

## بررسی روند تغییر بیان ژن‌های یونجه یکساله در پاسخ به باکتری

### *Ralstonia solanacearum*

احمد یامچی<sup>۱</sup>، سیدرضا زارعی<sup>۲</sup>، آقافخر میرلوحی<sup>۳</sup>، Laurent Gentzbittel<sup>۴</sup>،  
بدرالدین ابراهیم سیدطباطبایی<sup>۵</sup>، کتایون امیدی<sup>۶</sup>

۱- دانشجوی دکتری دانشگاه صنعتی اصفهان و دانشگاه INP-Toulouse

فرانسه

۲ و ۵- به ترتیب استادیار و دانشیار گروه بیوتکنولوژی، دانشکده کشاورزی،

دانشگاه صنعتی اصفهان

۳- استاد گروه بیوتکنولوژی، دانشکده کشاورزی، دانشگاه صنعتی اصفهان

۴- استاد دانشگاه INP-Toulouse فرانسه

۶- کارشناسی ارشد دانشگاه Toulouse-III فرانسه

\*نویسنده مسئول مکاتبات، پست الکترونیکی: mirlohi@cc.iut.ac.ir

(تاریخ دریافت: - تاریخ پذیرش: )

### چکیده

خانواده بقولات از لحاظ تغذیه انسان‌ها و جانوران جز گیاهان مهم زراعی هستند که قادرند با باکتری تثبیت کننده ازت در خاک و همچنین قارچ‌های میکوریزا همزیستی داشته باشند. باکتری *Ralstonia solanacearum* باکتری خاکزی است که از طریق ریشه به بیش از ۲۰۰ گونه گیاهی از جمله تعدادی از بقولات حمله کرده و باعث مسدود شدن آوندهای گیاهی از یک طرف و از طرف دیگر هضم پروتئین‌ها و آنزیم‌های کلیدی سلول گیاهی از طریق سیستم ترشحی بیماری‌زایی نوع سوم می‌شود که نتیجه آن پژمردگی و از بین رفتن گیاهان است. در این تحقیق از گیاهان مقاوم و حساس *Medicago truncatula* به عنوان گیاه مدل جهت مطالعه تغییر بیان ژن‌ها بر علیه باکتری *R. solanacearum* استفاده گردید. مطالعات پروتئومیکس و ترانسکریپتومیکس نشان داد که بیان دو گروه از ژن‌ها در هر دو نوع گیاهان حساس و مقاوم افزایش می‌یابد. گروه اول شامل ژن‌هایی که محصول آنها بصورت مستقیم با پاتوژن واکنش می‌دهد مانند پروتئین‌های PI، PR و پراکسیداز و گروه دوم شامل ژن‌هایی که محصول آنها بعنوان فاکتورهای رونویسی یا پروتئین‌های شرکت کننده در مسیر انتقال پیام و یا به عنوان چپرون در تعیین شکل فضایی پروتئین‌های دیگر دخالت می‌کنند مانند پروتئین‌های HSP90 و (PPI) peptidyl-prolyl cis-trans isomerase. مطالعات ترانسکریپتومیکس نشان داد که افزایش بیان ژن‌ها در گیاهان مقاوم پایدار ولی در گیاهان حساس ناپایدار است به این ترتیب که بیان ژن‌ها در یک روز پس از تلقیح با باکتری در هر دو نوع گیاهان حساس و مقاوم افزایش یافت ولی در ادامه و سه روز پس از تلقیح با باکتری بیان ژن‌ها در گیاهان حساس کاهش یافته ولی در گیاهان مقاوم ثابت و یا حتی افزایش یافت.

### واژه‌های کلیدی

*Ralstonia solanacearum*

*Medicago truncatula*

پروتئومیکس،

ترانسکریپتومیکس،

ژن

*M. truncatula*

DNA ( )

( )

*Medicago truncatula*

DD- *R. solanacearum*

RT-PCR

( )

*R. solanacearum* ( )

ESTs ( )

*Ralstonia solanacearum*

*R. solanacearum* ( )

*R. solanacearum* ( )

*R. solanacearum* ( )

*M. truncatula* ( )

(F8300.5) (A17)

*R. solanacearum* QTL ( )

*M. truncatula* QTL ( )

*Orobanche PR10* ( ) *Aphanomyces euteiches* ( ) *crenata* ( )

<sup>3</sup> - Chaperones

<sup>4</sup> -Membrane antigen

<sup>1</sup> -Express sequence tags

<sup>2</sup> - Recombinant inbred lines



S

cyclophilin

( )  
( )  
( )  
( )  
( )

mRNA

*M. truncatula*

*R. solanacearum*

(w/v)

( )

$\mu \text{ mol m}^{-2}$

$\text{s}^{-1}$

mRNA

*R. solanacearum*

*M. truncatula*

( )

( )

*R. solanacearum*

GMI1000

(F<sub>9</sub>) RILs

R.)

