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Differential Expression of Pathogenesis Related Protein Genes in Tomato during Inoculation with *A. Solani*

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Abstract

To further increase our understanding of responses in tomato to early blight pathogen, we studied a microarray analysis using Affymetrix Tomato Gene chip array, representing approximately 10,000 genes. Our goal was to understand the pattern of expression of pathogenesis related proteins, which have important roles during interaction between host and pathogen. We found that total thirty two genes in this category showed significant changes in resistant and susceptible genotypes i.e. EC-520061 and CO-3. Amongst these thirty two genes, twenty genes were up regulated in case of resistant genotype whereas no significant up regulation in fold change (FC) was observed in case of the susceptible genotype. This study might be useful for further improvement of resistance in agronomically accepted tomato variety.

Keywords: Tomato; *A. solani*; Expression profiling; Microarray; Pathogenesis related proteins

Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most economically important crops worldwide; however, it is susceptible to over 200 pathogens that cause severe destruction for this plant and consequent great reduction in the yield. Many strategies to control these diseases and others on tomato have been developed. However, the major component used in integrated pest management (IPM) studies was the chemical fungicides. The implication of chemical fungicides in soil and water pollution has mandated the search for alternative approaches to disease control management. One of these approaches could be development of resistant cultivar against the disease [1]and for that there is need to know the mechanism behind the resistance reaction i,e. proteins and genes involved in checking the disease in the plant.

Early blight (EB) is one of the most damaging diseases in many tomato production areas worldwide [2,3] incited by *Alternaria solani*. It reduces the quality and market value of tomato. This disease becomes serious when the season begins with abundant moisture or frequent rainfall by warm and dry weather which are unfavorable for the host and help rapid disease development. Measures to control the outburst of the diseases include a 3 to 5 year crop rotation, routine application of fungicides, and the use of disease free transplants [3,4]. Among the three control measures, fungicide treatments is generally the most effective but they are not economically feasible and might be ineffective when weather conditions are favorable for epidemics. The pathogen is also known to infect tomato fruit on maturity therefore fungicidal spraying during period will effect fruit quality and render it unfit for human consumption due to residual effect of the fungicide. Growing resistant varieties is the most effective and feasible technique for EB management in tomato. Progress in breeding for EB resistance has been, however,limited by the lack of effective resistance genes in cultivated tomato [5-7] and by quantitative expression and polygenic inheritance of the resistance [8-10].

When plant is attacked by pathogens, they defend themselves with an arsenal of both passive and active defense mechanism [11].The passive or pre-existing defense mechanism involves structural barriers or strategically positioned reservoirs of antimicrobial compounds which prevent colonization in the tissue. The active or induced defense mechanism include the hypersensitivity response, the production of phytoalexin, lignifications and the reinforcement of the cell wall, as well as the bio-synthesis of pathogenesis related proteins.

Pathogenesis-related proteins (PRs) are plant species-specific proteins produced in response to infection with viruses, fungi or bacteria. Several monocot and dicot plants have been found to produce PRs through a ubiquitous reaction during pathogen attack [12,13]. They have been associated with systemic acquired resistance and incipient anti-pathogen effects. These pathogenesis related responses and inhibition of fungal growth because of these proteins proved their defensive functions in the plant [14-16].They are produced in large quantities in hypersensitive and resistant reactions.Association of PRs in tissue-specific expression during development, consistent localization in the apoplast and vacuolar compartment, and their differential induction by endogenous and exogenous signaling compounds suggests that many PRs may also be involved in other important functions beside plant defense.

Genes responsible for the expression of pathogenesis related proteins has been significantly improved the resistance against various pathogens in different crops [17,18].Understanding the molecular interaction between the EB pathogen (*A. solani*) and tomato plant might give more insight into the resistance mechanism of tomato to *A. solani*. Thus, this study aimed to identify PRs and others genes differentially expressed in the tomato host plant upon attack from the EB pathogen (*A. solani*).

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Material and Methods

Plant material

Two tomato genotypes; EC-520061 resistant to early blight, and CO-3 susceptible to early blight were selected on the basis of their performance against EB pathogen during screening experiment [19] for transcription profiling in order to study their response against early blight. The seeds of these varieties were obtained from Indian institute of Vegetable Research, Varanasi, Uttar Pradesh, India. The plants were grown in growth chamber under temperature–controlled condition at 25°C.

