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Research note

Comparative analysis of gene expression of Ty-1 hybrid and non-hybrid tomatoes exposed to tomato yellow leaf curl virus strains

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Abstract

Tomato (*S. lycopersicum*) is an economically important vegetable food crop and threatened by TYLCV, a devastating pathogen throughout the world. In the present study, we analyzed differential gene expression in response to TYLCV-IL and TYLCV-Mld (*Begomovirus, Geminiviridae*) viral strains infection in the Ty-1 tomato hybrids (Esns1) and non-hybrid tomato (P1788) accessions. cDNA microarray hybridization was used to detect a group of genes that were differentially expressed in Esns1infected with TYLCV strains at 18 days post inoculation. The microarray analysis recognized significantly changed expression of different gene targets. Additional analysis confirmed that 43 genes were significantly up-regulated and 7 were down-regulated in the Esns1against compared to P1788 with TYLCV strains. Overall, major differences in gene expression were characterized by major physiological functions containing pathogen, defense, photosynthesis, cell wall or growth, metabolic, stress, biosynthetic and signaling-related responses. This is the first comparative genome-wide transcriptional study of the expression of Esns1plants depicting diverse responses to biotic stress induced by TYLCV-IL and TYLCV-Mld. Additionally, this study also provides sufficient knowledge into the identification of key defense-related genes in tomato for TYLCV disease management.

Key words: tomato, microarrays, gene expression, begomovirus, TYLCV.
Abbreviations: TYLCV_*Tomato yellow leaf curl virus*; *S. lycopersicum_Solanum lycopersicum*; TYLCV-IL_*Tomato yellow leaf curl virus*-Israel strain; TYLCD_Tomato yellow leaf curl disease; TYLCV-Mld_*Tomato yellow leaf curl virus*-Mild; *S. chilense_Solanum chilense*; *S. habrochaites_Solanum habrochaites*; *S. peruvianum_Solanum peruvianum;* R_Resistant; PVX_Potato virus X; BCoMV_Bean common mosaic virus; *S. pimpinellifolium_ Solanum pimpinellifolium*; ESTs_Expressed sequence tags ; ANOVA_Analysis of variance; FDR_False discovery rate; DEGs_Differentially expressed genes; GO_gene ontology; *Pepper golden mosaic virus*_PGMV; JA_Jamonic acid; *Pseudomonas syringae_p. syringae*; *A. thaliana_Arabidopsis thaliana*; NOS1-Nitric oxide synthase; ABA_abscisic acid; GS_Glutamine synthetase; PSY_phytoene synthase; PDS_Phytoene desaturase; GGPP_Geranylgeranyl diphosphate; *L. chilense_Lycopersicon chilense*; CIAP_Calf intestine alkaline phosphate; *A. tumefaciens_Agrobacterium tumefaciens*; ERF1_Ethylene-responsive factor 1.

Introduction

Tomato (*S. lycopersicum*) is world's most commonly used agricultural crop, and its production is increasing every year. In the meantime, due to excessive transfer of breeding material among diverse countries have increased the chances of incidence of TYLCV, which has caused serious limitation to tomato crops production (Hanssen et al., 2010; Moriones and Navas-Castillo 2000). TYLCV (*Begomovirus* family *Geminiviridae*), which is transmitted by the whitefly (*Bemisia tabaci*) has spread worldwide through its origin from Middle East (Lefeuvre et al., 2010). Many *begomoviruses* are important crop pathogens, among them TYLCD is the greatest devastating and causes ample damages to the tomato production in Japan and the casual agents responsible for TYLCD are TYLCV-IL-[Toc] and TYLCV-Mld-[Tok] strains of TYLCV. Over the past few years entirely all conventional breed cultivars/verities are susceptible to TYLCV, nevertheless a high resistance was found in certain wild tomato species. In recent years, genetic studies have succeeded in the mapping of five TYLCV resistance and/or tolerance genes that are being exploited for breeding resistance tomatoes. However, in last decade many major loci resistant to TYLCV (Ty-1, Ty-2, Ty-3, Ty-4 and Ty-5) have been introgressed into tomatoes isolated from diverse wild tomato accessions. Among them, Ty-1, Ty-2 Ty-3 and Ty-4 derived from different *S. chilense* accessions originated from *S. habrochaites* and Ty-5 was identified in *S. peruvianum*, respectively (Zamir et al., 1994; Hanson, et al., 2006, Ji et al., 2007 and Ji et al., 2009; Anbinder et al., 2009). Compare to classical and/conventional breeding R genes, none of the resistances to TYLCV described so far are related with a hypersensitive cell death response. Additionally, more or less in all TYLCV resistant cultivars, viral replication occurs (Narasegowda-Maruthi et al., 2003; Pico et al., 2000). This has also been proved for Ty-1 with the donors *S. chilense* (LA1969 or LA1932), and it's also true for Ty-1 introgression into the commercial line. TYLCV can