) A17

) F83005.5

(*solanacearum*)

(*R. solanacearum*)

$\mu$

$\text{mol m}^{-2} \text{ s}^{-1}$

INP-ENSAT

INRA

F<sub>9</sub>

( )

RILs

RILs

( )

/ / /

2-D Quant kit (Amersham Bioscience) ) (

18 cm 3-10 NL, Immobline dry strip, ) IPG :

(Bio-Rad

IPGphor (Amersham Bioscience) . ( ) ( )

(passive form)

active ) / TCA %

(form

rehydration ×g

IPGphor (focusing)

:

PMSF /

IPG

6M urea, 50 mM Tris-Cl pH

8.8,) 30% (v/v) glycerol, 2% (w.v) SDS, 1% DTT, a trace (of bromophenol blue

2% SDS, 30 mM Tris-Cl pH )

(8.5, 60 Mm DTT

iodoacetamide

IPG DTT ×g

SDS-PAGE

SDS-PAGE

2D clean SDS

up (Amersham Bioscience)

SDS-

colloidal CBB G-250 PAGE

40%) . ( )

(methanol, 10% acetic acid

10% phosphoric acid, 10% )

(w/v) ammonium sulfate, 20% methanol, 0.12% (w/v) ×g

(CBB G-250

epson perfection 4990 (Epson, Japan)

Image master 2D platenium ver.6 (Amersham



*M. truncatula* sport-trembl (All species) sport-trembl (Bioscience)  
Gene Index (MtGI)

carbamidomethyl (C) : MASCOT  
oxidation (M) deamidated (NQ)

ESI-MS/MS MALDI-MS

MtGI

cDNA mRNA

( mRNA ) 3' UTR : (v/v)

primer express v.2 (Applied Biosystem)

q-RT-

real time PCR

blast

Sequencing grade modified trypsin, )

(Promega

: real time q-RT-PCR RNA

Trizol (Invitrogen) RNA 10%)

(formic acid, 62.5% acetone, 27.5% H<sub>2</sub>O

Trizol (Invitrogen)

×g

Applied )

MALDI-MS (Biosystem

Applied )

×g

ESI-MS/MS (Biosystem

ESI-MS/MS

RNA

Q-Trap

(LC)

%

×g

ESI-MS/MS

(Viridiplantae)

MASCOT

/ / /

PCR  
(Applied Biosystems User  
Bulletin #2, <http://docs.appliedbiosystems.com/search>)  
q-RT-PCR  
ANOVA  
PCR  
RNase  
DNA RNA  
Nanodrap (Germany)  
DNase (Promega) RNA  
Agilent RNA RNA  
Agilent 2100 Bioanalyzer 600 Nano Kit  
(Agilent technologies, Massy, France)  
nanodrap DNA RNA  
DNA RNA  
(Oligo(dt)<sub>15</sub>) T RT (Promega)  
*M.*  
*truncatula*  
RILs F  
( )  
*R.*  
*solanacearum*  
( )  
( )  
( )  
(dissociation step)  
ABI 7900 (Applied Biosystem)  
(Applied Biosystem) PCR  
SDS 2.2  
elongation factor cDNA  
PCR 1α  
cDNA :  
q-RT-PCR  
SDS 2.2 PCR  
( + E)= slop  
(2D-PAGE) cDNA PCR  
LinReg v.4



)

position / pI ( Image master 2D platenium ver.6  
 pH shift  
 inhibitor ST1-like pI  
 / pI kunitz (Proteinase inhibitor 20)  
 .( )

:

( )

MALDI-TOF

( )

(Mass finger printing)

ESI-MS/MS

.( )

(mock-

.( )

inoculation)

.( )

M.

R.

*truncatula*

( )

*solanacearum*

.( )

.( )

(MtGI) *M. truncatula*

ESI-MS/MS

.( )

*M. truncatula*

( )

.( )

M.

NCBI

.( )

*truncatula*

)

Real time q-RT-PCR

peptidyl-prolyl

.(

q-RT-PCR

cDNA

pI 8.5

cis-trans isomerase

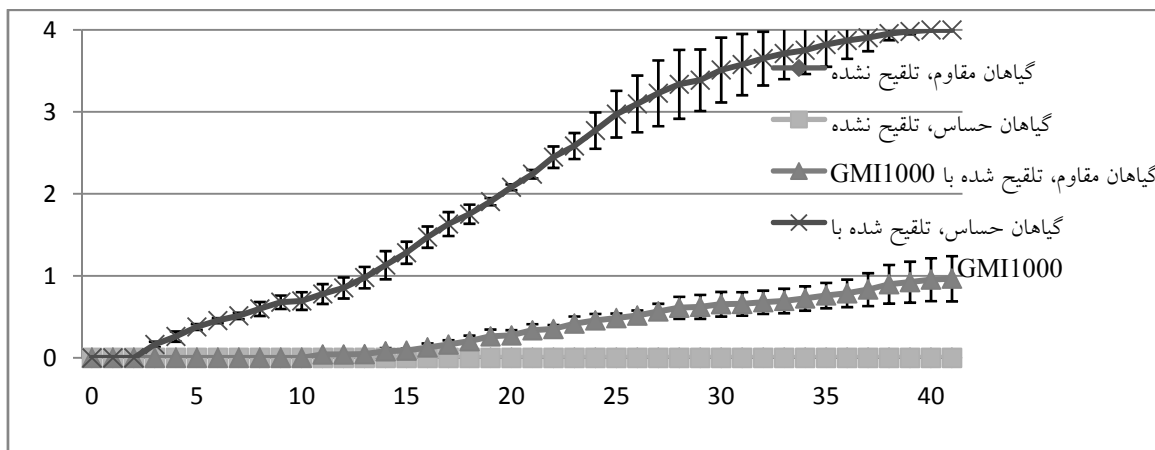
( )

/

/ / /

cold stress response PR5  
 Proteinase  
 Kunitz trypsin  
 peptidyl-prolyl cis-trans isomerase  
 protease inhibitor  
 peroxidase  
 Proteinase inhibitor 20  
 cold stress response PR5

