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PATTERN-TRIGGERED IMMUNITY (PTI) INDUCTION AND TRANSCRIPTIONAL REPROGRAMMING IN PERSISTANT ALLEXIVIRUS INFECTION

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Abstract

In virus—plant interactions, one of the major mechanisms for plant antiviral immunity relies on RNA silencing, which is often suppressed by co-evolving viral suppressors, thus enhancing viral pathogenicity in susceptible hosts. However RNA silencing should not only be viewed as an antiviral mechanism that must be counteracted. In fact, many viruses encode weak or transiently active suppressors and probably do not use these viral proteins for control RNA silencing; for example, Shallot virus X (ShVX) do not code the active silencing suppressor and consequently use the another molecular mechanism to overcome the silencing immune barrier, establish the persistent infection and prevent catastrophic damage to its host. We hypothesized that this "non-suppressor" mechanism is the process of transcriptomic reprogramming (TRP) induced by the PTI (pattern-triggered immunity), the first layer of plant defence, which is triggered by specific recognition of conserved microbe- or pathogen-associated molecular patterns (MAMPs, or PAMPs, respectively) by pattern recognition receptors (PRRs) at the plasma membrane and the induction of defense signaling. Recently a role of PTI in antiviral defence has been demonstrated in *Arabidopsis* by showing that mutants in the PRR (PRRs, Pattern recognition receptors) coreceptor kinases exhibit increased susceptibility to different RNA viruses. Our preliminary results confirm this hypothesis and show that there is a negative correlation between the ShVX reproduction rates and the levels of RNA-dependent RNA-polymerase (RDR) and DCL proteins in roots and leaves of infected shallot plants. The task of this study is the experimental verification of our PTI-induced TRP hypothesis by quantitative real-time PCR (Comparative CT experiment, delta-delta CT algorithm; calibrator: healthy shallot seedlings; normalizer: 18S RNA; The 7500/7500 Fast Real-Time PCR Systems, Applied Biosystems, USA) to evaluate in vivo expression levels of transcripts coding PTI markers, factors of RNA-silencing, NB-LRR receptors and complex of TCTP-PIRL-GRF6-DBP1 proteins in the healthy and ShVX-infected shallot (*Allium cepa* L. var. *aggregatum* L.G. Don) plants. In this study for the first time we obtained the convincing data about PTI and TRP induction in ShVX-infected shallot plants. As result of TRP, repression of all factors of RNA-silencing, some NB-LRR receptors (e.c., *Tm2²*) and some proteins of TCTP-PIRL-GRF6-DBP1 complex take place in this virus—plant system. On the other hand, group of defense genes with high expression levels has been discovered in this system: *SOBIR* — log10RQ ~ 1.0; *ARM* (genes encoding armadillo protein family) — log10RQ ~ 2.0; Pathogenesis-related protein 1, *PR1* — log10RQ ~ 2.0; Pathogenesis-related protein 5, *PR5* — log10RQ ~ 4.0 (!); Pathogenesis-related protein 14 = nsLTP — log10RQ ~ 2.0. So, in leaves and roots of infected plants ShVX programs dynamical and coordinated process of TRP and downregulation of genes coding for core RNAi components and disease resistance proteins might be correlated with successful virus reproduction and persistent virus infection establishment. We are of opinion that plant viral-specific PRRs identification, plant viral PAMP-triggered PTI and PRR (Pattern recognition receptors)-mediated transcriptomic reprogramming mechanisms ascertainment are the main tasks of coming antiviral plant immunity research period. Cloning of plant PRRs involved in plant virus PAMPs recognition, and the inter-species transfer of plant virus-sensing PRRs are the promising future technologies for broad spectrum antiviral resistant plants creation (D. Bao et al., 2017).

Keywords: allexiviruses, Shallot virus X, *Allium cepa* L. var. *aggregatum* L.G. Don, persistant infection, RNA-silencing, plant innate immune system, Pattern-triggered immunity (PTI), transcriptomic reprogramming.