replicate, however the level does not above than 10% that in susceptible tomato cultivars (Pérez de Castro et al., 2013; Shahid et al. 2013). To defend themselves from pathogen attack, plants use different defense mechanisms. The gene-based resistance is the utmost studied phenomenon in plants, and is governed on the ability of the host plant to recognize a pathogen and lastly initiate the hypersensitive response (Jones et al. 2006). Meanwhile, a large number of R genes have been known to responsible for the indirect recognition of viruses, for example in tomato *Sw-5* for tospo-viruses, *Rx2* for *PVX* and the I locus for BCoMV (Brommonschenkel et al. 2000; Vallejos et al. 2006). In recent time, the success in sequencing of tomato and its wild accession *S. pimpinellifolium* genomes have provided massive information into the genetic and genomics in tomato, and this has helped in the identification of important resistant genes in the *Solanaceae* family (Zouine et al., 2012). Furthermore, it has also enhanced resistance in world tomato production to better combat the biotic and abiotic stresses and decrease productivity in this vegetable crop. Microarray is a powerful tool to detect differential gene expression and has discovered several differentially expressed genes in different plants in response to pathogen attack. This has helped in understanding the mechanism of host resistance and the complex nature of plant-pathogen interactions. In this present communication, microarray was used to compare gene expression changes in Esns1 and P1788 in response to TYLCV-IL and TYLCV-Mld infection. The higher proportion of up-regulated differentially expressed genes in the Esns1 was observed. Some sets of pathogen, defense, photosynthesis, cell wall or growth, metabolic, stress, biosynthetic and signaling-related responses were investigated.

Results and Discussions

In this study, microarray analysis was carried out for the global gene expression profiling of Esns1and P1788, exposed to TYLCV-IL and TYLCV-Mld strains by agroinoculation. The successful establishment of infection of TYLCV-IL and TYLCV-Mld were confirmed in Esns1 and P1788 after two weeks of inoculations. After that the systemic leaves of Esns1 and P1788 were harvested, isolated RNA and RNA library was prepared. The data base containing microtome tomato genes cDNA technology allowed the simultaneous analysis of altered gene expression of approximately 56,000 non-redundant ESTs that correspond to 43,000 independent tomato loci on the microtome, a total of 125 tomato genes represented by the spotted probes were significantly differentially expressed at ANOVA ($P<0.05$) between the Esns1compared with P1788 tomato plants exposed to TYLC-IL and TYLCV-Mld infection. Only genes with known functions, 50 genes whose expression was significantly different among the three treatments were considered. Selected putative tomato gene targets belonging to six different Pathogen, defense, photosynthesis, cell wall or growth, metabolic, stress and biosynthetic, signaling-related responses (Table 1). Six putative genes grouped as defense-related genes with four genes having two to three fold expression levels, whereas only one gene has expression level below to one fold. For the comparison of gene expression levels an absolute value of $\log_2$ fold change >1 and the FDR < 0.05 was set to declare DEGs involved in the response of Esns1 and P1788 TYLCV strains infection. For a better understanding of DEGs intricate in the response of Esna1to TYLCV strains infection, the functional classes of DEGs were subjected to GO analysis with blast2go software. Blast2go software placed a large number of DEGs in the biological process, cellular component and molecular functions (Figure 1). Though various diversities between the Esns1 and P1788 were revealed by the DEGs and GO analysis, we were particularly interested in those up-regulated DEGs in the Esns1. After going through references available, at least 43 annotated genes out of the 50 up-regulated DEGs in the Esns1, while 7 were down-regulated compared to P1788 at 18dpi. McKenzie et al. (2005) also confirmed the similar results who revealed numerous genes upregulated at 25 dpi. Recently, the response of resistant and susceptible tomato lines against TYLCV was discovered by Chen et al., (2013), they found that the defense responses of these two tomato lines (resistance and susceptible) to TYLCV infection are quite different. Similarly another study on the begomovirus-vector harboring the PGMV discovered different gene expression in tomato plants in which genes were differentially expressed being infested by viruliferous compared with nonviruliferous whitefly (Musser et al., 2014). The ongoing study revealed the agroinoculation of hybrid and non-hybrid tomato with different TYLCV strains infection and a sample of the gene expression results are discussed below according to assigned biological function.