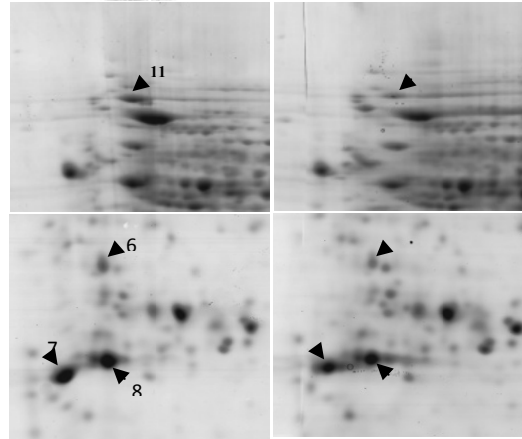
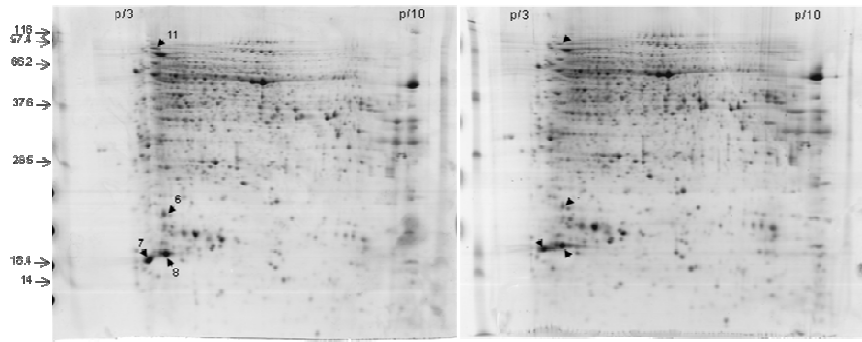
5 -days post inoculation



*M. truncatula*

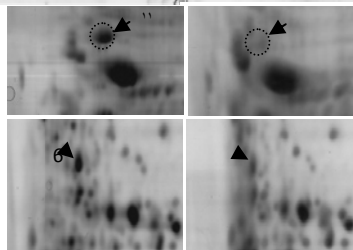
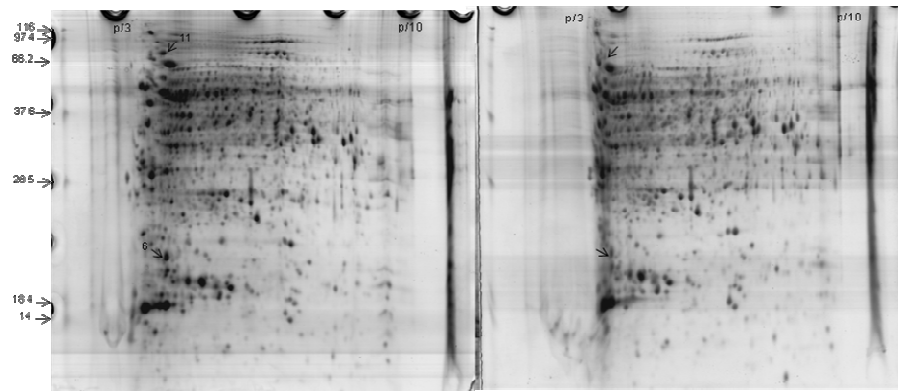
*R. solanacearum*





*M. truncatula*

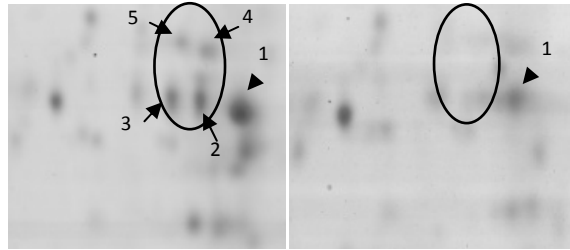
( ) *R. solanacearum*



*M. truncatula*

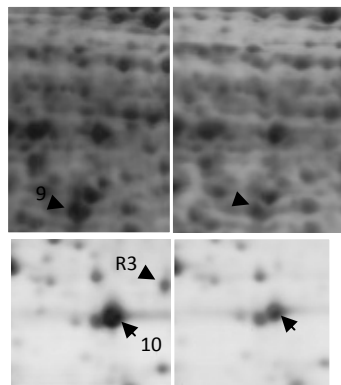
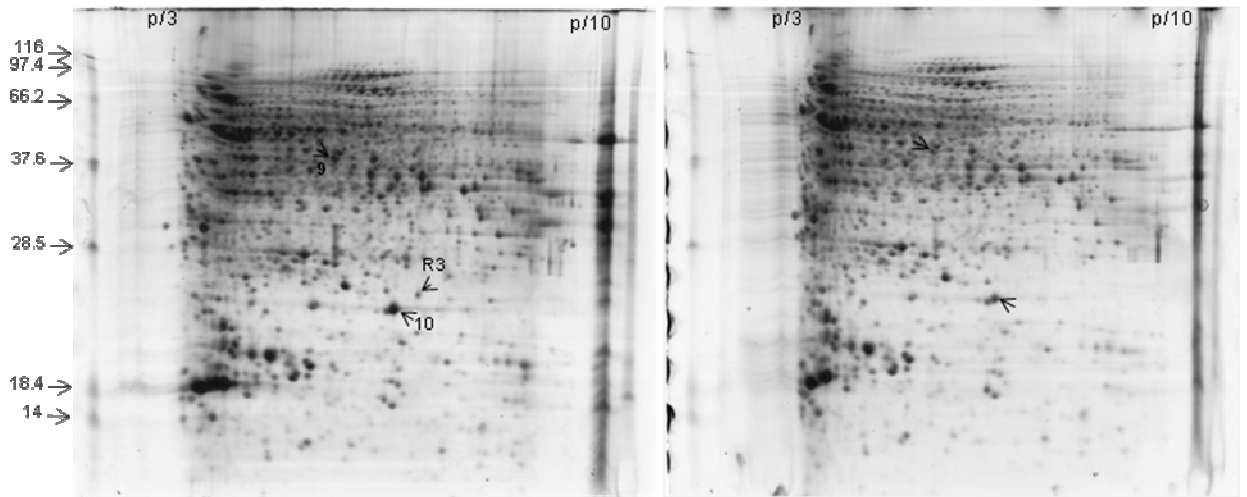
( ) *R. solanacearum*





