 plants confirm the predicted genotype of the selected F2 plants and thus confirmed the narrowed genome location of *bs6*.

The genome sequences of Zunla-1 and CM334 pepper plants were analyzed to reveal candidate genes, primarily resistance (R) genes for *bs6* using the online software FGENESH (http://www.softberry.com/). The sequence analysis and comparison with the draft sequence of the pepper genomes, all of them are *Xcv* sensitive lines, (Zunla-1, CM334, *Capsicum baccatum*, *C. chinense*) revealed six genes that may condition resistance to *bs6*. In order to obtain the genomic sequence from *bs6* resistant genomic background, we designed oligonucleotide primers to amplify the alleles of the candidate genes and the *bs6* region. The sequence of the amplified products were determined and used to assemble the sequence of the *bs6* region from the resistant genomic background. In order to identify the *Cabs6* gene, the sequence of the candidate genes from the resistant background (*C. annuum* PI271322) were compared to the reference gene sequences (susceptible parent). According to our preliminary analysis there are no large genomic rearrangements or any additional gene that controls the *Xcv* resistance have been identified in the continuous ~40 kb region. To validate the gene content and predict the transcriptionally active genes, total RNA samples from mock Xcv (*Cabs6*) resistant pepper plant (PI271322 USDA) have been prepared and sequenced (RNA-seq) in collaboration with the Seqomics Ltd. (Mórahalom, Hungary). The Illumina reads were mapped to the resistant and sensitive reference sequence to determine the expression pattern of the candidate genes in normal condition. Further analysis of the RNA seq data is under way. In advance, based on the predicted function and transcriptional activity, four candidate genes have been selected to introduce these genes into tobacco plants using *Agrobacterium tumefaciens* mediated transformation experiments. The preparation of four construct for transformation is progress. Stable transformed plants will be inoculated with *Xcv* and infected leafs will be analyzed and scoring the plants for resistance and susceptibility to bacterial spot disease.

**Task 3 Functional analysis of *CaBs5/Cabs5* genes and their gene products**

The Cabs5 gene was identified prior this grant proposal but the function of the CaBs5 has not been explored. Preliminary observations showed that pale-green sectors developed on infected leaves without the appearance of HRs. we planned to further analyze *CaBs5/Cabs5* genes and its gene product to better understand the mechanism of the resistance to *Xcv*.

Transcriptional analysis of Xcv resistant (*Cabs5*) and sensitive (*CaBs5*) plants

To analyze the activated signaling pathways in *Cabs5* (Xcv resistant) and *CaBs5* (Xcv sensitive) plants, transcriptome analysis of these plants was carried out post inoculation with Xcv. RNA samples were prepared from *bs5* resistant and *Bs5* susceptible pepper plants. 48 individuals of the F2 segregating population were genotyped by gene specific markers to prove resistant and susceptible plants. These plants were inoculated with Xcv 'Race10', a highly virulent race lacking effectors and inducing hypersensitive reaction. Total RNA from mock and Xcv inoculated bulks from eight resistant (*bs5/bs5*) and eight homozygote sensitive (*Bs5/Bs5*) plants was prepared and shipped for transcriptome sequencing. The RNA sequencing generated ~115 million 50bp reads. After validation of the reference sequences (~32000 EST and TC) by filtering non-protein coding, or repetitive elements and normalisation by housekeeping genes like actin, RNA seq analysis was carried out using CLC genomic workbench software package. The analysis identified large number of genes showing altered (regulated up/down in resistant bulk) expression pattern following Xcv. infection. To verify the RNAseq data, we selected and tested the expression of 50 genes (e.g. genes of a pathogen-induced membrane protein, choline/ethanolamine kinase, ABA receptor, heat shock protein, lipid-transfer protein) predicted to be involved in plant defense responses and resistance processes using RT-PCR (Figure 7). We used the single homozygous and heterozygous sensitive and homozygous

resistant plants and bulk (10 plants) isolates of the original population to confirm alteration in gene expression detected by RNAseq. Only 25% of the genes including the resistance related like PR1, PR5, Cellulose synthase, chitinase genes could be validated by RT-PCR due to expressional discrepancies found on heterozygous sensitive samples.