Defense related response

With respect to defense-related responses, arginase 1and 2 expression was significantly induced in Esns1with both TYLCV-IL and TYLCV-Mld compared with the susceptible P1788. However, previous studies showed that arginase expression is due to a JA signal from application of JA and also in tomato plants infected with *p. syringae* pv. Tomato (Chen et al. 2004). Arginase is mainly present in seeds and other storage organs of several plant species and breakdowns to arginine, to urea and ornithine, a nitrogen-rich storage amino acid, (Van Etten et al. 1967, Polacco and Holland 1993), and its activity is frequently improved during germination in many plants including arabidopsis and soybean (Zonia et al. 1995; Matsubara and Suzuki 1984, Kang and Cho 1990). Nevertheless, environmental stress induces accumulation of some nitrogen containing compounds including arginine, proline, glutamine, asparagine, ammonium, and three polyamines (Kao 1997). The increase in transcripts of proteins associated with nitrogen accumulation is possibly due to the early tissue senescence resulting from TYLCV strains infection as the plant attempts to defend itself and control the spread of the infection. Several other PR-protein, including subtilisin-like endoprotease, pi1 protein, ethylene receptor homolog were significantly upregulated in the TYLCV-IL and TYLCV-Mld-infected Esns1, compared with P1788 tomato plants.

Pathogen-related responses

In the pathogen-related category, pathogenesis-related protein P2 and leucine aminopeptidase expression was significantly upregulated in the Esns1tomatoes. While the expression of these transcripts were not significant with TYLCV-IL as it was observed with TYLCV-IL, however the effect was not significantly different compared with the control plants. Although it is not well understood how the PR proteins affect the response of plants to viral infection, PR protein expression is mediated by the SA pathway and induced by pathogens. These results are consistent with those of other similar studies.

Table 1. Genes with altered expression in TYLCV-IL and TYLCV-Mld-agroinoculated leaves of Esns1and P1788 tomatoes at 18 days after inoculation .