*M. truncatula* ( ) ( ) -  
 peptidyl-prolyl cis-trans isomerase *R. solanacearum*

pH / position shift / pI  
 / pI inhibitor ST1-like (Proteinase inhibitor 20) kunitz  
 position shift



*M. truncatula* -  
 ( ) ( ) *R. solanacearum*  
*R. solanacearum* R3





Spot <sup>a</sup>	Best matching gene product <sup>b</sup>	Chromosome <sup>c</sup>	MW (KD)Theoretical	pI Theoretical	MW(KD) on gel	pI on gel	Inductions ratios postinoculation <sup>d</sup>				<i>M. truncatula</i> (Accession Number)	Identified peptide sequences <sup>e</sup>	Protein Score (MASCOT) LC-MS/MS <sup>f</sup>	% Coverage <sup>g</sup>
							Susceptible Plant		Resistant Plant					
							Day 1	Day 3	Day1	Day 3				
1	Peptidyl-prolyl cis-trans isomerase	?	18.1	8.3	17.3	8.5	-	-	-	-	TC127550	FDMSVGGNPAGR-GGESIYGSKFADENFIK-KHTGP GILSMANAGPPTNGSQFFICTAKTEWLDGKHVVFQ	335	41
2	Peptidyl-prolyl cis-trans isomerase	?	18.1	8.3	17.3	8	Absent	Absent	Present	Present	TC127550	FADENFIK-VVEGLDIVKEIEKVGSGSGKTSKPVV IAD	191	22
3	Peptidyl-prolyl cis-trans isomerase	?	18.1	8.3	17.3	7.5	Absent	Absent	Present	Present	TC127550	IIFELFADVTPRTAENFRALCTGKEKGVGRSGKPL-F ADENFIK	260	25
4	Proteinase inhibitor 20	6	23.4	7.6	19.8	8	Absent	Absent	Present	Present	AC122730_2	CPVTVLQDR	40	4
5	Proteinase inhibitor 20	6	23.4	7.6	19.8	7.5	Absent	Absent	Present	Present	AC122730_2	CPVTVLQDR	40	4
6	Kunitz trypsin protease inhibitor	3	22	5.1	20.7	4.8	4.21±0.18	-	2.01±0.15	-	CU019604_15	TCPLDVIR-NPDAIGTPVYFSASGLDYIPTLTDLT IEIPILGSPCNEPK-LAGEHAYEISYFK-FCPSVP GVLCAVGVTFVDTDGTK-VMAVGDGIEEPYYVR	155	46
7	PR10 protein	?	16.6	4.5	14.3	4.3	1.42±0.09	-	2.39±0.17	-	gi 1616609	GVNFDETTISIVAPAR-ALVTDSDNLIPIK-VIDAI QSIEIVEGNGGAGTIK-TFVEGETK-LSAGPDGG SIK-GDAAPSEEEIK-ALEGYVLANPDY	523	60
8	Pprg2 protein	?	16.0	5.8	14.8	4.8	1.68±0.13	-	2.78±0.16	-	gi 22266001 <i>M. sativa</i>	GVFTFNDEHVSIVAPAK-VISAAQSVEIVEGNGGP GTIK-TNFVLHK	219	28
	Cold responsive protein	?	16.0	5.8	14.8	4.8	1.68±0.13	-	2.78±0.16	-	gi 37542526 <i>T. repens</i>	DADEIVPK-LTVVEDGK	102	10
9	Peroxidase	?	38.2	5.8	36.9	5.8	-	2.25±0.19	-	3.52±0.20	gi 537317 <i>M. sativa</i>	DTCPNVHSIVR-GLDVVNQIK-VLTGSQGEIR	195	8
10	Thaumatococin-like protein PR-5	5	26.2	5.8	21.9	6	-	1.02±0.09	-	3.25±0.12	CU326391_11	GQTWNLWVNPGTAMAR-TGCNFDGSGR-ISCTADI NGQCPNELR-TQGGCNPCTVFK-CHDSYSYPQDD PTSTFTCPAGSNYK	487	40
11	Molecular chaperone Hsp90	5	80.5	4.9	84.3	4.7	8.72±0.34	-	4.5±0.28	-	CT573078_38	ELISNASDALDK-FEGLTDK-LDSQPELFIHIIIPD K-TNNTLTIIDSGIGMTK-ADLVNNGLTAR-DTTG EALGR-EDQLEYLEER-EVSNEWSLVNK-SLTNDWE EHLAVK-APFDLFDTK-VFIMDNCEELMPEYLSFV K-GIVDSEDLPLNISR-FYEAFSK-LGIHEDSQNK -EGQNDIYYITGESK-AVENSPFLEK-LGLSIDED AAEADAMPPLLEADADAEGSK	450	31

*R. solanacearum* .