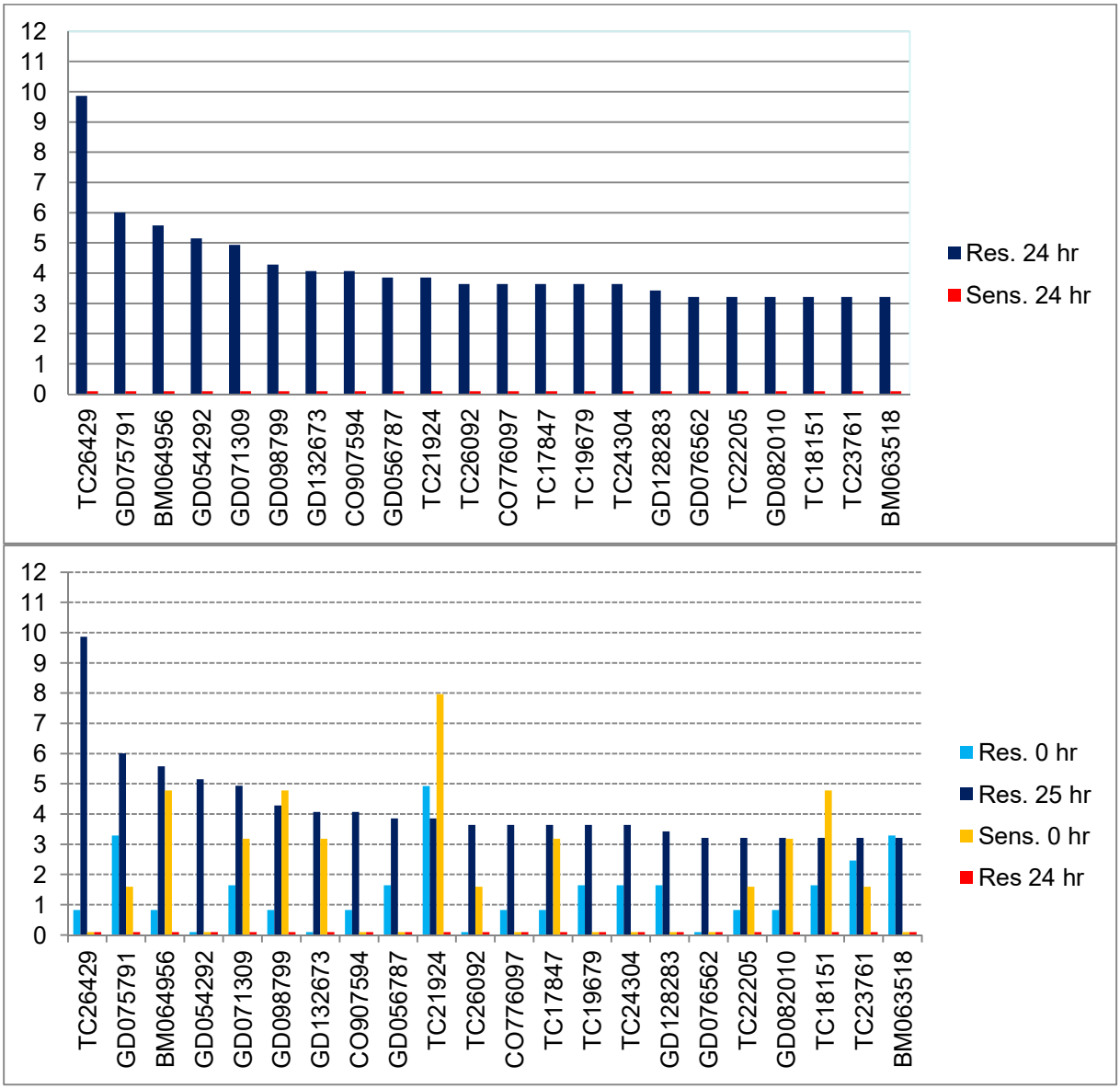


Figure 7.: Gene expression differences and changes of pathogen- related genes upon Xanthomonas infection.