Plants possess complex protective antiviral system with the key role of RNA silencing (the molecular mechanism induced by viral double-stranded RNA) controlling virus replication and manifestation of the symptoms [1, 2]. In this context the activity of viral suppressor proteins which prevent viral RNA fragmentation or blocking of the viral RNA translation during silencing has been highlighted as an indispensable condition for successful reproduction of phytoviruses [3, 4]. It is known, however, that many phytoviruses encode inactive, very weak or transiently active suppressors [5]. In particular, as we have shown earlier [6], Shallot virus X (ShVX), the prototype of the genus *Allexivirus*, can successfully multiply and establish persistent symptomless infection in the absence of an active suppressor protein; therefore, it overcomes the immune barrier of silencing using some others mechanism(s). We suggest that such a mechanism could be transcriptomic reprogramming (TRP) [7, 8] caused by PTI (Pattern-triggered immunity), the first "defense line" of the innate immune system of plants [9]. As a result of TRP, expression of a number of target genes involved in the reproduction of phytoviruses can be selectively changed, in particular, expression of the RNA silencing key factors, i.e. DCL proteins, Argonaute proteins (Ago), and cellular RNA-dependent RNA polymerases (RDR), can be suppressed to a critical level. The results we obtained earlier testify in favor of this assumption: DCL and RDR genes' transcription have been repressed in roots and leaves of ShVX infected plants [10].

Experimental data obtained in the past 2-3 years in several foreign laboratories indicate that phytoviruses, like bacterial pathogens, induce a process similar to classical PTI [11-13], and in this context the double-stranded replicative forms of viral RNA act as virus-specific PAMPs (pathogen-associated molecular patterns) [12, 13]. Thus, at least two antiviral mechanisms triggered by double-stranded RNAs, i.e. RNA silencing and PTI, function in a plant cell. In Russia, the studies of the molecular mechanisms of antiviral phytoimmunity have not yet received proper development.

In is commonly deemed that the pattern recognition receptors (PRRs), RLKs (receptor-like kinases) or LRR-RKs (leucine-rich repeat receptor kinases), localized on plant cell plasma membrane, specifically recognize conservative PAMPs, such as bacterial flagellin, and thus initiate PTI [14-18]. During the interaction of molecular patterns with PRRs, immediate and intensive induction of transcriptomic reprogramming occurs. This particularly results in differential expression of a number of proteins which are the PTI markers [19]. Specific antiviral PRRs of a plant cell have not been identified to date, although their existence is quite convincingly proved by the recent study of the role of multifunctional co-chaperone Hop/Sti1 in the symptoms of potato virus Y infection [20].

This paper is the world's first attempt to obtain the experimentally evidence in witness of PTI induction in shallot plants under persistent viral infection and the participation of this mechanism in transcriptomic reprogramming. The objective was to experimentally test the authors' hypothesis [10] of the transcriptomic reprogramming key role as a mechanism of RNA silencing suppression, as well as the assumption that transcriptomic reprogramming is associated with PTI and, as a result, the expression of many genes involved in reproduction of phytoviruses (e.c. TCTP-complex) can be selectively changed [21]. In particular, our findings show that, due to the TRP, expression of all silencing factors and some R-genes, e.c. the *Tm2²* gene homologs, is suppressed.

Techniques. Shallot plants (*Allium cepa* L. var. *aggregatum* L.G. Don) were

grown from bulbs under ShVX persistent infection. In each experiment, 5-6 bulbs of one shoot cluster were planted, and in 3 days and 2 weeks the bulk samples of roots, seedlings and leaves were collected.

Total RNA isolation and ShVX detection by PCR method were described earlier [10].

Nucleotide sequences encoding homologs of selected target genes of *A. cepa* L. transcriptome (the species which is the closest to shallot plants) were searched using tblastn, tblastx software and the TSA database (Transcriptome Shotgun Assembly, https://www.ncbi.nlm.nih.gov/gen-bank/tsa/). The primers specific for the shallot homologs of the examined target genes were designed as described earlier [10, 23]; a set of primers was generated with Primer3 v.4.1.0 program (http://primer3.ut.ee/).