( )

a

NCBI.

*M. truncatula* (MtGI)

(ESI-MS/MS)

b

*M. truncatula* (MtGI).

c

= :

d

absent present

(ESI-MS/MS).

e

*M. truncatula* (MtGI)

MASCOT

(ESI-MS/MS)

f

NCBI.

g

*R. solanacearum*

Spot <sup>a</sup>	Best matching gene product <sup>b</sup>	Susceptible Plant <sup>c</sup>		Resistant Plant <sup>c</sup>		<i>R. solanacearum</i> Accession Number	Identified peptide sequence <sup>d</sup>	Protein Score MASCOT LC- MS/MS <sup>e</sup>	% Coverage <sup>f</sup>
		Pre- Inoculation d	Post- Inoculationd	Pre- Inoculationd	Post- Inoculationd				
R1	Putative uncharacterized protein	Absent	++	Absent	+	gi 17544951	DTLDVLAK - LINSIVGLYDGVSQQDK- YLVLSLIR – FELCFP ELAYETV	311	42
R2	Putative uncharacterized protein	Absent	++	Absent	+	gi 17544951	SVFLAEQTSFDSAAAVGDFLGK- GEAIYSDPR DTLDVLAK- YV EEIDNTP FFHLLTHDGNR-LINSIVGLYDGVSQQ DK-YLVLS LIR-QTIIGWTK-FELCFPE L AYETV	423	44
R3	Putative uncharacterized protein	Absent	++	Absent	+	gi 17546446	WHAHEHAVMTVEQR-ALASLAEKQLADAPQIK	79	16
R4	Alkyl hydroperoxide reductase (Subunit c) oxidoreductase protein	Absent	++	Absent	+	gi 17548466	FVEVTEQNLK-AGAEVYIVTTDTHFSHK-GTFVINPEGVVKTSE VHDNAIAR-AAQYVASNPGQVCPAK	199	35
R5	Polyhydroxybutyrate granule- associated protein (Phasin) phap1	Absent	++	Absent	+	gi 17546324	MLTQEIQIAAAQKANLETFGLTNK-DA QELLAVHTA AVQP LAEK-HLYEIFS DTQTEFGKVAETQIAEGSR-NAPAGSESAVA LVKSALSAANNAYDSVQK	118	51
R6	Polyhydroxybutyrate granule- associated protein (Phasin) phap1	Absent	++	Absent	+	gi 17546324	MLTQEIQIAAAQKANLETFGLTNK-DAQELLAVHTAAV QPL AEKVLAYNRHLYEIFSDTQTEFGKVAETQIAEGSR-SALSA AN NAYDSVQK-QAVELAESNFHAAANAASK	451	56

*R. solanacearum.*

a

NCBI.

(ESI-MS/MS)

b

= ++

= +

: Absent =

c

(ESI-MS/MS).

d

NCBI.

MASCOT

(ESI-MS/MS)

e

f

NCBI.

*M. truncatula* (MtGI)

:

Name	Accession no.	Forward primers	Reverse primers
Thaumatococcus-like protein PR-5	CU326391_11	TCAATGGTGTGTTCTTTCAATAAGTTG	CACACAATACACACAGCAAAATACAGTAA
Kunitz trypsin protease inhibitor	CU019604_15	GCCCAAATCAGACGAGGTATGT	GAGACTTCCTGCATGAGACACAA
Proteinase inhibitor 20	AC122730_2	TGCTGCATCAACAAAATGGTTAA	TTTTCAGGACCACCAATACCAAT
Peptidyl-prolyl cis-trans isomerase	TC127550	TCTTCCTCCACCGTGTGATC	ATTCCAGGACCGGTGTGCTT
Pprg2 protein	gi 22266001	CACACCAAAGGTGATGCAGCTC	ATGGCCTTGATAAGTCCAGTTCC
Cold responsive protein	gi 37542526	GGAACAGGGTTGGATGAAAGTTT	CACTGAAATCTTAACAATGGATCCA
Molecular chaperone Hsp90	CT573078_38	TATTAGATGCAATTTTCTTGACCAGTGT	CGAACCAACAACAGTCAACAAATAA
PR10 protein	gi 1616609	CACAGTGGAGAAGATCTCCTTTGA	ACAGTAAGCTTTGCAATGGATCCT
Peroxidase	TC114533	TAGAGGTCAATGCAGATTCTTCGTT	GTTGAGAGTTGGATCGGGATTTC
Elongation factor 1 $\alpha^a$	gi 217072267	GAGGTTGGTTCGTGAAATTGC	CAGCATGGCTCTGGAAACG

real time q-RT- PCR.