Preparation of fungal inoculums

An *A.solani* isolate obtained from infected tomato leaves in Indian Institute of Vegetable Research, Varanasi was propagated on Potato Dextrose Agar (PDA) in 90-mm Petri dishes. The dishes were incubated at25°C under a cool-white fluorescent diurnal light with 12 h photoperiod for 10-15 d. A 15-d-old culture was scraped and macerated together in a pestle and mortar. This culture was free from conidia but thickening of conidiogenous hyphae and chlamydo spore-like structures were observed in a 15 dold culture. Before the formation of these structures, cultures did not have their usual aggressiveness and potential for infection. Tomato plants were sprayed with an inoculum (157 $cfuml^{-1}$) to induce infection.

Treatment of tomato plants with *A. solani* inoculum and RNA isolation

Seeds of the two genotypes, EC-520061 and CO-3, were germinated and transplanted into a small pot with soil and places in the growth chamber. During the late seedling stage (6-7 weeks old), plants were individually inoculated with *A. solani* isolate. All inoculated plants were kept moist by maintaining >95% relative humidity (RH) and 20-25°C temperature for 5 d. The plants were closely observed each day after inoculation. After the 5d infection period, the RH was reduced to ~85% and plants were maintained under 12h photoperiod. The RH, light and temperature were controlled by a microprocessor regulated system. Plants were individually evaluated for disease symptoms after 7 d of inoculation.The stress treatments were performed on resistant and susceptible tomato genotypes in three biological replications. The experiment was conducted in reference design, where respective tissues from unstressed plants served as control. The samples for RNA isolation was collected at 24h after inoculation and kept at -80°C till RNA isolation was performed. RNA was isolation using TRIZOL method using manufactures protocol (Invitrogen, USA).

Expression profiling and data analysis

Affymetrix gene expression kit (Affymetrix, USA) was used for profiling of tomato genome in response to

A. solani infection. Raw microarray data were analyzed to find genes with significantly different expression profiles under early blight stress relative to control conditions. The data was analyzed using Gene Spring 10.1 GX software from Agilent Technologies, Inc. 3501 Stevens Creek Blvd. Santa Clara, CA 95052 USA.

Microarray validation: quantitative real-time RT-PCR analysis

The total RNA was isolated using TRIZOL (Invitrogen, USA). First strand cDNA for each sample was synthesized by using Superscript™III first-strand synthesis system for RT- PCR (Invitrogen, Carlsbad, CA, USA) following the manufacturer's instructions. Primers for quantitative real-time RT-PCR were designed using web based primer designing tool from IDT (http://eu.idtdna.com/Scitools/Applications/Primerquest/default.aspx). The sequences of all primers are enlisted in the table 1.

cDNA of each sample was diluted to 200 $ng\mu l^{-1}$ before amplification. The mRNA expressionlevels of selected probes were analyzed by quantitative real-time PCR (qPCR) using iQ-SYBR Green Supermix (Bio-Rad, CA, USA) according to the manufacturer's protocols on iQ5 thermo cycler (Bio-Rad, CA, USA) with iQ5Optical System Software version 2.0 (Bio-Rad, CA, USA). For calculation of the threshold cycle (C_T) values, the auto-$C_T$function was used. Each sample was analysed in three technical replicates and for further calculations, the mean value of each triplicate was used. To normalize the target gene expression, the difference between the $C_T$of the target gene and the $C_T$of Actin (constitutive control) for the respective template was calculated (ΔC_Tvalue). To calculate fold changes (FC) in gene expression, the ΔC_T value was calculated as follows: $\Delta C_T = C_T$(target gene)$-C_T$ (constitutive control gene). Relative transcript levels were calculated as: $1000\times2^{-\Delta CT}$.

