Supplementary Material

Transcriptome-based analysis of candidate gene markers associated with resistance mechanism to *Phytophthora melonis* that causes root and crown rot in pumpkin

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Supplementary Table S1.

Gene description, accession number, primer name, forward and reverse primers sequence, GC%, annealing temperature and amplicon size of the oligonucleotide primers used in qRT-PCR.

Gene description	Accession number[a]	Primer name		Sequence primer (5′………3′)	GC%	Annealing temperature (°C)	PCR product length (bp)	Gene reference
Basic Helix loop-helix transcription factor	XM_023691996.1	*bHLH87*	F	TGACCCAGAAGCCATAGCACA	52.38	60	165	Guo et al. 2020
			R	CTGATTCTCTCTCTCCTTTGCCT	47.83			
Ethylene response factor	XM_023669808.1	*ERF014*	F	CTCAGCCTCTTTTGCCCTTTC	52.38	59	172	Guo et al. 2018
			R	CCAACTCCTCATTCTAACACCCT	47.83			
Heat stress transcription factorA	XM_023690139.1	*HSF*	F	GGTCCGCCTCCATTTCTCACTA	54.55	60	181	Shen and Yuan. 2020
			R	TGAGCTGACGAACGAAACTAGAG	47.83			
Transcription factors	XM_023682473.1	*MYB*	F	ACCTGCCTCCATCCATTTCCTT	50.00	60	188	Guo et al. 2018
			R	ATGGCTCTTTGTCTTCTGTTTCCC	45.83			
Pathogenesis-related protein 1	JF332040.1	*PR1*	F	ACGCTACCCAATATGCCAATGAAC	45.83	58	131	Zeighaminejad et al. 2016
			R	ATCCACCCACATTTGAACTGCT	45.45			
Transcription factors	XM_023698327.1	*WRKY21*	F	GCAGCATGAGAGGTTGTCCAG	57.14	58	221	Guo et al. 2018
			R	AAAGAAGCACCAACACCAATCC	45.45			
Cysteine proteinase inhibitor 1	XM_023664493.1	*CPI*	F	CTCGTGGCTTCTCTTCTCCTCTA	52.17	59	208	Zinovieva et al. 2021
			R	TTTGCATTTCTCCACCAATCACT	39.13			
Peroxidase	XM_023676901.1	*POD*	F	AGCTCGTGTCCAAACCTTC	52.63	58	165	Vicente-Do´lera et al. 2014
			R	AAATTGCTCCCCATCACTTCC	47.62			
Phytosulfokine receptor 2	XM_023696730.1	*PSK*	F	AAGACCTGAAACCGACACTTACTC	45.83	60	156	Zhang et al. 2020
			R	TCAAAACTGTTACTCCGATCTCCA	41.67			
Suppressor of G-two allele of skp1	XM_023697769.1	*SGT*	F	ATAGGCTCCGTTTTGGGCTCAG	54.55	60	133	Guo et al. 2018
			R	CTGCATCCCCATATAATGTCACCT	45.83			
Actin	XM_023676306.1	*Act*	F	GCTCTACCCCATGCTATCCTCC	59.59	60	115	Bezold et al. 2005
			R	TTTCCCGTTCAGCAGTGGTTGT	50.00			

[a] The accessions are presented in https ://www.ncbi.nlm.nih.gov/gene/?term

Supplementary Table S2.
Mean squares analysis of variance for the relative expression level of defense genes, for susceptible and resistant genotypes against *P. melonis.*