Transcripts, encoding target proteins, were detected by real time PCR using the Comparative CT experiment (delta-delta CT algorithm; virus-free shallot seedlings as a calibrator; 18S RNA as a normalizer) which seems to be optimal for dynamic process of transcriptomic reprogramming [22]. An amplifier was 7500/7500 Fast Real-Time PCR Systems or QuantStudio (Applied Biosystems, USA), a set of reagents was SYBR® Green Reagents (OAO Syntol, Moscow), 3-fold biological and 4-fold technical repetitions were used.

Target gene expression levels were calculated, statistical processing made and histogram constructed with embedded software Design & Analysis Software v.1.4.3 (Applied Biosystems, USA). The figures show the mean logarithmic RQ values and their deviations (RQ_{min} and RQ_{max}).

Results. Table 1 shows the PTI markers, and Table 2 shows the corresponding primers. In the first series of experiments, the profiles of transcripts of 10 classical PTI markers (Fig. 1) were investigated.

1. Target genes which expression was investigated in shallot (*Allium cepa* L. var. *aggregatum*) plants persistently infected by shallot virus X

Gene	Protein	Plant species, ortholog ID
	PTI marker genes	
ARM	Armadillo repeat family proteins	*Arabidopsis thaliana* AT3G02840
RBOHD	Respiratory Burst Oxidase Homologue D	*Arabidopsis thaliana* AT5G47910
EDS5	Mate Efflux Family Protein (Enhanced Disease Susceptibility 5)	*Arabidopsis thaliana* AT4G39030
LOX3	Lipoxygenase 3	*Arabidopsis thaliana* AT1G17420
BRI1	Bri1-like 3 (Brassinosteroid insensitive)	*Arabidopsis thaliana* AT3G13380
SOBIR	Leucine-Rich Repeat Protein Kinase Family Proteins (Suppressor Of Bir1-1/Evershed)	*Arabidopsis thaliana* AT2G31880
CRK4	Calcium-dependent protein kinase (CDPK) family proteins (Cysteine-rich receptor-like kinase 4)	*Arabidopsis thaliana* AT5G24430
SERK1	Somatic embryogenesis receptor-like kinase 1	*Arabidopsis thaliana* AT1G71830
PR1	Pathogenesis-related proteins group 1	*Arabidopsis thaliana* AT2G14610
PR5	Pathogenesis-related proteins group 5	*Arabidopsis thaliana* AT1G75040
NHL10	Late embryogenesis abundant (lea) hydroxyproline-rich glycoprotein family	*Arabidopsis thaliana* NP_181142.1
ACRE31	Avr9/cf-9 rapidly elicited protein 31	*Nicotiana tabacum* AAG43547.1
ACRE132	Avr9/cf-9 rapidly elicited protein 132	*Nicotiana tabacum* AF211532.1
	Other target genes	
DCL	Dicer-Like proteins	*Allium sativum* EPP005KGAA12S003959
RDR 6	RNA-dependent RNA polymerase 6	*Arabidopsis thaliana* NP_001327617.1
AGO	Argonaute family proteins	*Triticum aestivum* AGB34311.1
Tm2²	ToMV resistance protein	*Solanum lycopersicum* AAQ10736.1
PR6	Pathogenesis-related proteins group 6	*Arabidopsis thaliana* NP_199170.2
LTP	Lipid transfer proteins (Pathogenesis-related proteins group 14), (Antimicrobial protein Ace-amp1 precursor mRNA)	*Allium cepa* AF004946.1
WRKY	WRKY transcription factors family proteins	*Arabidopsis thaliana* AEC09374.1
TCTP	Translationally controlled tumor proteins	*Jatropha curcas* EF091818.1
PIRL	Plant intracellular Ras group-related LRR proteins	*Arabidopsis thaliana* NP_196204.1
DBP1	DNA-binding protein phosphatase 1	*Arabidopsis thaliana* NP_001324148.1
CBP60g	CBP60G (Calmodulin-binding protein 60 G)	*Arabidopsis thaliana* OAO89604.1
GRF6	G-box regulating factor 6	*Arabidopsis thaliana* NP_001190276
FRK1	FRK (Fertilization-related kinase 1)	*Arabidopsis thaliana* OAP09570.1

Note. The orthologs are indicated which allow us to construct corresponding primers (see Tables 2, 3).