Results

To identify host responses to*A. Solani* infection, we analyzed the expression profiles of 10,308 genes represented on the Affymetrix Tomato Genome Array Gene Chip. After 24 h of inoculation of plants

	Forward Primer	Reverse Primer
Down regulated Genes		
Pathogenesis related protein (PRB-1)	GCAGACTCATACACTCTGGTGG	ACTCCATTGCACGTGTTCGCAG
Auxin response factor 2 (ARF-2)	TCGCTACAGATGCAGCGTGTTCTA	TGAGCCCTCAGCAACAGAAGAAGT
Salicylic acid binding protein-2 (SAB-2)	GGCTGCTTTCATGCCTGATTCTGT	AGCTTGTGAGCCAAGAACTTTGGG
Peroxidase (PA-2)	TGGAGGTCCAACATGGCAAGTTCT	TGCCACATCTTGCCCTTCCAAATG
Gibberellin 20-oxidase-1 (20 ox-1)	GGGCCTCATTGTGATCCAACATCA	GGAACGCCATTCATCGTCCACAAA
Abscission polygalacturonase (TAPG-2)	TGCATCTCTATTGGCCCTGGAACT	CCCAACTCTTGTTGTTTCCAGCCT
Lipid binding (LADH-SF)	ATCCATCCCACCACACTCGTCAAT	TCCATGTCAAGTCACTCCCAGTGT
Up regulated Genes:		
Ethylene-responsive transcription factor 3 (RAP-2)	AAAGAACCATCTGTGGCGTGTGAG	CGAATCTTGTAAGCGGCTTGGTCA
Xyloglucan endotransglycosylase (XET-2)	TGGAGGAGATTCTGCTGGTGTTGT	TCTGTCTCCTTTGCCTCCTGTGAA
Class IV chitinase (CHI-14)	ATGTCACGCATGAGACTGGACACT	AATCCTTCCCGGACACACATGGAT
Ascorbate peroxidise (APX-1)	ATGTCACGCATGAGACTGGACACT	AATCCTTCCCGGACACACATGGAT
Catalytic hydrolase -2 (ACS-2)	TTCCATCACTGCAGCTTTGCTTCG	TTTGTTTGGGCCAGCTTCTCTCTC

Table 1: The nucleotide sequences Primers of Real Time PCR.

with *A. solani*, when the infected leaves werenot curled and the only disease symptom detected as the initial appearance of black spots, the leaves were sampled for RNA isolation. *A. solani* inoculated tomato leaves were compared with those in mock-inoculated plants in three independent biological replicates. Genes showing at least a 2-fold change were considered as differentially expressed. In this study, we have observed differentially expressed defense related genes (Table2).

Differential expression of pathogenesis related proteins

Total thirty two genes in category of pathogenesis related proteins involved in defense mechanism to *A. solani* were differentially expressed in this experiment.Amongst these thirty two genes, twenty genes were up regulated in case of resistant genotype whereas no significant up regulation in fold change (FC)was observed in case of the susceptible genotype. The up-regulated genes in the resistant genotype were Arginine decarboxylase (FC 16.29), DnaJ-like protein (FC 12.42), Xyloglucan endo-transglycosylase (FC 11.74), Putative acyl-CoA synthetase (FC 10.59, 7.28), Patatin-like protein 3 (FC10.01), 17.6 kDa class I heat shock protein (FC 8.83), Tuber-specific protein (FC 8.29), Ethylene-responsive late embryogenesis-like protein (FC 7.28), Glutathione S-transferase (FC 6.88), Plastidicaldolase (FC 6.67), Rhodanese-like (FC 6.43), Cytosolic ascorbate peroxidase (FC 6.03), Pathogenesis-related protein-like protein (FC 5.57), Heat shock cognate 70 kDa protein 1(FC 5.56), 101 kDa heat shock protein (FC 5.24), Unknown proteins (FC 7.96, 5.18) , ChaC-like family protein-like(FC 5.12) and Iron superoxide dismutase (FC 5.01). The change in expression of these genes was insignificant in susceptible genotype.

