SUPPLEMENTARY INFORMATION

Proteomic analysis of proteins related to defense responses in *Arabidopsis* plants transformed with the *rolB* oncogene

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Supplementary Table S1. Sequences of the PCR primers used in this study

UniProtKB code	Genbank accession number	Primer name	Primer sequences (5'-3')	Ref.
P20402 (ROB1_AGRRH)	X03433	*rolB*-780-D	ATGGATCCCAAATTGCTATTC	[1]
		rolB-780-R	TTAGGCTTCTTTCTTCAGGTT	
		rolB-D	ACATCATAGGGGCGGTTTTCAGT	[2]
		rolB-R	TTTCGCAAGTTCCTTGTTCATTC	
Q84K90 (RHIP1_ARATH)	BT002964	*RHIP1*-D	GAGCTGAAGTGGCTTCAATGAC	[3]
		RHIP1-R	GGTCCGACATACCCATGATCC	
O24456 (GBLPA_ARATH)	AY035007	*RACK1A*-D	GCTGAAAAGGCTGACAACAGT	[4]
		RACK1A-R	GCTCCAGTTAAGGCTTGTGC	
Q9C4Z6 (GPLPB_ARATH)	AY059723	*RACK1B*-D	TGTTGAGGATTTGAAGGTTGA	
		RACK1B-R	CCAGTTCAAGCTTGTGCAGTA	
Q9LV28 (GPLPC_ARATH)	AY050338	*RACK1C*-D	GAGGCAGAGAAGAATGAAGGTG	
		RACK1C-R	CCAGTTCAAGCTTGTGCAGTA	
P34790	AY093227	*CYP18-3*-D	GAACGGAACAGGCGGTGAG	

(CP18C_ARATH)		*CYP18-3*-R	TCAAGCCAATCGGTCTTCACG	
Q38867 (CP19C_ARATH)	AY072128	*CYP19-3*-D	CTTTCACCGTATAATCCCAG	[5]
		CYP19-3-R	CCTGTCAAGATCAACCCACCC	
Q38900 (CP19A_ARATH)	AY048215	*CYP19-1*-D	GGTCAAGTTGTTGAGGGATTG	
		CYP19-1-R	ACACAAAGCTACCATTGGATC	
P34791 (CP20C_ARATH)	AF325026	*CYP20-3*-D	CAATGCATCGGTCAATAGTG	[5]
		CYP20-3-R	CTGGTGAGAAGAAATACGGG	
Q42406 (CP18D_ARATH)	AY054468	*CYP18-4*-D	ATCTACGGTGCTAAGTTCAAGG	
		CYP18-4-R	GTGCTTACCATCTAACCACGAC	
A8R7E6 (CERK1_ARATH)	AB367524	*CERK1*-D	TTATAGGAGTGATTGTGGCTTTG	
		CERK1-R	CCTTAGTAGACAACGGAATAGAAGA	
Q39023 (MPK3_ARATH)	AF386961	*MPK3*-D	GCCCTTAGCTAAACTTTTCTC	[6]
		MPK3-R	CGTGCAATTTAGCAAGGTACT	
Q39024 (MPK4_ARATH)	AF360231	*MPK4*-D	AGCAGACGCATCACAGTTGA	
		MPK4-R	TGAACGGCCTCACACATACC	
Q39026 (MPK6_ARATH)	AY120737	*MPK6*-D	CGTTTGTTCGGCTATGAATTCTG	[7]
		MPK6-R	GTGGCGGGATAATATCTCTGATTG	
Q9XI87 (Q9XI87_ARATH)	BT026491	*VIK*-D	ATGGCTCCTGAAGTATTCAAGC	[8]
		VIK-R	TCTTGAGAATGTCCAGAAACGACG	
P43082 (HEVL_ARATH)	AF370536	*HEL*-D	TGTTCTCCGACCAACAACTG	
		HEL-R	GTTCTTCACCCTTAAACACTTGC	
O49195 (VSP1_ARATH)	AF386930	*VSP1*-D	CATCTCATACTCAAGCCAAACG	
		VSP1-R	AGTATCCTCAACCAAATCAGC	
O82122 (VSP2_ARATH)	AY048282	*VSP2*-D	ATGGATACGGAACAGAGAAGACC	
		VSP2-R	CGAGAGTGACATTCTTCCACAAC	
Q9SR37 (BGL23_ARATH)	AF386967	*BGLU23* -D	CGTCAATGCTAAATGCCAAGA	
		BGLU23 -R	CGAACCAAGCAGGACTATGTG	
O04314	AF370488	*PBP1*-D	TACCAAAGGCCGTGTTCTCC	