Elongation factor 1 $\alpha$ 

a

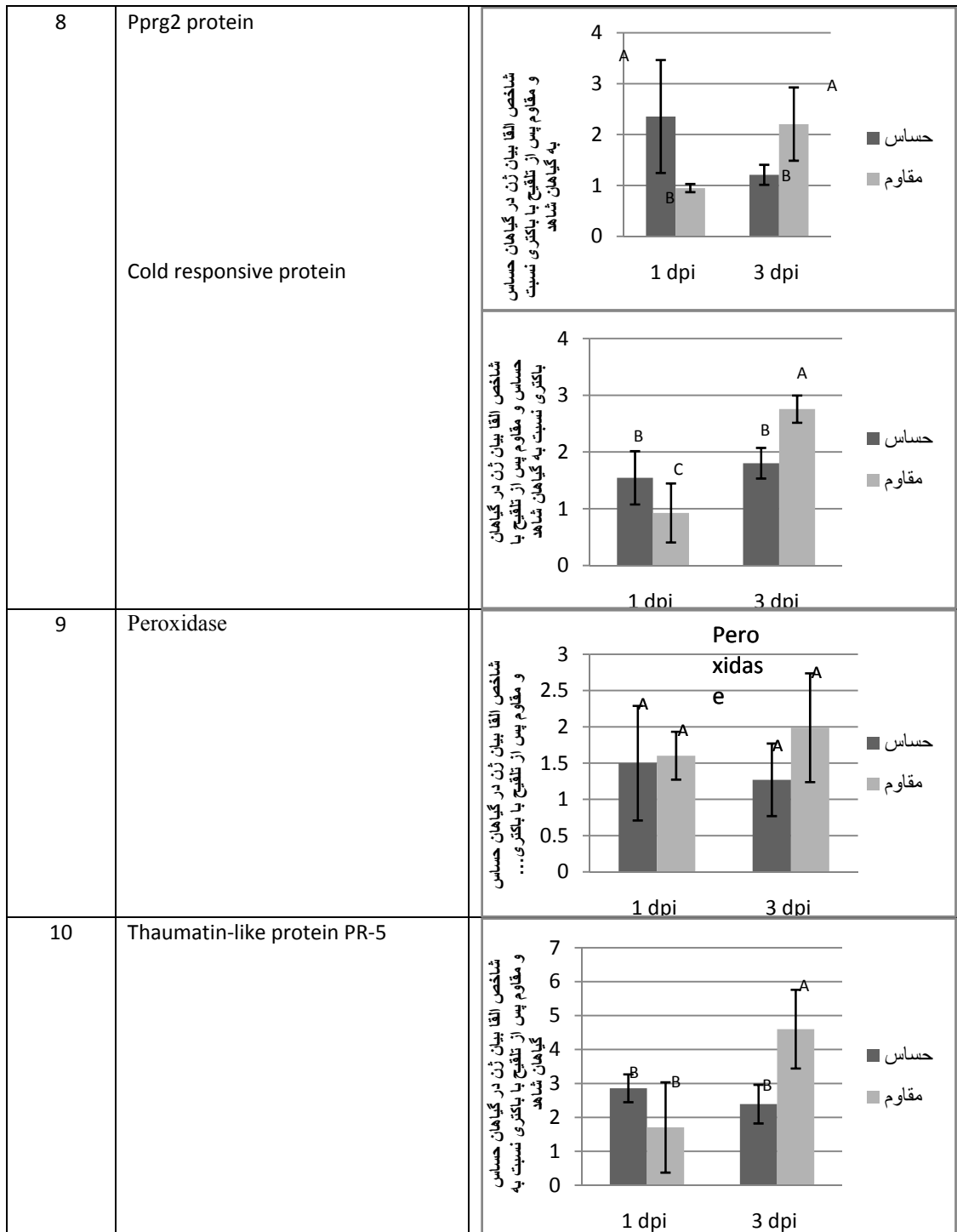
*M. truncatula*

mRNA

*R. solanacearum*

Spot <sup>a</sup>	Best matching gene product	q-RT-PCR <sup>b</sup>
1,2,3	Peptidyl-prolyl cis-trans isomerase	
4,5	Proteinase inhibitor 20	
6	Kunitz trypsin protease inhibitor	
7	PR10 protein	





)

a

*R. solanacearum*.