On the other hand, after 24hrs of inoculation with *A. solani*, 12 genes found to be down regulated in the resistant genotypes wereEndo-beta-1,4-D-glucanase (FC -2.48), Gibberellin 20-oxidase-1(FC -5.22), Peroxidase (FC -5.24, -7.30), Abscission polygalacturonase (FC -5.51), Aspartic-type endopeptidase/ pepsin A (FC -5.67), Lipid binding protein (FC -5.77), Ethylene-responsive catalase (FC -5.92), Catalytic hydrolase (FC -8.28), Polygalacturonase precursor (FC -10.24), Salicylic acid-binding protein 2 (FC-17.09), Glucan endo-1,3-beta-glucosidase precursor (FC -18.63). Interestingly, the three genes (lipid binding protein, gibberellin 20-oxidase-1, and endo-beta-1,4-D-glucanase) were down-regulated in the resistant and up-regulated in the susceptible genotype

Microarray validation

The microarray data was validated by quantitative Real-time PCRand the relative fold change in expression was analyzed by the $2^{-\Delta\Delta CT}$method. The quantification was relative because the gene expression value analyzed was normalized in relation to the expression of internal reference gene (endogenous control) in the same cDNA

Affymetrix Probe ID	Gene ID	Description	Fold change in resistant genotype	Fold change in susceptible genotype
Les.4004.1.S1_a_at	Les.8179	17.6 kDa class I heat shock protein	8.83	-1.10
Les.3766.1.S1_at	Les.3766	Ethylene-responsive late embryogenesis-like protein	7.28	-1.69
Les.3125.1.S1_at	Les.3125	Xyloglucan endotransglycosylase	11.74	1.45
Les.4819.1.S1_at	Les.4819	Heat shock cognate 70 kDa protein 1	5.56	-1.10
Les.4225.1.S1_at	Les.11471	Plastidic aldolase	6.97	-1.12
LesAffx.3099.1.S1_at	Les.8243	Putative acyl-CoA synthetase	10.59	1.22
LesAffx.14776.1.S1_at	Les.7742	Unknown protein	7.96	1.62
LesAffx.3918.1.S1_at	Les.11394	Cytosolic ascorbate peroxidase	6.03	1.25
LesAffx.47187.1.S1_at	Les.208	101 kDa heat shock protein	5.24	1.17
LesAffx.64902.2.S1_at	Les.9517	ChaC-like family protein-like	5.12	1.02
LesAffx.46815.1.S1_at	Les.5864	Unknown protein	5.18	1.79
LesAffx.3606.1.S1_at	Les.12361	Tuber-specific protein	8.29	-1.65
Les.2677.1.S1_at	Les.8243	Putative acyl-CoA synthetase	7.28	1.82
LesAffx.55504.1.S1_at	BG124298	Pathogenesis-related protein-like protein	5.57	1.63
Les.179.1.S1_at	Les.12505	Arginine decarboxylase	16.29	-2.06
LesAffx.68054.1.S1_at	Les.11984	DnaJ-like protein	12.42	1.34
Les.2721.2.S1_at	Les.2721	Rhodanese-like protein	6.43	1.15
Les.1724.3.A1_at	Les.1724	Glutathione S-transferase	6.88	1.30
Les.4233.2.A1_at	Les.3014	Iron superoxide dismutase	5.01	1.19
LesAffx.3554.1.A1_at	CK720570	Patatin-like protein 3	10.01	1.60
Les.3665.1.S1_at	Les.3665	Polygalacturonase precursor	-10.24	1.10
Les.3647.1.S1_at	Les. 3647	Abscission polygalacturonase	-5.51	1.16
Les.3549.1.A1_at	Les.3549	Ethylene-responsive catalase	-5.92	1.14
Les.1044.1.A1_at	Les.1044	Catalytic hydrolase	-8.28	1.35
LesAffx.39.1.S1_at	Les.6908	Peroxidase	-5.24	-1.03
LesAffx.60831.1.S1_at	Les.10237	Peroxidase	-7.30	1.80
LesAffx.62698.2.S1_at	Les.8987	B-1,3 Glucanase	-18.63	-1.26
LesAffx.58326.1.A1_at	Les.10796	Salicylic acid-binding protein 2	-17.09	1.14
LesAffx.51348.1.S1_at	Les.9863	Aspartic-type endopeptidase/ pepsin A	-5.67	1.03
LesAffx.50170.1.S1_at	Les.12226	Lipid binding protein	-5.77	2.51
Les.3732.1.S1_at	Les. 3732	Endo-β-1,4-D-glucanase	-2.48	5.67
Les.64.1.S1_at	Les.64	Gibberellin 20-oxidase-1	-5.22	3.75

*The basis of significance is these genes which shows fold change >2.0 at p-value <0.05

Table 2: Differential expression of pathogenesis related genes in resistant (EC-520061) and susceptible (CO-3) genotypes of tomato against *A. solani.*

sample [20]. In this study, Actin (a housekeeping gene) was used as endogenous control since its expressionremained uniform during the experiments (Table 1).