(JAL30_ARATH)		*PBP1*-R	CATCCCATACAGTTCCGTCATC	
Q03250 (RBG7_ARATH)	AF428381	*RBG7*-D	GCGACGTTATTGATTCCAAG	
		RBG7-R	TTGTCCGTTCATTCCCTCA	
Q9LKR3 (BIP1_ARATH)	BT000453	*BIP1/2*-D	TCACTTGGGAGGTGAGGACTTT	[9]
Q39043 (BIP2_ARATH)	BT002392	*BIP1/2*-R	CTCACATTCCCTTCGGAGCTTA	
Q9LTX9 (HSP7G_ARATH)	BT000919	*HSP70-7*-D	CAACTCAAGAAATCAAAGACAC	[5]
		HSP70-7-R	CGTCAATCACATCACCGCCG	
Q9SIF2 (HS905_ARATH)	AF436826	*HSP90-5*-D	CTTCTCCTTGTGTGCTTGTC	
		HSP90-5-R	CTTGTTGCTTCAGTGCTCTC	
O65282 (CH10C_ARATH)	AJ010818	*CPN20*-D	CCGAGACTACCAAAGAGAAG	
		CPN20-R	CTAAGAAAGTATAGCCATCAC	

Supplementary Table S2. Comparison of protein expression and expression of the corresponding genes; data from the present study and previous study for *rolB* transformed calli.

Protein Name	**UniProtKB code**	***rolB*-plants**		***rolB*-calli**
		Protein (2DE)	*mRNA (qPCR)*	*Protein (2DE)* [5]
RACK1-associated proteins				
RACK1A	O24456 (GBLPA_ARATH)	**1,48* down**	**1,44** down**	**1,5* down**
RACK1B	Q9C4Z6 (GPLPB_ARATH)	**3,8* down**	**1,7* down**	Not found
RACK1C	Q9LV28 (GPLPC_ARATH)	**4,2* down**	**3,55* down**	Not found
Defense reactions and plant immunity				
PBP1 (JAL30)	O04314 (JAL30_ARATH)	**10* up**	**2,18* up**	Not found
PYK10 (BGL23)	Q9SR37 (BGL23_ARATH)	**10* up**	**2,24* up**	Not found
GRP7	Q03250 (RBG7_ARATH)	**1,98* down**	**3,31* down**	Not found
HEVL	P43082 (HEVL_ARATH)	**10* up**	**9,04* up**	Not found
VIK	Q9XI87 (Q9XI87_ARATH)	Not found	1,03 down	**2* down**
VSP1	O49195 (VSP1_ARATH)	**3,4* up**	**2,73** up**	Not found

VSP2	O82122 (VSP2_ARATH)	**6,7* up**	**2,58** up**	Not found
CERK1-associated proteins.				
CERK1	A8R7E6 (CERK1_ARATH)	Not found	1,05 up	Not found
MPK3	Q39023 (MPK3_ARATH)	Not found	1,06 down	Not found
MPK4	Q39024 (MPK4_ARATH)	Not found	1,43 up	Not found
MPK6	Q39026 (MPK6_ARATH)	Not found	1,28 up	Not found
Chaperone-type proteins.				
BIP1/2	Q9LKR3 (BIP1_ARATH) Q39043 (BIP2_ARATH)	**2,8* up**	**2,55* up**	Not found
HSP70-7	Q9LTX9 (HSP7G_ARATH)	Not found	1,104 up	**2.9* up**
HSP90-5	Q9SIF2 (HS905_ARATH)	Not found	1,13 up	**2.4* up**
CPN10	O65282 (CH20_ARATH)	Not found	**2,43* up**	**2* up**
ROC1 (CYP18-3)	P34790 (CP18C_ARATH)	**2,0* up**	**1,84* up**	**6,6* down**
ROC2 (CYP19-3)	Q38867 (CP19C_ARATH)	**3,8* up**	**1,65* up**	**10* down**
ROC3 (CYP19-1)	Q38900 (CP19A_ARATH)	**1,4* up**	**1,41* up**	Not found
ROC4 (CYP20-3)	P34791 (CP20C_ARATH)	Not found	**4,96* down**	**10* down**
ROC5 (CYP18-4)	Q42406 (CP18D_ARATH)	**1,7* up**	**1,72* up**	Not found