Gene Name (Classification)	Gene Symbol	Entrez Gene ID	GenBank Accession	Esns1*	P1788**	Regulation
Defense-related annotation						
Arginase 1 (*L.esculentum*)	ARG1	543944	AY656837	3.469	-2.011	Up
Ethylene receptor homolog (*L. esculentum*)	ETR4	543588	AF118843	0.515	-0.818	Up
Pi1 protein (*L. esculentum*)	PI1	543758	BT012973	2.106	-1.420	Up
PR protein (*L. esculentum*)	PR1B1	544123	Y08804	2.199	-3.230	Up
Subtilisin-like endoprotease (*L. esculentum*)	PR-P69	544111	X95270	1.563	-0.004	Up
Arginase 2 (*S.lycopersicum*)	ARG2	544271	AK321112	3.542	-2.264	Up
Pathogen-related annotation						
Pathogenesis-related protein P2 (*L. esculentum*)	PR-P2	544069	BT013355	0.536	-2.080	Up
Leucine aminopeptidase (*L. esculentum*)	LAP2	544017	U50152	3.946	-1.904	Up
Signaling-related annotation						
Ethylene-responsive factor 1 (*L. esculentum*)	ERF1	606712	AY044236	2.924	-0.547	Up
TDR3 protein (*L. esculentum*)	TDR3	544075	X60756	0.645	-1.741	Up
Ethylene receptor (*L. esculentum*)	NEVER-RIPE	544279	BT013741	0.204	-0.876	Up
Photosystem-related annotation						
Photosystem II 10 kD polypeptide (*L. esculentum*)	LOC780564	780564	Z75521	-0.383	0.436	Down
Threonine deaminase (*L. esculentum*)	TD	543983	M61914	1.177	-5.602	Up
Subtilisin-like protease (*L. esculentum*)	P69B	544296	Y10149	2.277	-0.586	Up
Ribulose-1,5-bisphosphate carboxylase precursor (*S. lycopersicum*)	RBCS-1	543973	AK319577	-0.587	0.450	Down
33kDa precursor protein of oxygen-evolving complex (*L. esculentum*)	PSBO	778353	DQ539439	-0.146	0.654	Down
Cell wall, growth-related annotation						
Glucan endo-1,3-beta-D-glucosidase (*S. lycopersicum*)	TOMQ`B	544092	AK323220	0.484	-2.572	Up
Ornithine decarboxylase (*L. esculentum*)	ODC	544209	AF029349	1.681	-1.128	Up
Carbonic anhydrase (*L. esculentum*)	CA1	543802	AJ849375	0.594	-0.781	Up
Phospholipase (*S. lycopersicum*)	PLDA2	544251	AY013253	1.644	-0.379	Up
S-adenosylmethionine decarboxylase (*S. lycopersicum*)	SAMDC	100134880	EF550528	1.211	-0.557	Up
Gluaredoxin (tomato)	LOC544298	544298	GO372328	1.069	-0.153	Up
Metabolic process-related annotation						
Eli3 protein (*S. lycopersicum*)	ELI3	543602	AK329686	1.592	-0.615	Up
2-oxoglutarate-dependent dioxygenase (*S. lycopersicum*)	LEODD	100125906	AK324589	3.374	-0.897	Up
Iron superoxide dismutase (*S. lycopersicum*)	FESOD	544259	AK329391	1.441	-0.769	Up
Succinic semialdehyde dehydrogenase (*L. esculentum*)	LESSADH	100147726	BT013982	0.729	-0.320	Up
Nitrite reductase (*L. esculentum*)	NII2	778326	BT014587	2.345	-0.928	Up
Gibberellin 2-oxidase (*S. lycopersicum*)	SLGA2OX4	100134889	GO376166	2.627	-0.587	Up
Ss-galactosidase (*L. esculentum*)	TEG3	543736	AJ012798	0.994	0.421	Up
Glutamine synthetase (*S. lycopersicum*)	GS	543998	AK319584	-0.108	0.969	Down
Xyloglucan endoglucanase inhibitor protein precursor (*S. lycopersicum*)	XEGIP	543853	AK323482	1.206	-1.764	Up
Ethylene-inducible CTR1-like protein kinase (*L. esculentum*)	TCTR1V	544218	AF110519	0.989	-0.140	Up
Aromatic amino acid decarboxylase 2 (*S. lycopersicum*)	AADC2	778256	AK323831	1.461	-0.015	Up
Peroxidase (*S. lycopersicum*)	CEVI16	544293	AK325152	2.096	-0.346	Up
Beta-fructosidase (*S. lycopersicum*)	AIV-1	100125905	D11350	0.369	-0.649	Up
Sucrose synthase (*S. lycopersicum*)	SUS3	543731	AK325807	0.084	-1.488	Up
Xyloglucan endotransglycosylase (*S. lycopersicum*)	LEXET2	543619	AK321633	1.264	-0.776	Up
Acid invertase (*L. esculentum*)	AI	543992	S70040	0.393	-0.708	Up
Stress-related annotation						
Nitric oxide synthase (*S. lycopersicum*)	NOS1	778272	AK327734	-0.223	1.100	Down
SPM1 protein (*S. lycopersicum*)	SPM1	543894	AK324059	2.281	-1.248	Up
TSW12 protein (*L. esculentum*)	TSW12	544066	BI933938	2.814	-1.765	Up
PR1 protein (*S. lycopersicum*)	LOC10019111	100191111	AK324158	3.040	-3.884	Up
TSI-1 protein (*S. lycopersicum*)	TSI-1	544134	AK247106	1.820	-1.092	Up
Formate dehydrogenase (*S. lycopersicum*)	FDH	544250	AK321203	0.959	-2.766	Up
Hero resistance protein 1 homologue (*L. esculentum*)	HELP1	544247	AJ457048	1.218	-1.376	Up
Chitinase (*S. lycopersicum*)	LOC544148	544148	AK322999	1.979	-1.139	Up
Wound-induced proteinase inhibitor II prepeptide (tomato)	LOC543955	543955	K03291	3.032	-4.850	Up
Fatty acid/ biosynthetic-related annotation						
Lycopene epsilon-cyclase (*S. lycopersicum*)	CRTL-E-1	544129	AK321362	0.022	1.010	Down
Lipoxygenase (LOX) (*L. esculentum*)	CEVI34	100125903	X94945	0.635	-1.391	Up
Geranylgeranyl pyrophosphate synthase 2 (*S. lycopersicum*)	GGPS2	778360	DQ267903	0.787	1.300	Down