Subcellular localization of Cabs5

The subcellular localization of Cabs5 was identified using *C. annuum* 'Fehérözön' SCAMP1, Rab5, Rab7, Rab11 marker proteins showing specific localization in subcellular compartments (ER, Golgi, vacuoles, plastids and cell membrane). The corresponding gene sequences were fused to mCherry to produce N-terminally tagged fluorescent proteins. The constructs were introduced into *A. tumefaciens* by three parental mating. C- or N- terminal GFP fusions of CaBs5 and Cabs5 proteins were transiently expressed in *N. bentamiana* by co-infiltration with P14 silencing suppressor.

Compared to the nuclear localized control, Bs5 and bs5 were detected by LEICA confocal laser microscopy in the plasma membrane of intact cells and protoplasts. We were unable to detect co-localization CaBs5 and Cabs5 proteins with other sub-cellular compartment-specific proteins located in the endoplasmatic reticulum, the post-Golgi pathways, endocytotic compartments (FM-64 dye tested parallel), the lysosomes or the peroxisomes. The C- terminal GFP fusion of CaBs5 and Cabs5 proteins could be detected in the cytoplasm indicating that the GFP fluorescent protein tag abolished the plasma membrane targeting of the CaBS5 and Cabs5 proteins (Figure 8.).

Figure 7.: Transient expression of C- or N- terminal GFP fusions of CaBs5 in N. benthamiana leaves

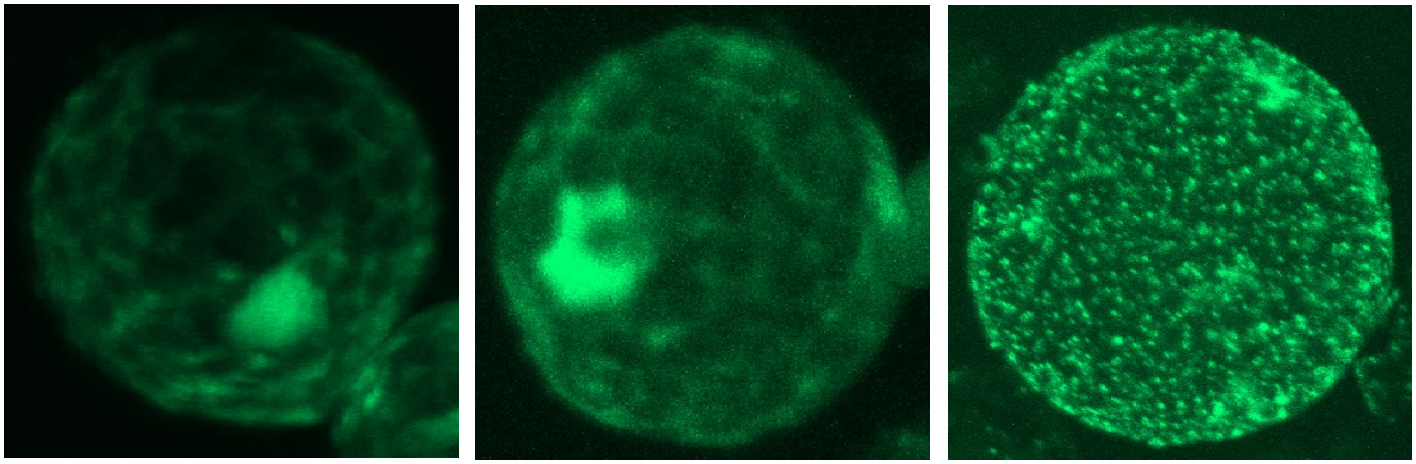

Figure 8.: Transient expression of C- or N- terminal GFP fusions of CaBs5 in *N. benthamiana* protoplasts