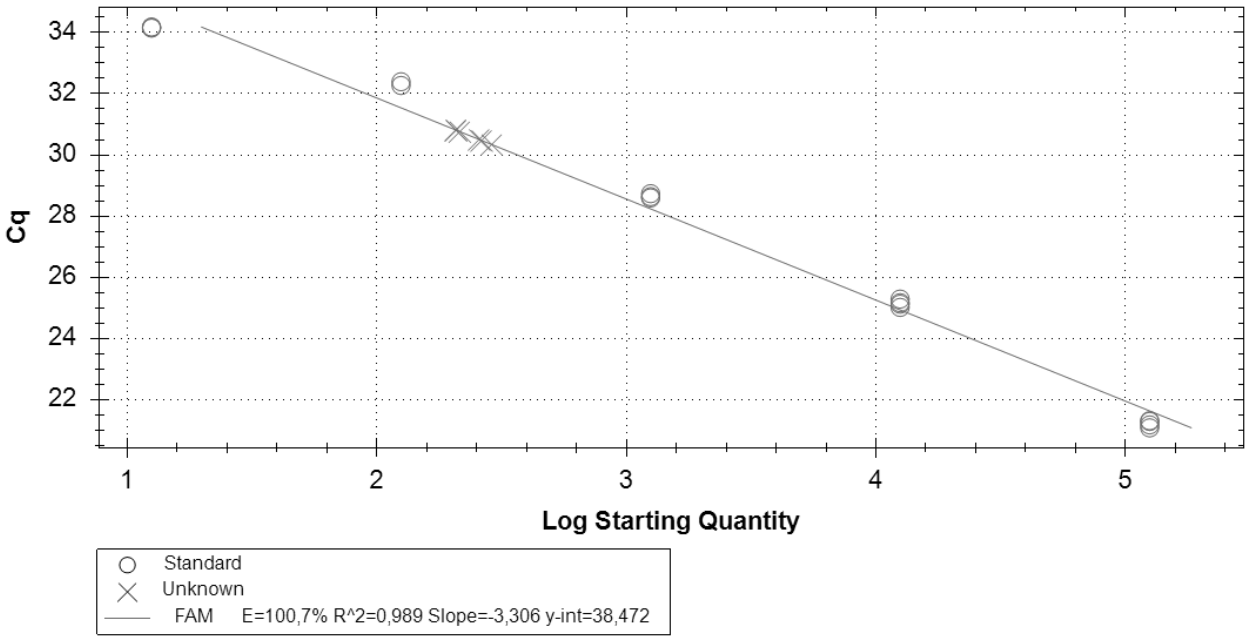
Asterisks indicate statistically significant differences of means (*P <0.05; **P <0.01), Fisher's LSD.

References

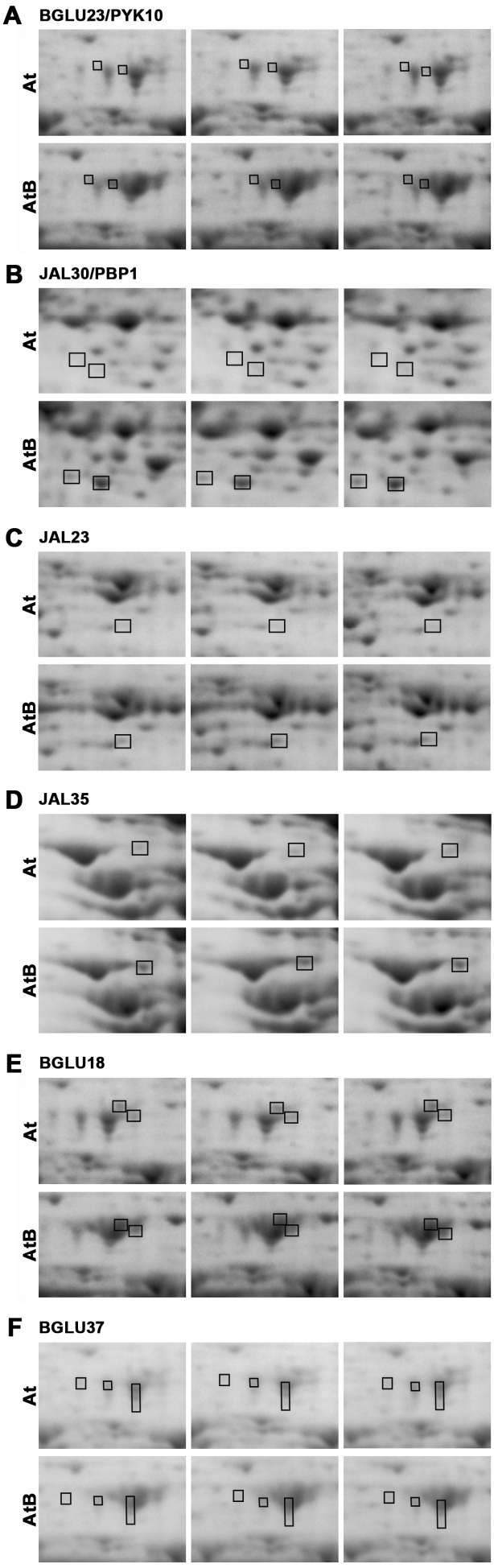
1. Bettini, P.P.; Marvasi, M.; Fani, F.; Lazzara, L.; Cosi, E.; Melani, L.; Mauro, M.L. *Agrobacterium rhizogenes rolB* gene affects photosynthesis and chlorophyll content in transgenic tomato (*Solanum lycopersicum L.*) plants. *J. Plant. Physiol.* ***2016****, 204*, 27-35, doi: 10.1016/j.jplph.2016.07.010.
2. Grishchenko, O.V.; Kiselev, K.V.; Tchernoded, G.K.; Fedoreyev, S.A.; Veselova, M.V.; Bulgakov, V.P.; Zhuravlev, Y.N. *RolB* gene-induced production of isoflavonoids in transformed *Maackia amurensis* cells. *Appl. Microbiol. Biotechnol.* **2016***, 100*, 7479-7489, doi: 10.1007/s00253-016-7483-y.
3. Brzezinka, K.; Altmann, S.; Czesnick, H.; Nicolas, P.; Gorka, M.; Benke, E.; Kabelitz, T.; Jähne, F.; Graf, A.; Kappel, C.; Bäurle, I. *Arabidopsis* FORGETTER1 mediates stress-induced chromatin memory through nucleosome remodeling. *Elife* **2016***, 5, e17061*, doi: 10.7554/eLife.17061.
4. Cheng, Z.; Li, J.F.; Niu, Y.; Zhang, X.C.; Woody, O.Z.; Xiong, Y.; Djonović, S.; Millet, Y.; Bush, J.; McConkey, B.J.; Sheen, J.; Ausubel, F.M. Pathogen-secreted proteases