*Ty-1 hybrid tomato; **non-hybrid tomato

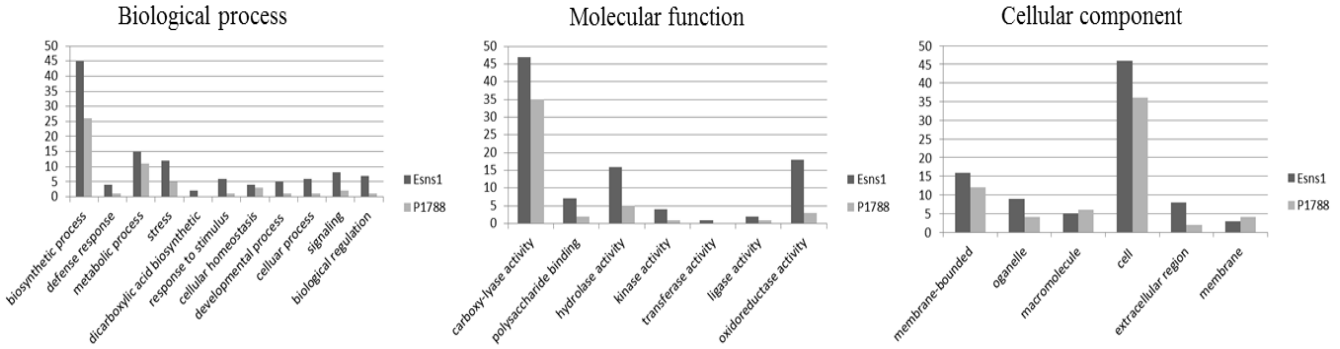

Fig 1 Functional categories (GO) distribution of different expressed transcripts in the Esns1 and P1788 tomatoes. Esns1: Ty-1 hybrid tomato accession P1788: non-hybrid tomato (susceptible). The percent of DEGs which belong to three major functional categories (biological process, molecular function and cellular component) are shown. Percent of transcript is reported for each functional category on y-axis.