S.O.V	df	*ERF014*	*bHLH87*	*WRKY21*	*HSF*	*MYB*	*POD*	*PR-1*	*PSK*	*SGT1*	*CPI*
Treatment (A)	1	10.922**	7.539**	9.734**	10.076**	5.124**	8.885**	7.989**	6.946**	6.880**	9.730**
Tissue (B)	1	0.013 ^{ns}	0.405**	0.000 ^{ns}	0.080**	0.181**	0.065**	0.138**	0.162**	0.000 ^{ns}	0.026**
Time (C)	2	0.054**	0.081**	0.444**	0.200**	0.007 ^{ns}	1.401**	0.405**	0.200**	0.409**	0.299**
Cultivar (D)	3	3.231**	2.850**	3.966**	4.750**	2.507**	3.212**	2.610**	2.580**	2.071**	3.761**
AB	1	0.000 ^{ns}	0.063**	0.012 ^{ns}	0.014 ^{ns}	0.018**	0.036**	0.067**	0.148**	0.004 ^{ns}	0.003 ^{ns}
AC	2	0.077**	0.035**	0.172**	0.052**	0.023**	0.425**	0.155**	0.081**	0.175**	0.252**
AD	3	2.877**	2.562**	3.980**	4.230**	2.326**	2.824**	2.166**	2.479**	1.686**	3.183**
BC	2	0.037**	0.172**	0.019*	0.053**	0.076**	0.098**	0.146**	0.193**	0.016**	0.024**
BD	3	0.043**	0.080**	0.013*	0.022**	0.112**	0.111**	0.089**	0.046**	0.017**	0.054**
CD	6	0.036**	0.066**	0.110**	0.127**	0.025**	0.278**	0.057**	0.135**	0.055**	0.095**
ABC	2	0.036**	0.081**	0.009 ^{ns}	0.052**	0.018**	0.100**	0.061**	0.056**	0.063**	0.008 ^{ns}
ABD	3	0.006 ^{ns}	0.036**	0.053**	0.017*	0.015**	0.032**	0.025**	0.098**	0.018**	0.003 ^{ns}
ACD	6	0.026**	0.060**	0.128**	0.032**	0.022**	0.187**	0.056**	0.057**	0.087**	0.075**
BCD	6	0.025**	0.111**	0.017**	0.028**	0.029**	0.139**	0.053**	0.045**	0.042**	0.019**
ABCD	6	0.025**	0.064**	0.025**	0.051**	0.008**	0.047**	0.043**	0.017**	0.038**	0.004 ^{ns}
Error	96	0.003	0.003	0.005	0.004	0.002	0.003	0.004	0.003	0.003	0.003
C.V.		36.32	35.53	40.35	41.54	32.26	39.82	34.35	34.64	31.74	38.23

* = Significant at 5% probability level, ** = Significant at 1% probability level and - ns= Not significant

Supplementary Table S3.

Correlation between roots and leaves genes levels expression for susceptible and resistant pumpkin genotypes to *P. melonis*.

Leaf \ Root	ERF014	bHLH87	WRKY21	HSF	MYB	POD	PR-1	PSK	SGT1	CPI
ERF014	0.952**									
bHLH87		0.857**								
WRKY21			0.960**							
HSF				0.950**						
MYB					0.916**					
POD						0.902**				
PR-1							0.900**			
PSK								0.911**		
SGT1									0.916**	
CPI										0.974**

** = Significant at 1% probability level.

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Supplementary Table S4- Analysis of variance in relation to quantification of CAT and POX activities in resistant and susceptible pumpkin cultivars as compared to controls (non-inoculated ones).

S.O.V		Mean square (M>S)			
		CAT (U mg^{-1})		PAL (U mg^{-1})	
		Activity	Specific activity	Activity	Specific activity
Cultivar (A)	3	37.54**	114.4**	495.1**	854.6**
Time (B)	2	5.19**	15.6**	284.3**	682.1**
Treatment (C)	1	49.43**	37.0**	874.1**	984.9**
AB	6	7.00*	13.2**	351.9**	531.1**
AC	3	6.24**	13.7**	407.6**	1092.8**
BC	2	2.72**	3.7**	273.1**	604.4**
ABC	6	4.12**	7.1**	303.0**	508.2**
Error	48	0.35	0.7	2.2	3.7
C.V%		18.0	13.0	15.2	10.2

[ns] Not significant, **Significant at p<0.05.

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Supplementary Table S5.

Mean squares of variance analysis of the individual effect of inoculation treatment for the evaluated growth parameters including, stem length (SL), leaf length (LL), root length (RL), root volume (RV), stem diameter (SD), root diameter (RD), root fresh weight (RFW), stem fresh weight (SFW), root dry weight (RDW), stem dry weight (SDW) in Tanbal, Ghelayni, Marmari and Khoreshti 7 days after inoculation with *P. melonis.*

SOV	df	SL	LL	RL	RV	SD	RD	RFW	SFW	RDW	SDW
					Mean square (MS)						
Genotype(A)	1	39**	26**	3.4^{ns}	0.65**	0.025*	0.069**	5.35**	6.57**	0.25**	0.44**
Inoculation treatment(B)	3	6.9**	0.2^{ns}	1.8^{ns}	0.09^{ns}	0.001^{ns}	0.023^{ns}	0.00^{ns}	0.41**	0.05**	0.08**
Interaction AB	3	2.7*	11.1**	3.1^{ns}	0.44**	0.003^{ns}	0.003^{ns}	0.89**	9.15*	0.028**	0.06**
Error	32	0.6	0.9	1.3	0.10	0.006	0.0076	0.01	0.05	0.0005	0.001
CV%		11.0	9.3	15.2	24.1	16.8	18.0	7.1	6.5	10.6	7.4

* = Significant at 5% probability level, ** = Significant at 1% probability level and - ns= Not significant