(

mRNA

b

real time q-RT- PCR

*R. solanacearum*

*M. truncatula*

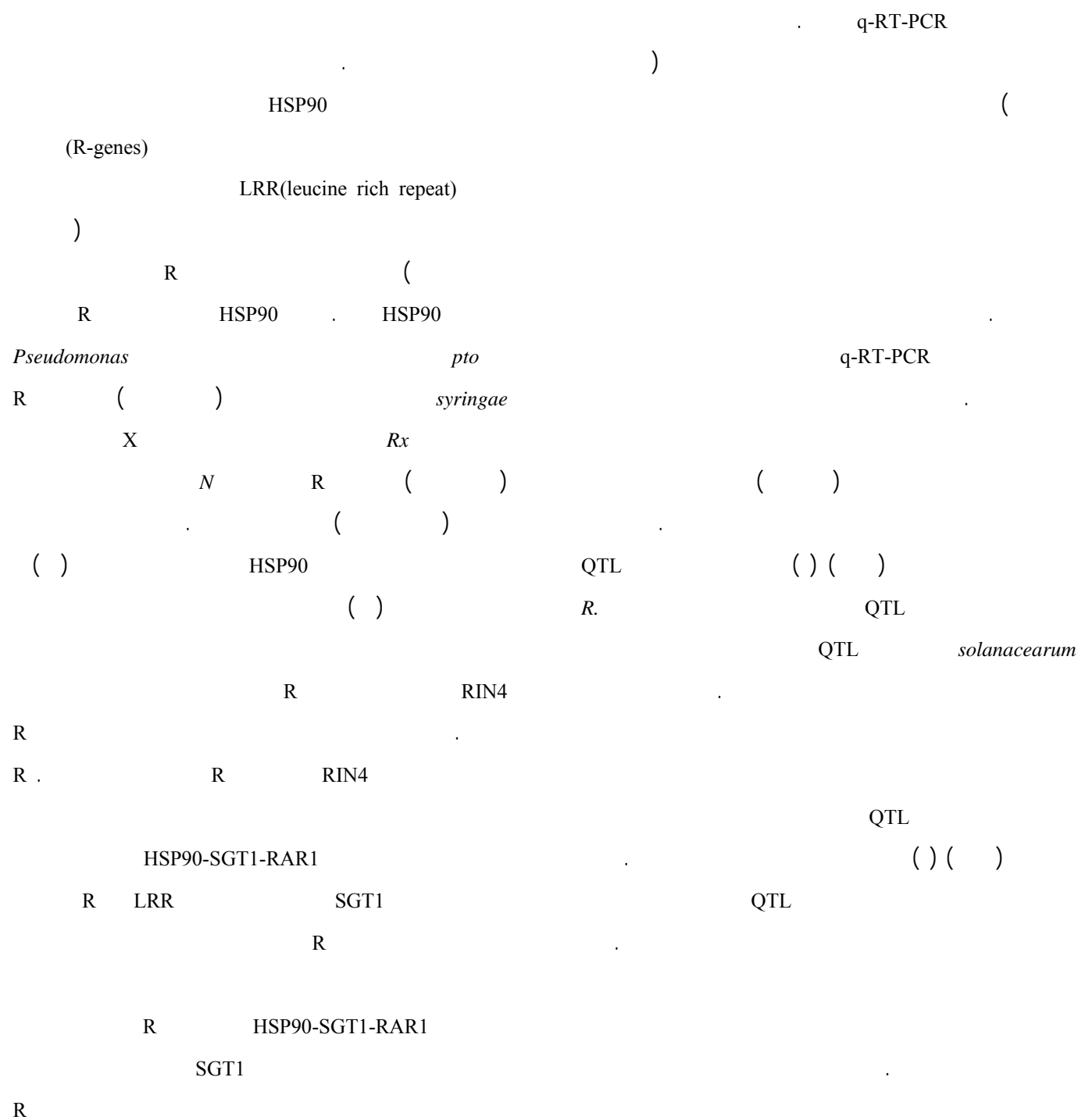
mRNA

( $p < .$ )

.







<sup>9</sup> -avirulence factor

<sup>8</sup> -Transcription factor



kunitz

inhibitor ST1-like (Proteinase inhibitor20)

signal P3.0 server

mRNA

N

signal peptide

bp

( )

( )

pI

M.

*R. solanacearum*

*truncatula*

pI

peptidyl-prolyl cis-trans isomerase

( )

PAMP-

A17 *M. truncatula*

( )

triggered immunity (PTI)

*Aphanomyces euteiches*

( )

RILs

:

A17

) *R. solanacearum*

(

)

pI

(

RILs

( )

F83005.5

)

(

pI

( )

peptidyl-prolyl cis-trans isomerase

/

/

pI

( )

M.

(PTI)

AvrRpt2

*P. syringae*

*truncatula*

( )

Net phos 2.0 server

<sup>10</sup> -virulence

/ / /

*solanacearum*-tobacco interaction. Plant Biotechnol. 24: 409-416

11. Afroz A, Khan MR, Ahsan N, Komatsu S (2009) Comparative proteomic analysis of bacterial wilt susceptible and resistant tomato cultivars. Peptides 30: 1600-1607

12. Dahal D, Heintz, D, Dorsselaer, AV, Braun, H.P, Wydra, K (2009). Pathogenesis and stress related as well as metabolic proteins are regulated in tomato stems infected with *Ralstonia solanacearum*. Plant Physiol. Biochem. 47: 838-846

13. Trapphoff T, Beutner, C, Niehaus, K, Colditz, F (2009). Induction of Distinct Defence-Associated Protein Patterns in *Aphanomyces euteiches* (Oomycota)-Elicited and -Inoculated *Medicago truncatula* Cell-Suspension Cultures: A Proteome and Phosphoproteome Approach. MPMI 22:421-436

14. Castillejo M, Maldonado AM, Dumas-Gaudot E, Fernández-Apariciol M, Susin R, Diego R, Jorrín JV (2009) Differential expression proteomics to investigate responses and resistance to *Orobanche crenata* in *Medicago truncatula*. BMC Genomics 10: 294-311

15. Fahraeus G (1957). The infection of white clover root hairs by nodule bacteria studied by a simple slide technique. J. Genet. Microbiol. 16:374-381

16. Damerval C, de Vienne D., Zivy M., Thiellement H (1986). Technical improvements in two-dimensional electrophoresis increase the level of genetic variation detected in wheat-seedling proteins. Electrophoresis 7: 52-54

17. Candiano G, Bruschi M, Musante L, Santucci L, Ghiggeri GM, Carnemolla B, Orecchia P, Zardi L, Righetti PG (2004). Blue silver: A very sensitive colloidal Coomassie G-250 staining for proteome analysis. Electrophoresis 25: 1327-1333

18. Chinnasamy G, Rampitsch C (2006). Efficient solubilization buffers for two-dimensional gel electrophoresis of acidic and basic proteins extracted from wheat seeds. Biochim. Biophys. Acta 1764: 641 - 644

19. Song J, Braun G, Bevis E, Doncaster K (2006). A simple protocol for protein extraction of recalcitrant fruit tissues suitable for 2-DE and MS analysis. Electrophoresis. 27: 3144-3151

20. Gygi SP, Rochon Y, Franza BR, Aebersold R (1999). Correlation between protein and mRNA abundance in yeast. Mol. Cell. Biol. 19:1720-1730

21. Fitcher B, Latter GI, Monardo P, McLaughlin CS, Garrels JI (1999). A sampling of the yeast proteome. Mol. Cell. Biol. 19: 7357-7368