Amplification of gene-specific products was analyzed by melting-curve analysis, followed by agarose gel electrophoresis. In the dissociation curves (temperature versus fluorescence), the Tm values of the PCR products ranged between 82.4° and 88.6°C for each target gene, and 89.8°C for Actin. During the 40 amplification cycles, neither nonspecific amplification nor primer-dimer peaks were detected, indicating the specificity of the primers tested. The specificity of gene products were confirmed by the presence of one amplicon per primer-pair tested by agarose gel electrophoresis.

Further change the data from q-RT PCR was analysed using bar graph (Figure 1) and it showed approximately the same pattern in expression of the genes in susceptible and resistant genotypes as it was observed in expression profiling experiment using microarray. The microarray approach produced accurate picture of differences, with suitable sensitivity to identify differentially regulated transcripts.

Discussion

Plants have evolved numerous defensive strategies to perceive and cope with aggression by pathogens, including insects and viruses. The domesticated tomato is extremely susceptible to *Alternaria solani*. Breeding efforts have resulted in the development of resistant lines and commercial cultivars that present ameliorated symptoms and yields and a lower titer of *A. solani* than susceptible plants [21-24]. Only few studies have been conducted providing limited insight in the molecular background of *A. solani* resistance in tomato.

In the present investigation, the genes that possibly contribute to the establishment of disease resistance in host plant were identified. Role of the pathogenesis related genes either induced or suppressed during the interaction between tomato and the early blight agent *A. solani* have been described. By a judicious choice of the control treatment and use of the microarray technique, a data have been generated which shows that these genes have played an important role in this interaction.We have compared the patterns of proteins known to be involved in defense-like (PR proteins) responses upon *A. solani* infection in tomato genotypes EC-520061, resistant to early blight and CO-3 susceptible to early blight, issued from the breeding program.

There are few studies related to the application of microarraytechnique to identify genesexpressed during plant–pathogen interactions [29]were the first to publish microarray in studying plant–fungal interactions. Because microarray can provide information aboutpreviously known genes, it is a robust approach for detecting differentially expressed and potentially important genes. On one hand, knowledge of the expression patterns of specific genes can provide important information with regard to genes required for resistance and their function. While on the other hand, it might help to develop suitable molecular markers to identify resistant cultivars and donor varieties as has been shown by Butterfield et al. [30] and Elansky et al. [31].

To understand molecular basis of specific plant-pathogen interactions, it is important to identify the plant genes that respond to the pathogen attack. Most of the PR proteins involved in recognition processes release defense-activating signal molecules from the walls of invading pathogens [32,33]. Study by Takeuchi et al. [34], Ham et al. [35] and Balasubramanian et al. [36] showed that glucanendo β-1, 3-glucanase induced in soybean seedlings by infection or chemical stress releases elicitor-active fragments from cell wall preparations of the fungus *Phytophthora megasperma* f. sp. *Glycinea* helping to stimulate defense responses in adjacent cells, as well as induce acquired resistance to further infection. However, the glucanendoβ-1, 3-glucanase and PR-2 induction might reduce callose accumulation