2. Nucleotide sequences of primers used in determining the expression levels of Pattern-Triggered Immunity (PTI) markers in shallot (*Allium cepa* L. var. *aggregatum*) plants persistently infected by shallot virus X (qPCR)

Primer	Sequence	ID of the transcript *Allium cepa* L. in the database TSA (NCBI)
ARM 530-L	5′- ATGATGCGGGCCTAGTAGAC -3′	GETF01031504.1
ARM 766-R	5′- CTCCCTCGATCAGTCCACTC -3′	
RBOHD 2641-L	5′- GTTTGATCCTAGACGACGCG -3′	GBRN01002659.1
RBOHD 2868-R	5′- TCAACATACCCGACCCGAAA -3′	
EDS5 303-L	5′- TCGCTTGGTCTTGGCTTCTA -3′	GBRQ01023449.1
EDS5 549-R	5′- CGTCTGAGAATCCAACGACG -3′	
LOX3 1753-L	5′- ATGCCACTCGTACGCTTTTC -3′	GBRQ01024907.1
LOX3 1943-R	5′- GACGCCTGCATCATTAGAGC -3′	
BRI1 1541-L	5′- TGTTCCCGCTCAGCTGATTA -3′	GBRQ01031765.1
BRI1 1749-R	5′- TACTTTCGGTGGCAATGGGA -3′	
SOBIR 812-L	5′- CAAGTCATGCAAGCTTCCGT -3′	GBRQ01012958.1
SOBIR 1041-R	5′- CTGGAAAGATGATCGCGGTG -3′	
AtCRK4 1496-L	5′- CTTTCTTGACCTTGGCCTCG -3′	GBGJ01061169.1
AtCRK4 1731-R	5′- TCCCCAGCTAAGCACATCAA -3′	
SERK1 1291-L	5′- CTTCTTCAGCGGGAACATCG -3′	GBGJ01064935.1
SERK1 1478-R	5′- TCCACCTCCTCCATTTGTCC -3′	
PR1 443-L	5′- GTCAAGATCGGTTGCGCTAG -3′	GBJZ01171295.1
PR1 660-R	5′- CCAAGCAAACTCTCATCGCA -3′	
PR5 616-L	5′- ACTGTCTACGGGCCCAAAAT -3′	GBGJ01079964.1
PR5 803-R	5′- ATATGCTGCCTCCGGAACTC -3′	
18s rRNA-L	5′- CATCAGCTCGCGTTGACTAC -3′	[22]
18s rRNA-R	5′- GATCCTTCCGCAGGTTCAC -3′	

3. Nucleotide sequences of primers used in determining the expression levels of RNA silencing factors, NB-LRR receptors and genes involved in viral reproduction in shallot (*Allium cepa* L. var. *aggregatum*) plants persistently infected by shallot virus X (qPCR)