22. Varshavsky A (1996). The N-end rule: functions, mysteries, uses. Proc. Natl. Acad. Sci. USA 93:12142-12149

23. Harford JB, Morris D. R (1997). Post-transcriptional gene regulation. Wiley-Liss, Inc., New York

24. Wang R, Prince JT, Marcotte EM (2008). Mass spectrometry of the *M. smegmatis* proteome: Protein expression levels correlate with function, operons, and codon bias. Genome Res. 15: 1118-1126

*R. solanacearum*

( ) R-gene *M. truncatula*

1. Samaj J, Thelen J (2007). Plant proteomics. Springer-Verlag, New York

2. May G, Dixon R (2004) *Medicago truncatula*. Curr Biol 14:R180-181

3. Frugoli J, Harris J (2001) *Medicago truncatula* on the move! Plant Cell 13:458-463

4. Choi H-K, Kim D, Uhm T, Limpens E, Lim H, Mun J-H, Kalo P, Penmetsa RV, Seres A, Kulikova O, Roe BA, Bisseling T, Kiss GB, Cook DR (2004). A sequence-based genetic map of *Medicago truncatula* and comparison of marker colinearity with *M. sativa*. Genetics 166:1463-1502

5. Young N D, Cannon, SB, Sato, S, Kim, D, Cook, DR, Town, CD, Roe, BA, and Tabata, S (2005). Sequencing the gene spaces of *Medicago truncatula* and *Lotus japonicus*. Plant Physiol. 137:1174-1181

6. Hayward AC (1991). Biology and epidemiology of bacterial wilt caused by *Pseudomonas solanacearum*. Annu. Rev. Phytopathol. 29:65-87

7. Thoquet P, Olivier J, Sperisen C, Rogowsky P, Laterrot H, Grimsley N (1996a) Quantitative trait loci determining resistance to bacterial wilt in tomato cultivar Hawaii 7996. Mol Plant-Microbe Interact 9: 826-836

8. Deslandes L, Olivier J, Theulieries F, Hirsch J, Feng DX, Bittner-Eddy P, Beynon J, Marco Y (2002). Resistance to *Ralstonia solanacearum* in *Arabidopsis thaliana* is conferred by the recessive RRS1-R gene, a member of a novel family of resistance genes. Proc Natl Acad Sci USA 99: 2404-2408

9. Vaillau F, Sartorel, E, Jardinaud, MF, Chardon, F, Genin, S, Huguet, T, Gentsbittel, L, Petitprez1, M (2007). Characterization of the Interaction Between the Bacterial Wilt Pathogen *Ralstonia solanacearum* and the Model Legume Plant *Medicago truncatula*. MPMI. 20: 159-167

10. Kiba A, Maimbo, M, Kanda, A, Tomiyama, H, Ohnishi, K, Hikichi, Y (2007). Isolation and expression analysis of candidate genes related to *Ralstonia*

<sup>11</sup> -Effectors-triggered immunity

25. Urlinger S, Kuchler K, Meyer TH, Uebel S, Tamp'e R (1997). Intracellular location, complex formation, and function of the transporter associated with antigen processing in yeast. *Eur. J. Biochem.* 245:266–272
26. Altenbach D, Robatzek S (2007). Pattern Recognition Receptors: From the Cell Surface to Intracellular Dynamics. *MPMI.* 20: 1033-1039
27. Muskett P, Parker J (2003). Role of SGT1 in the regulation of plant R gene signalling. *Microb. Infec.* 5: 969–976
28. Boter M, Amigues B, Peart J, Breuer C, Kadota Y, Casais C, Moore G, Kleanthous C, Ochsenbein F, Shirasu K, Guerois R (2007). Structural and Functional Analysis of SGT1 Reveals That Its Interaction with HSP90 Is Required for the Accumulation of Rx, an R Protein Involved in Plant Immunity. *The Plant Cell.* 19: 3791–3804
29. Azevedo C, Betsuyaku S, Peart J, Takahashi A, Noel L, Sadanandom A, Casais C, Parker, J, Shirasu K (2006). Role of SGT1 in resistance protein accumulation in plant immunity. *EMBO J.* 25: 2007–2016
30. Peart JR (2002). The ubiquitin ligase-associated protein SGT1 is required for host and nonhost disease resistance in plants. *Proc. Natl. Acad. Sci. USA* 99: 10865–10869
31. Sangster TA, Queitsch C (2005). The HSP90 chaperone complex, an emerging force in plant development and phenotypic plasticity. *Curr. Opin. Plant Biol.* 8:86–92
32. Coaker G, Falick A, Staskawicz B (2005). Activation of a Phytopathogenic Bacterial Effector Protein by a Eukaryotic Cyclophilin. *Science* 308 : 548-550
33. Zipfel C (2008). Pattern-recognition receptors in plant innate immunity. *Curr. Opin. Immunol.* 20:10–16
34. Desender S, Andrivon D, Val F (2007). Activation of defence reactions in Solanaceae: where is the specificity? *Cellular Microbiol.* 9: 21–30
35. Nomura K, Melotto M, He SY (2005). Suppression of host defense in compatible plant–*Pseudomonas syringae* interactions. *Curr Opin Plant Biol.* 8:361–368
36. Musser RO, Hum-Musser SM, Eichenseer H, Peiffer M, Ervin G, Murphy JB, Felton GW (2002). Caterpillar saliva beats plant defences. A new weapon emerges in the evolutionary arms race between plants and herbivores. *Nature* 416:599-600