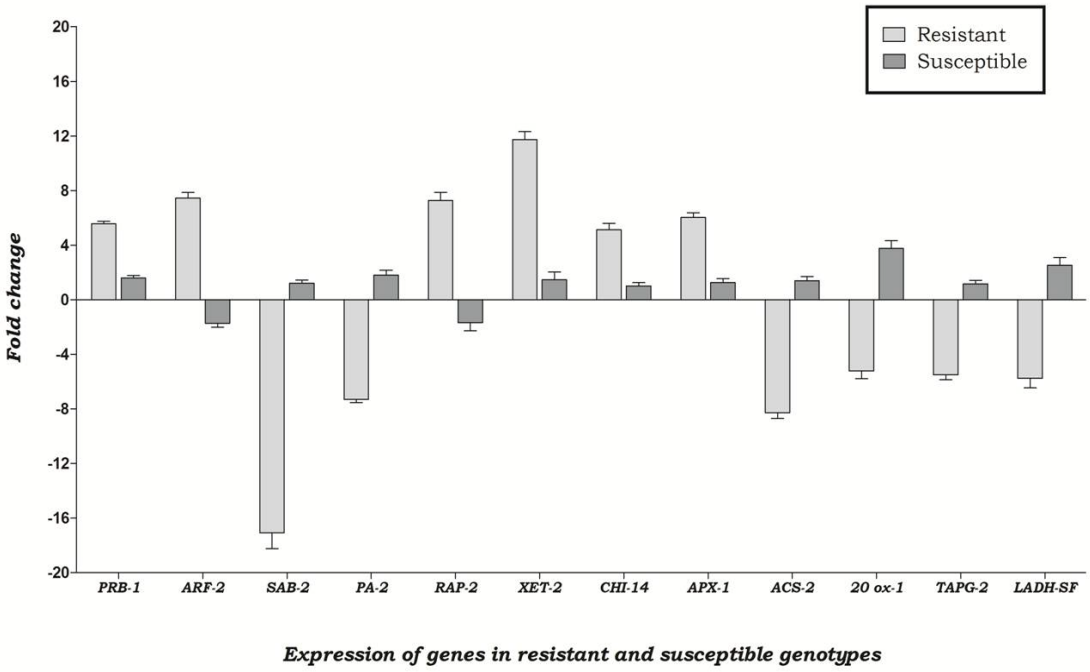

Figure 1: Graphical representation of PR proteins genes behavior in resistant and susceptible genotypes of tomato at 24 hrs after inoculation with *A. solani* pathogen.

against virus infection in tobacco [37-39]. In the present investigation, expression of glucanendoβ-1, 3-glucanase gene was found to be down regulated which is contradictory with the Takeuchi et al. [34] and Ham et al. [35]. It's down regulation may be responsible for blocking the fungal mycelia during penetration of the cells and which results in defense response.

Gene for arginine decarboxylase was found up regulated in the resistant genotype. This result is in accordance with the results observed by Prabhavathi et al. [40]where it has been seen that the egg plant genotypes with more expression of arginine decorboxylase was more resistance to *Fusarium* wilt. In plants, polyamines can generally be synthesized by the ornithine decarboxylase and arginine decarboxylase pathways.Polyamines (PAs) are small aliphatic amines whose synthesis is tightly regulated. PAs are involved in the modulation of different cellular processes, including functioning of ion channels, chromatin organization, DNA replication, gene transcription, mRNA translation, cell proliferation and apoptosis [41,42]. In the present investigation, polyamine homeostasis might be maintained by induction of the arginine decarboxylase pathway, resulting in the higher hydrogen peroxide accumulation. This ultimately increased resistance of the tomato plants against the fungal pathogen.

The peroxidase enzymes are heme-containing glycoproteins that catalyze the oxidation of a wide range of organic and inorganic substrates by hydrogen peroxide, such as cytochrome c, nitrite, leuco-dyes, ascorbic acid, indole amines, and iodide ion. Peroxidases occur in numerous isoforms in plants and animals. Plant peroxidases have been implicated in a wide range of physiological processes, such as auxin metabolism, ethylene biosynthesis, lignin formation, respiration, light-mediated processes, growth, andsenescence. In addition, peroxidase activity has been correlated with plant defense against pathogens [43-45].

Plant cell wall constitutes one of the first lines defense against pathogen invasion, and peroxidases are key enzymes in the wall-building processes. These processes include peroxidase-mediated oxidation of hydroxycinnamyl alcohols into free radical intermediates [46], phenol oxidation [47], polysaccharide cross-linking [48], cross-linking of extensin monomers [49], lignifications, [50,51]and suberization (Quiroga 2000) [51]. Although direct involvement of any one type of peroxidase in defense has not been demonstrated conclusively, extracellular or wall-bound peroxidases have been proposed to enhance resistance by the construction of a cell wall barrier that may impede pathogen ingress and spread [46,52-54]. The accumulation of cell wall-strengthening materials following infection might be expected to correlate with enhanced resistance [45]. The accumulation of lignin and phenolic compounds have been correlated with the resistance in a number of plant–pathogen interactions.