Primer	Sequence	ID of the transcript *Allium cepa* L. in the database TSA (NCBI)
Ago-L-672	5′-AACTCCCAAGAAGCTTTGCG-3′	GBGJ01050630.1
Ago-R-851	5′-CCCTCCTTGAGCAGTTCTGA-3′	
$Tm2^2$-L-3013	5′- TCGTGGGCTCTTTCACTGAT-3′	GBRN01023560
$Tm2^2$-R-3257	5′- CACCCGCTTCATTGGTGTAG-3′	
FRK1-L-421	5′-AGTCACGCTCAATGGCAATG-3′	GAAO01012059.1
FRK1-R-635	5′-CTGCCGCAACATCATAGCAT-3′	
NHL10-L-327	5′-TGCTCCTCACATCGTTCACA-3′	GBRQ01023138.1
NHL10-R-572	5′-AGCTCACCACCTTCACTCTC-3′	
ACRE31-L-473	5′-GCAGTTCTTCGAAAGCAGGA-3′	GBRO01073928.1
ACRE31-R-699	5′-ATTGAGCACATCCTCCCCTT-3′	
ACRE132-L-272	5′-GCCATGCCTCAACCTGATTT-3′	GBRQ01011764.1
ACRE132-R-451	5′-CCTTCTTGATCGGGAAAGCG-3′	
PR6-L-100	5′-ATGAGGGGTACATGGCAGAC-3′	GBRQ01165078.1
PR6-R-274	5′-AAGCATCGGAAGCGAAGAAG-3′	
LTP pcr-59- L	GCA-GTC-CGT-ATG-CAA-AT	AF004946.1
LTP pcr-274-R	TAG-GGT-TTC-GTC-TCA-GAC-CG	
WRKY-L-1367	5′-ACGTGGAAAGGGCATCAAAC-3′	GBRO01047677.1
WRKY-R-1550	5′-GCGACCGGTCTTTGAACATT-3′	
TCTP-L-172	5′-AGGGCAAGTGGGTAGTTCAA-3′	JR844934.1
TCTP-R-382	5′-TCCAATTTCGAAGGCAGAAGT-3′	
PIRL-L-84	5′-ATCATGGATCCAAGCCCCAA-3′	GAAN01019083
PIRL-R-377	5′-CTTTGCGAGGTCAACAGCTT-3′	
DBP-L-392	5′-AGGGTCGTTGTGCTCTTGTA-3′	GBRO01024689.1
DBP-R-623	5′-CAACGGTCAGCTCAACGTAG-3′	
CBP-L-429	5′- GAAGCAGAGGGAAAGCAACC-3′	GBRO01059419.1
CBP-R-673	5′- AAGCCAACACCATCATGCAG-3′	
GRF6-L-607	5′-TCCAGTCTTGAATTCGGCCA-3′	GAAN01023832.1
GRF6-R-829	5′-TTCGATCGAGCAGAAGGAGG-3′	
18S rRNA-L	CATCAGCTCGCGTTGACTAC	[22]
18S rRNA-R	GATCCTTCCGCAGGTTCAC	

Our results show (see Fig. 1) that, to some extent, viral infection affects expression of all the examined genes; however, a group of genes with very high expression both in leaves and in roots of the infected plants is revealed, viz.

SOBIR with log10RQ ~ 1.0, ARM with log10RQ ~ 2.0, PR1 with log10RQ ~ 2.0, and PR5 with log10RQ ~ 4.0 (!). Therefore, it can be concluded that ShVX during the initiation of infection interacts with the factors involved in PTI and TRP is triggered by the PTI induction.