Identification of interacting partners of Cabs5

Proteins interacting with Bs5 were screened in yeast two hybrid screens using the Arabidopsis homolog of CaBs5. Homologs of *CaBs5* were identified by similarity search with CaBs5 protein and nucleotide sequence in the Arabidopsis genome. The most homolog gene, *AtBs5-1* was amplified from *Arabidopsis thaliana* 'Columbia' and cloned into pGADT7 AD bate vector of Clontech Matchmaker Gold Yeast Two-Hybrid System. Normalized Mate & Plate Library of *A. thaliana* was screened with AtBs5-1 and the 117 positive clones were selected from 50 plates for further test and sequencing. Positive clones from the yeast two-hybrid screen were rescued from yeast to *E. coli* and 37, probably non-technical replicates have been sequenced. We have selected the clones carrying the genes of At3g52940 (encoding a sterol C-14 reductase), At3g16530 (a lectin like protein),

At4g00430 (plasma membrane intrinsic protein) At4g17730 (syntaxin23) and At5g06860 (a polygalacturonase inhibiting protein) for a second confirmation test to prove positive interactions. Positive clones selected from C-terminal truncated (without transmembrane domain, TMD) Y2H screens were tested in assay experiments with full, C-terminal truncated (without TMD) and N-terminal truncated (only TMD segment) Bs5 protein constructs. Interactions with TMD domain containing proteins could not be verified so we focused on potentially TMD interacting partners. We created full and N-terminal truncated Bs5 protein constructs in mating-based split-ubiquitin system (mbSUS). Preliminary results indicate no interaction between Bs5 proteins ( no homodimer formation). Interaction tests are still in progress to assay the interaction with the known homolog (*Xcv2*) and tetraspanin protein like *Capsicum annuum Tet5* (*TORNADO2*).

N-terminal GFP fusions of full and truncated versions of CaBs5 and Cabs5 proteins with P14 silencing suppressor were transiently expressed in *N. bentamiana*. Localization of the truncated proteins was compared to the non-truncated version using confocal laser scanning microscopy. All N-terminal GFP fusion CaBs5 and Cabs5 proteins were detected in the plasma membrane of intact cells and protoplasts indicating no other domains than the C-terminal transmembrane domain responsible for the protein localization. Interaction tests in mating-based split-ubiquitin system (mbSUS) showed no interaction between Xcv2 and tetraspanin protein like *Capsicum annuum* Tet5.

<u>Studies using the CaBs5 antibody</u>

In order to localize the Bs5 protein within the pepper leaf cells and analyze its function, we planned to develop antibody against the Bs5 protein. To find the most antigenic region on the Bs5 protein, different antibody epitope prediction online tools were used. The analysis was carried only on those regions of the Bs5 protein sequence which did not show high similarity to other proteins. Two predicted antigens (P1: Bs5 _18-31 and P2: Bs5_53-70) were chosen and the production of antibodies were ordered from Thermo Scientific Biopolymers. Polyclonal antibody against the mixture of the two selected epitopes (peptides) was produced. As a first step, we tested the obtained antibodies in different dilutions on the antigenic peptides with dot blot. The antibody showed 50 times more sensitivity to P1 peptide, than P2. Upon these results we continued the test on crude protein extracts purified from tobacco plants transiently expressing GFP-CaBs5 and CaBs5-GFP fusion proteins.

The method to generate Xcv resistance plants by applying the bs5 gene was patented previously (Identification of a Xanthomonas euvesicatoria resistance gene from pepper (Capsicum annuum; WO2014068346A3). The manuscript presenting the identification and characterization of the bs5 locus has been prepared by our side and sent to our collaborator partners (2Blades Foundation)to develop the study further and submit the manuscript for publication.

**Other conducted projects related to virus resistance**

Another subproject related to the analysis of TSWV resistance was the observation of the unique event of a simultaneous infection of the P0 and P1 pathotypes of TSWV on a single fruit of a TSWV resistant pepper plant. The result of the characterization of the symptoms and verification of the infection of both pathotypes on this single fruit was presented in a talk on the 8th International Plant Protection Symposium at University of Debrecen (17-18 Oct 2018). The preparation of the manuscript presenting the result of this study is in progress.

In addition to the identification of new P0 and P1 pathotypes of TSW in Hungary, we were able to identify a new Tobamovirus, belonging to sub-group 3. The natural host range tests and pathological properties alongside the molecular characterization of the new tobamovirus will be presented in a separate publication planned to submit in 2020.