The resistance response in wheat cultivar relude-Sr5, against an a virulent race of the stem rust fungus *Puccinia Graminis* F. sp. *Tritici,* was correlated with rapid lignification in penetrated host cells [55]. In tomato, resistance to the fungal pathogen *Verticilliumalbo-atrum* was correlated with a more rapid deposition of suberin and lignin in a resistant isoline than a susceptible isoline [56,57]. Similarly, infiltration of rice leaves with suspensions of *Xanthomonas oryzae* Pv. *oryzae*, the bacterial blight pathogen, caused the deposition of lignin-like polymers at the site of inoculation during resistant interactions. In rice, the spatial and temporal patterns of phenolic polymer deposition were correlated with resistance, that is, the decrease in bacterial multiplication rates and onset of bacteriostasis [58]. Expression of stress-response proteins upon whitefly-mediated inoculation of tomato yellow leaf curl virus in susceptible and resistant tomato plants showed that there was an elevation of peroxidase in susceptible plants but not in resistant plants [59]. The activity of this PR protein may reflect the massive early blight disease symptoms in susceptible leaves compared with the absence of symptoms in resistant tomato plants.

Endo-1, 4-beta-glucanase was down regulated in the resistant genotype by a 2.48 fold change while it was found to be up regulated in the susceptible genotype. Another gene Xyloglucan endo-transglycosylase (XET) was found up regulated by 11.74 fold change in the resistant genotype while its fold change was insignificant in the susceptible genotype. These two genes, Endo-1, 4-beta-glucanase and XET, function in cell wall. The gene endo-1, 4-beta-glucanase, here it is not involved in the resistance reaction while the gene XET is accountable for the cleavage of xyloglucan compounds, which are essential for the remodelling of the cell wall, and responsible for the cell wall architecture. In other cases, a direct involvement in the pathogen response could be demonstrated [60]. It was shown that XET was induced in different plants upon aphid infestation, and its role in cell wall modification as part of the plant defence was established. During the hypersensitive response in resistant tomato genotype upon *A. solani* attack, cell wall modifications resulting in the isolation of the affected tissues were also observed [61]. Such modifications prevent feeding by phylloxera as well as secondary infections in the penetration area. We assume that the specific XET up-regulation in resistant genotype observed during interaction between *A. solani* and tomato, hypersensitive response is part of such cell wall modifications leading to the isolation of the affected tissues.

PR proteins can function either directly on the pathogen or indirectly by creating physical barriers to the fungal infection process or on upstream intrinsic PR signaling. Most of the identified PR proteins act directly to disrupt the fungal/bacterial cell wall (endo-1, 4-beta-glucanase, basic endochitinase and glucanendo- 1, 3-beta-glucosidase), or inhibit fungal germination due to ribonuclease activity (hevein-like precursor) [62].It is possible that these proteins participate in the increased tolerance to early blight pathogen*A. solani*, not only in laboratory conditions but also in the field, by minimizing the damages due to pathogen, insect feeding and viruses [63].

In this study, several genes have been identified that might contribute to disease resistance in the resistant tomato genotypes. The potential application of these genes could be to over-express them in tomato in order to obtain disease-resistant lines. Markers could be developed to select tomato lines that show a high expression of the identified genes [30]. Although disease-resistant transgenic crops are commercially available, future product development seems likely as our current level of understanding of pathogenesis and plant defense improves [64].

In conclusion, the processes that determine the outcome of an interaction between a microbial pathogen and a host plant are complex. Understanding the molecular details of these interactions, such as the pathogen genes required for infection, effective host defense responses and mechanisms by which host and pathogen signaling networks are regulated, might be utilized to design new plant protection strategies. The analysis presented here identified novel pattern of genes for pathogenesis related proteins in stressed tomato that may be important in the response to parasitic threat. Studies of the pathways in which these genes are involved, will give more information about the physiology of early blight disease and may elucidate the mechanisms

of tolerance. In any case, the analysis of these genes will contribute to a more comprehensive view of the tomato–*A. solani* interaction. Nevertheless, further characterization and functional analysis of the genes, identified in this study, can lead to a more comprehensive understanding of tomato-pathogen interactions.

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