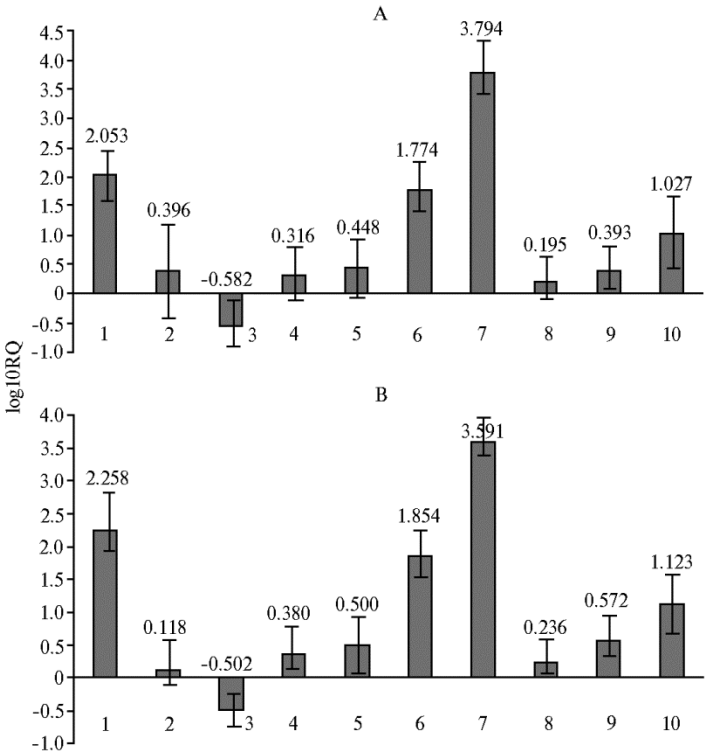

Fig. 1. Transcripts of the Pattern-Triggered Immunity (PTI) marker genes in leaves (A) **and roots** (B) **of shallot (*Allium cepa* L. var. *aggregatum*) plants persistently infected by shallot virus X 2 weeks after the bulb planting:** RQ — Relative Quantification (changes in mRNA expression level compared to the internal control RNA). Symbols of the PTI marker genes are given in Table 1.

At the initial stage of the infection in seedlings (Fig. 2, A), the expression of all target genes (Table 3) is activated (except for *AGO* and *PIRL*), including four additional PTI markers and nsLTP (= PR14, Pathogenesis-Related Proteins Group 14). After 2 weeks of infection, the opposite pattern is noted (see Fig. 2, B). The expression of most target genes, including all RNA silencing factors, three PTI markers, NB-LRR receptors and CBP60g proteins that control the synthesis of salicylic acid, is suppressed in the leaves (Argonaute proteins more than others). However, within the same time frames, the expression of ACRE 132 and LTP (PTI markers) in the leaves show a noticeable tendency to growth, while TCTP expression remaines quite high and stable.

In the roots (Fig. 3, A, B), inhibition of the target genes expression occurs at the initial stage of infection, and after 2 weeks most of the target genes are suppressed, but LTP expression is slightly reduced.

These results lead to the conclusion that in persistent infection ShVX induces the dynamic TRP in roots and leaves, a selective change in the expression of many target genes, including the genes encoding PTI marker proteins, RNA silencing factors, NB-LRR receptors, lipid transfer proteins, as well as the proteins involved in viral replication (TCTP complex). Ultimately, in the examined pathosystem, expression of all silencing factors and some NB-LRR receptors is

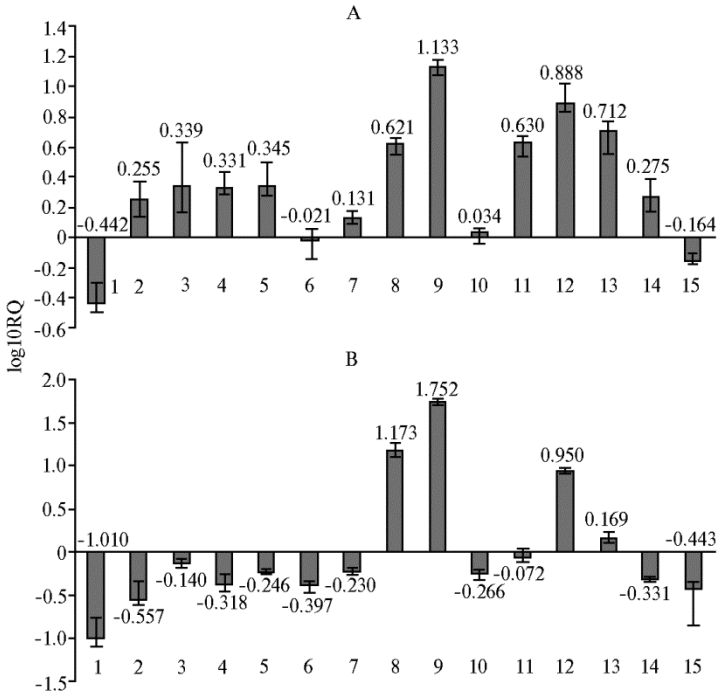

Fig. 2. Target gene transcripts in the above-ground organs of shallot (*Allium cepa* L. var. *aggregatum*) plants at different stages of shallot virus X infection: A — seedlings (3 days after the bulbs were planted), B — leaves (2 weeks after the bulbs were planted); RQ — Relative Quantification (changes in mRNA expression level compared to the internal control RNA).