Photosynthesis-related responses

In photosynthesis-related responses, photosynthesis-related protein genes, photosystem II polypeptide proteins, ribulose-1,5-bisphosphate carboxylase precursor and 33kDa precursor protein of oxygen-evolving complex were downregulated in the combined TYLCV-IL and TYLCV-Mld-treated Esns1 compared with the P1788 tomato plants. The 33-kDa polypeptide of the oxygen-evolving complex of photosystem II is nuclear encoded protein. Pradeep et al., (1998) studies the single psbO gene of *A. thaliana* that harbors two introns and encodes a precursor polypeptide of 332 amino acid residues; the first 85 amino acid residues represent the transit peptide and the following 247 amino acids constitute the mature polypeptide. It is expressed in a tissue-specific manner and the transcript levels being highest in the leaves and undetectable in the roots. Also, expression of the psbO gene is development-dependent and regulated by light in young arabidopsis seedlings. However, the expression was upregulated in case of threonine deaminase and subtilisin-like protease proteins in Esns1 compared to P1788 tomato plants. Generally, with respect to wound/infections the photosynthetic protein translation is turned off (Haldrup et al. 2000) because maintenance of the photosynthetic machinery signifies a major expenditure of cellular energy. Suppressing de novo synthesis of these proteins would save the plant energy following tissue damage (Zhou and Thornburg 1999). Our study demonstrate that gene expression involved in the photosynthetic machinery was reduced following the TYLCV-IL and TYLCV-Mld-infected and could possibly be due to reallocation of resources for defense protein synthesis.

Signaling-related responses

In signaling-related category genes encoding ERF1, TDR3 and Ethylene receptor protein were significally upregulated by the TYLCV-IL and TYLCV-Mld-infected Esns1tomatoes. Schenk et al. (2000) proved that *A. thaliana* treated with ethylene upregulated a protein kinase nearly fivefold. In our study, TYLCV-IL and TYLCV-Mld-infected significantly increased signal transduction associated with mediating the host plant response to disease, suggesting that tomato plants detected the subtle signals responded to viral pathogen attack.

Stress-related responses

NOS1 protein gene was significantly downregulated with TYLCV-Mld- infected Esns1compare to P1788. Plants produce four different nitric oxide synthase enzymes. Nitrite-dependent NO synthesis is catalyzed by cytoplasmic nitrate reductase/root plasma membrane enzyme and/or occurs non-enzymatically. Nitric oxide function in drought and ABA induction of stomatal closure requires nitrate reductase and NOS1. Nitric oxide synthase likely functions to produce sufficient NO to inhibit photosynthetic electron transport, allowing nitrite accumulation. Nitric oxide is produced during the hypersensitive responses outside of cells undergoing programmed cell death immediately prior to loss of plasma membrane integrity (Shapiro 2005)

A wound dehydrogenase and PR1 protein genes were also significantly upregulated in the TYLCV-IL and TYLCV-Mld-infected Esns1 tomato plants. These proteins are expressed under low temperature stress. In addition, a SPM1 (a stress-activated MAP kinase that regulates morphogenesis in S. pombe), TSW12 (Non-specific lipid-transfer protein 1), Formate dehydrogenase proteins were also upregulated in TYLCV-IL and TYLCV-Mld-infected Esns1 tomatoes. These proteins act as molecular escorts to aid organisms during stress by preventing denaturation of proteins acute to plant physiological processes. Their significant expression suggests that tomato plants detected and responded to virus infection, presumably, to protect themselves from damage.

Metabolic process-related responses

In the metabolic process categories Eli3 protein, 2-oxoglutarate-dependent dioxygenase, iron superoxide dismutase, succinic semi aldehyde dehydrogenase, acid invertase, gibberellin 2-oxidase, ss-galactosidase , xyloglucan-specific fungal endoglucanase inhibitor protein precursor, ethylene-inducible CTR1-like protein kinase, aromatic amino acid decarboxylase 2, peroxidase, beta-fructosidase, sucrose synthase, xyloglucan endotransglycosylase, nitrite reductase expression was significantly induced in Esns1. However, the expression of glutamine synthetase was significantly down-regulated in Esns1. GS is an enzyme that plays a vital role in the metabolism of nitrogen by catalyzing the condensation of glutamate and ammonia to form glutamine. However, the

metabolic responses related of GS was significantly down-regulated in Esns1 compared to P1788 tomatoes. Hence plants have two or more isozymes of GSII, one of the isozymes is translocated into the chloroplast. Glutamine synthetase uses ammonia produced by nitrate reduction, amino acid degradation, and photorespiration (Liaw et al., 1995). The amide group of glutamate is a nitrogen source for the synthesis of glutamine pathway metabolites (Jump et al., 1993).