activate a novel plant immune pathway. *Nature* **2015**, *521*, 213-216, doi: 10.1038/nature14243.

5. Bulgakov, V.P.; Vereshchagina, Y.V.; Bulgakov, D.V.; Veremeichik, G.N.; Shkryl, Y.N. The rolB plant oncogene affects multiple signaling protein modules related to hormone signaling and plant defense. *Sci. Rep.* **2018***, 8*, 2285, doi: 10.1038/s41598-018-20694-6.
6. Ye, L.; Li, L.; Wang, L.; Wang, S.; Li, S.; Du, J.; Zhang, S.; Shou, H. MPK3/MPK6 are involved in iron deficiency-induced ethylene production in *Arabidopsis*. *Front. Plant Sci.* **2015***,* 6, 953, doi: 10.3389/fpls.2015.00953.
7. Zhao, L.; Wang, C.; Zhu, F.; Li, Y. Mild osmotic stress promotes 4-methoxy indolyl-3-methyl glucosinolate biosynthesis mediated by the MKK9-MPK3/MPK6 cascade in *Arabidopsis*. *Plant Cell. Rep*. **2017**, 36, 543-555, doi: 10.1007/s00299-017-2101-8.
8. Wingenter, K.; Trentmann, O.; Winschuh, I.; Hörmiller, I.I.; Heyer, A.G.; Reinders, J.; Schulz, A.; Geiger, D.; Hedrich, R.; Neuhaus, H.E. A member of the mitogen-activated protein 3-kinase family is involved in the regulation of plant vacuolar glucose uptake. *Plant J*. **2011**, *68*, 890-900, doi: 10.1111/j.1365-313X.2011.04739.x.
9. Arraño-Salinas, P.; Domínguez-Figueroa, J.; Herrera-Vásquez, A.; Zavala, D.; Medina, J.; Vicente-Carbajosa, J.; Meneses, C.; Canessa, P.; Moreno, A.A.; Blanco-Herrera, F. WRKY7, -11 and -17 transcription factors are modulators of the bZIP28 branch of the unfolded protein response during PAMP-triggered immunity in *Arabidopsis thaliana*. *Plant Sci.* **2018***, 277*, 242-250, doi: 10.1016/j.plantsci.2018.09.019.



Supplementary Figure S1. Standard curve for absolute quantification of *rolB* expression. Standard curve was constructed using $1.25x10^1$ to $1.25x10^5$ copies/µl of *rolB* amplicon (three technical replicates for each standard dilution). The data represent a result from two biological replicates with cDNA samples (marked by a cross) analyzed in triplicate.


A
BGLU23/PYK10
At
AtB
B
JAL30/PBP1
At
AtB
C
JAL23
At
AtB
D
JAL35
At
AtB
E
BGLU18
At
AtB
F
BGLU37
At
AtB

Supplementary Figure S2. Induction of proteins of the PYK10 complex by the *rolB* gene. Fragments of 2D gels of the control (At) and *rolB* AtB-1 (AtB) plant protein fractions are presented. **A-F**: beta-glucosidases 23 (PYK10), PYK10-binding protein 1 (jacalin-related lectin 30)/PBP1, jacalin-related lectin 23, jacalin-related lectin 35, beta-D-glucopyranosyl abscisate beta-glucosidase/BGLU18, and myrosinase 2/Beta-glucosidase 37/ BGLU37