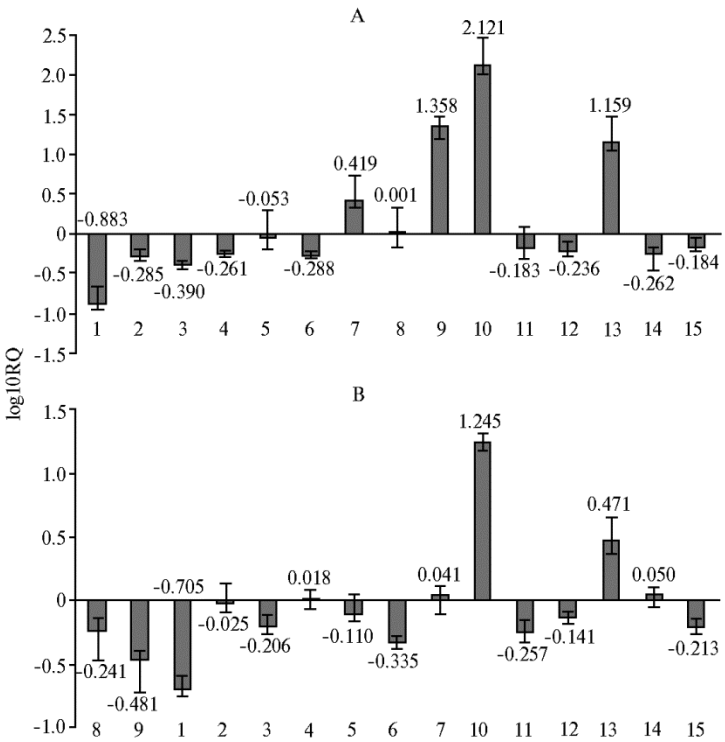

Fig. 3. Target gene transcripts in the roots of shallot (*Allium cepa* L. var. *aggregatum*) plants at different stages of shallot virus X infection: A — 3 days after the bulbs were planted, B — 2 weeks after bulbs were planted; RQ — Relative Quantification (changes in mRNA expression level compared to the internal control RNA).

suppressed, which confirms our initial hypothesis. High induction of the transcription factor WRKY (Group III) and the genes of PR proteins, e.c. PR1, PR5, and PR14 (nsLTP), should be noted as a characteristic feature of reprogramming.

We believe that the induction of PTI, ETI (Effector-triggered immunity) [9] and the resulting transcriptomic reprogramming are the mechanisms, common to all RNA-containing viruses. Due to receptor and signaling functions inherent to innate immune system factors, numerous and diverse immune responses are activated in plants, including RNA silencing. Their goal is to control the reproduction of the virus, but not completely eliminate it. For their part, viruses have developed mechanisms to counteract the host plant immune responses, in particular through suppressor proteins. Many phytoviruses encode very weak, transiently active or inactive suppressors. Thus, from the point of view of the virus, RNA silencing is not a perfect weapon of a cell: it can be ignored or weakened by the suppressor proteins or transcriptomic reprogramming. The virus's choice of the way to suppress the antiviral immunity depends on how the host plant resolves the dilemma of the concept of "growth-defense tradeoffs" [24].

The TRP process described in this paper has organ specific and a pronounced biphasic character. At early stages of the infection, the expression of most target genes is activated, and at later stages it is suppressed. Analysis of the experimental facts also leads to the conclusion that in seedlings, leaves, and roots of the infected plants the transcriptomic reprogramming is likely induced by PTI, but not silencing involving a paralog of Ago-protein family. This paralog is not identified in this work, but its expression, as follows from the data (see Figs. 2, 3), is intensely suppressed at all stages of the infection. However, it can be assumed that at some stage of infection, transcriptomic reprogramming may also be due to the activity of a specific complex of silencing factors (for example, Ago2 + DCL4 + RDR1), the expression of which was probably not suppressed under the conditions of this experiment. As a result of this activity, endogenous small interfering RNAs of an extensive class of virus-activated cellular siRNA (virus-activated siRNAs, vasiRNAs) are generated [25] the targets of which are different genes of a host plant. It is also possible that in persistent ShVX infection the induction of transcriptomic reprogramming may be due to RNA silencing involving small interfering RNA the source of which is the viral genome [26]. Moreover, a yet unknown mechanism of the studied pathosystem may change expression of certain types of microRNAs that control the TRP process [27].