Fatty acid/ biosynthetic-related annotation

In fatty acid or biosynthetic response category the expression of two genes were significantly downregulated while the expression of one gene was downregulated. The red color of tomato fruits is provided by the carotenoid pigment lycopene whose concentration increases dramatically during the ripening process. Ronen et al., (1999) proved that lycopene accumulation in tomato fruits is based on the differential regulation of expression of carotenoid biosynthesis genes. They also found that during fruit development, the mRNA levels for the lycopene-producing enzymes PSY and PDS increase, while the mRNA levels of the genes for the lycopene beta and epsilon-cyclases, which convert lycopene to either beta or delta-carotene, respectively, decline and completely disappear. GGPP is the precursor for the biosynthesis of gibberellins, carotenoids, chlorophylls, isoprenoid quinones, and geranylgeranylated proteins in plants. Okada et al., (2000) discover the GGPP synthase gene is expressed in different tissues during plant development and GGPP is synthesized by the organelles themselves rather than being transported into the organelles, furthermore they also predict there will be specific pathways of GGPP production in each organelle. Surprisingly, the expression of gene encoding lycopene epsilon-cyclase and geranylgeranyl pyrophosphate synthase 2 was strongly down-regulated, contrary to the expression of lipoxygenase which was up-regulated in the fatty acid/biosynthetic-related response.

Materials and Methods

Plant and virus source

Tomato hybrids produced based on Ty-1 tolerant gene (Esns1) isolated from wild tomato *L. chilense* (Kagome Co., Ltd., Tochigi, Japan) and non-hybrid tomato (P1788) was used as susceptible control. Two domestic TYLCV strains TYLCV-IL and TYLCV-Mld were used to agroinoculate Esns1 and P1788 tomato plants in the microarrays experiments.

Infectious clone constructions

For *Agrobacterium*-mediated inoculation partial repeat constructs of TYLCV-Mld was produced by general method (Amrao et al. 2010). pGreen0029 binary vector was digested with *SacI* and BamH*1*and subsequently an approx. 1075 bp (containing hair pin) fragment of TYLCV-Mld already cloned in PGEM-T vector was released and ligated to produce pGTYLCV-0.4 (binary vector and partial part). Next, full-length TYLCV-Mld insert was released by *Sac*1 digestion and ligated into pGTYLCV-0.4 already linearized and treated with CIAP to produce pGTYLCV-1.4 (a partial repeat construct). Infectious clone of TYLCV-IL was produced using the same strategies.

Agroinoculation and growth conditions of tomato plants

The partial repeat constructs were transformed into *A. tumefaciens* (GV3101) strain. Transformed *Agrobacterium* of recombinant plasmids of TYLCV-IL and TYLCV-Mld were grown in 50 ml LB liquid supplemented with the appropriate antibiotic selection. The bacteria were pelleted by centrifugation at 4000 rpm for 10 min at 4^{o}C and resuspended in infiltration solution (10 mM $MgCl_2$, 200 µM acetosyringone). Young plants of Esns1 and P1788 were agroinoculated at the 4th to 6th leaf stage, as described previously (Idris et al. 2011). The agro-infiltrated plants were monitored for virus infection in an insect free and secured growth rooms at 28^{o}C, daily cycle of 16h light and 8h dark for four weeks.

Preparation of total RNA

Total RNA was extracted from systemic tomato leaves tissues by using RNeasy Plant Mini Kit (QIAGEN Sciences, Maryland, and USA). The total RNA was quantified by spectrophotometry using NanoDrop™ 1000 Tem Scientific) and RNA quality was evaluated using Agilent 2100 Bioanalyzer (Agilent).

cDNA array synthesis and preparation of probe and hybridization

Three nylon membranes each of (8×12 cm) by a BIOMEK 2000 robotic workstation (Beckman nstruments, Inc., Fullerton, CA, U.S.A.) corresponding to ~9,000 genes used according to the manufacturer's instruction manual (Asamizu et al., 2000, Hirai et al., 2003). Preparation of the RNA and cDNA, and hybridization reactions were conducted according to Ishihara et al. (2004). Total RNA was reverse transcribed to synthesize [3P]dCTP-labeled cDNA probes followed the protocol established by Ishihara et al. (2004) with slight modifications in washings. In our experiment we increase the membranes incubation period from 20 h to 22 h and washed the membranes twice, with 1× SSC containing 0.1% SDS at 65°C for 14 min. Finally the membranes were enveloped with plastic film and exposed to an IP image plate (Fuji Photo Film, Tokyo, Japan) for 72 h.

Microarray analysis

With assistance the microarray suite version 5.0.1 software (Affymetrix) scanned GeneChip images were analysed). Normalization and analysis of microarray data were performed using GeneSpring GX 7.3 software (Agilent Technologies, URL: http://www. home.agilent.com/). The data were normalized per chip and per gene to the median value. IP image plates were scanned with FX (Bio-Rad, U.S.A.) for signals and quantified using Array Vision 5.1 software (IMAGING Research Inc., Ontario, Canada). Signal intensity was calculated by subtracting raw signal intensity (vol) with local background (bg) quantified in the corners around individual spots. For each membrane normalization of signal intensity was calculated using the following formula: normalized value = (vol – bg)/MED. Three replicate experiments for one set of membranes were conducted for consistency. The average of the normalized value of the signal intensity for each gene in three replicate experiments was accepted as the expression value of the gene.

Data analysis

To select differentially expressed genes among the treatments: TYLCV-IL, TYLCV-Mld-infected Esns1and P1788, stringent criteria were applied. When the value of gene expression was ≤0.3 in one of the treatments, the gene was eliminated for further analysis, because such low intensity data are less reproducible. Differentially expressed probes were recognized by linear models analysis (Smyth GK2004) using limma package and applying Bayesian correction, adjusted p-value of 0.05 and a $|FC| \geq 2$. Genes were grouped in main functional categories according to the "biological" terms of the GO (http://www.geneontology.org) assigned to each tomato TC or EST (Release 12.0) and manually curated annotation of differentially expressed transcripts was on the basis of the results of Blast P analysis against the UniProt database (UniProt Database http://www.uniprot.org/) (Figure 1) using terms of biological process of GO. Genes without significant BlastP results were classified as "no hits found" (Evalue < 1e-8; identity > 40%). (adjusted p-value ≤0.05; $|FC| \geq 2$). To identify the genes having similar altered expression patterns in the replicate experiment One-way analysis of variance of the expression values was performed. Gene which expression level was significantly different among all treatments was selected using the Fisher's least significant difference procedure. The expression value for TYLCV-IL or TYLCV-Mld-divided by that of P1788 indicated the ratio of induction or suppression. Ratios of <1 were transformed to –1/ratio. When the value of gene expression greater than 3-fold or decreased less than –3-fold in the TYLCV-IL or TYLCV-Mld - inoculated plant, we identified the gene expression as altered reproducibly between two treatments.

Conclusions

In this communication we demonstrated the first global microarray analysis performed on TYLCV-IL and TYLCV-Mld, a monopartite phloem-limited begomovirus strains infecting its natural host. Our findings highlight several genes have been shown to be differentially expressed in Esns1tomato plants at 18 dpi. Many of them were specifically associated with the TYLCV-Mld, compared with the P1788 tomato plants. Major different gene expression was observed for eight significant physiological function categories, containing pathogen, defense, photosynthesis, signaling, cell wall, metabolic process, stress and biosynthetic related responses. Several of these transcripts/ proteins encoded by them have been identified in studies involving TYLCV-infected Esns1tomato plants. This study also provides new insight into the biology of TYLCV-plant interactions and represents a step toward in the identification of candidate genes for future tomato breeding programs to abate the assault on key defense responses to the invading TYLCV complex.

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