In our opinion, identification of plant antiviral PRR involved in PTI and elucidation of the molecular mechanisms of interaction of these receptors with double-stranded replicative viral RNA will bring to better understanding of TRP induction and its role in antiviral phytoimmunity. The fundamental features of the PTI process induced by viral PAMPs, suggest that the cloning and interspecific gene transfer of plant antiviral PRRs may be promising for creating cultivars with high long-term resistance to a wide range of pathogenic viruses [28].

Thus, our experiments, for the first time in the world, shown that persistent infection of shallot virus X induces in the roots and leaves of shallot plants a dynamic process of transcriptomic reprogramming, resulting in a change in the expression of a wide range of target genes, including those encoding PTI marker proteins, RNA silencing factors, NB-LRR receptors, PR proteins, as well as the proteins involved in virus replication.

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Science events

10th ANNUAL RNA THERAPEUTICS CONFERENCE

(February 20-21, 2019, London, United Kingdom)

Undoubtedly, the field of RNA therapeutics is currently undergoing a major expansion, and the potential for using RNA drugs for personalised medicines and immunotherapy, as well as to address genetic, infectious and chronic diseases will ensure the continued development of RNA therapeutics for years to come.

Disciplines: chemistry, life science, computer science, health science

Information: http://www.therapeutics-rna.com/gel

7th EDITION OF INTERNATIONAL CONFERENCE ON PHARMACOGNOSY AND MEDICINAL PLANTS

(March 11-12, 2019, London, United Kingdom)

Subdisciplines: biology, biochemistry, botany, toxicology, biotechnology, complementary medicine, pharmacy & pharmacology

Information: http://pharmacognosy.euroscicon.com/

ICSD 2019: 7th INTERNATIONAL CONFERENCE ON SUSTAINABLE DEVELOPMENT

(September 4-5, 2019, Rome, Italy)

Subdisciplines: geology, soil science, environmental science, ecology, physiology, environmental engineering, environmental chemistry, earth science, life science, geography, agriculture, climatology, hardware & architecture, social & economic medicine, health science, sports medicine, energy, energy, carbon, education, business and management science, public health

Information: http://www.globaleventslist.elsevier.com/events/2016/09/4th-international-conference-on-sustainable-development-icsd-2016-rome-italy

EMBL COURSE: GENOME ENGINEERING: CRISPR/Cas

(September 17-21, 2018, Heidelberg, Germany)

Subdisciplines: biology, molecular biology, biotechnology

This course will provide hands-on training in genome editing and cell engineering in mammalian cells using the highly efficient CRISPR/Cas9 system. Participants will learn design of CRISPR targets using bioinformatics tools, generation of gene knockouts/knock-ins, and target validation using the most current technologies. This course is aimed at researchers who are familiar with basic molecular and cell biology techniques and want to learn how to create an engineered mammalian cell line using the most recent and advanced CRISPR/Cas9 system. No previous experience in genome editing is required.

Information: http://www.globaleventslist.elsevier.com/events/2018

SMi's 8th ANNUAL PHARMACEUTICAL MICROBIOLOGY UK

(January 21-22, 2019, London, United Kingdom

Addressing the key challenges, trends and strategies in contamination control

Subdisciplines: microbiology, medicine, chemical engineering, biomedical engineering, life science, process engineering, toxicology, biotechnology, general & internal medicine, medical computation & informatics, pharmacy & pharmacology, health science, knowledge transfer, medical ethics / bioethics, medical technology, systems engineering

Information: http://www.globaleventslist.elsevier.com/events/2016/02/parallel-trade/