

Supplemental Figures

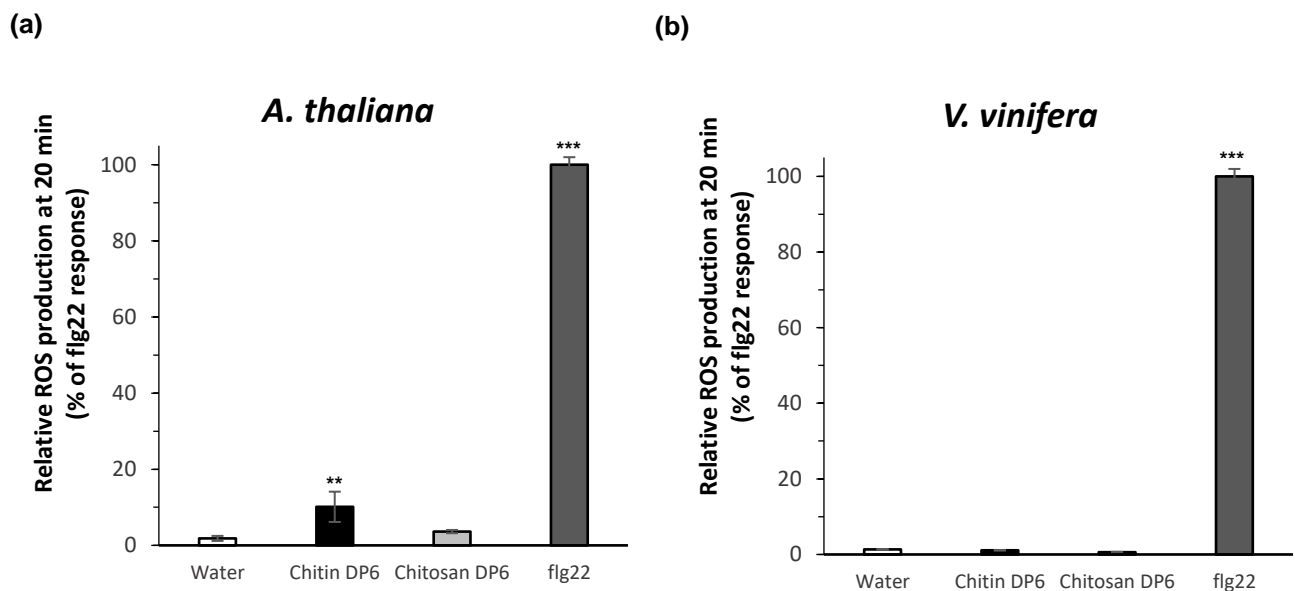


Fig. S1. Chitin and chitosan DP6 did not induce similarly ROS production in *Arabidopsis* and grapevine cells. Relative H₂O₂ production in (a) *A. thaliana* or (b) *V. vinifera* cells 20 min post-treatment with chitin DP6 (100 µg mL⁻¹), chitosan DP6 (100 µg mL⁻¹) and flg22 (1 µM) treatments or water (negative control). ROS production was measured by chemiluminescence of luminol according to Trdá et al. (2014) and results are expressed relatively to flg22 (positive control=100%). Values are means ± SE from three independent experiments (n=3). Asterisks indicate a statistically significant difference between control and the elicitor treatment (Tukey's pairwise test; *, P < 0.05, **, P < 0.01, ***, P < 0.001).

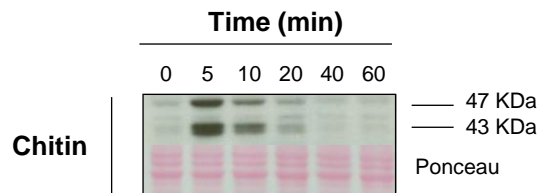


Fig. S2. The crab shell chitin also induced defense responses in grapevine cells. Activation kinetics of two mitogen-activated protein kinases (MAPKs) in grapevine cells treated with $100 \mu\text{g.mL}^{-1}$ of the unpurified crab shell chitin NA-COS-Y (Lloyd et al., 2014). MAPKs were detected by immunoblot with an antibody raised against the human phosphorylated extracellular regulated protein kinase 1/2 ($\alpha\text{-pERK1/2}$). Fifteen μg of proteins were loaded in each lane. Homogeneous loading was checked by Ponceau red staining. This result shows one representative experiment out of three.

		Signal peptide	
AtCERK1/LYK1	1	MKIKIS-LIAPILLI-----FSFFFVAVESKCR	RTSCPLALASYYLENGITLTSVINQNLS
OsCERK1	1	MEASTS-LLVLLVLAFAAF-AAGTVTEAAGDGC	SAGCDLALASFYVTPNQNVTNMADIFGI
VvLYK1-1	1	MKQKV-----GLGFFVLLSVFCVAVDSQC	SRGCDLALGSYYVWQGSNLTFTISQLFQT
VvLYK1-2	1	MVTSNSRNATQILAFGFHFLVLLCSKANAKC	SRGCDLALASYVVDGNSLTYIRKIFGR
VvLYK1-3	1	MLVFRISRFEMLLV---FVSLVIFLSIGVESK	CRGCDLALASYNINWNGITLSFIATAFST
		LysM1	
AtCERK1/LYK1	54	SIAPYDQINFDPILRYNSNIKDKDRIQMGSR	VLPF-PCFCQP-----GDFLGHNFYS
OsCERK1	59	GAAN-----YRSIAPYNPNIPLDFINVGGR	VNYF-TGCRSLPGSPGATYLAGAFPFQ
VvLYK1-1	52	TI-----SEILSYNSQIANQDSVEADTRIR	VPYSSDCIN-----GEFLGKVFNYT
VvLYK1-2	61	EI-----SEILKYNPQINQDSIDTGSRIN	VPF-RCDCLN-----GDFLGHTEFYT
VvLYK1-3	58	SI-----SEIQSFNPQINDIDLIIIVDTRL	NIPF-SCSCID-----GEFLGHTEFYT
		LysM2	
AtCERK1/LYK1	107	VROEDTYERVAISNYANLTTMESLQARNPFP	PATNIPL-SATLNVLVNVCSCGDES
OsCERK1	113	MSRGOITYTSVAA-NYNNLTTAEWLQATNSYP	ANNIPD-TAVINATVNCSCGDASIS
VvLYK1-1	98	VQSGDTYLVAETIYNSLTTSAWLQNFNSYA	ANLIPDTDAYLNVTLNCSCGNST
VvLYK1-2	106	TQFGDTYRIRIAERAFSNLTTEDWVHRVNE	YPPTRIPD-DVQINVTVNCS
VvLYK1-3	103	VDSNDTYNIIARTFYANLTTVEWLERFNRYE	ATEIPV-NAIINVTVNCS
		LysM3	
AtCERK1/LYK1	166	LFVITYPLRPEDSLSSIARSSGVSAD--ILQ	RYPNGVNFNSGNGIVYVPRDPNGA
OsCERK1	171	LFLTYPLRAEDTLASVAATYGLSSQLDVVRR	YNPMEASATGSGIVYIPVKDPNGS
VvLYK1-1	158	LFLSYPLRPEDNLSVAESEGLNAS--LLQSY	NPDSNFSAGSGLVYIPTKDTSGS
VvLYK1-2	165	LFATYPLRDGENLSTVAAAAGITDD--LVRR	YNPAADFSAIGLVFVPAKQDQET
VvLYK1-3	162	LFVITYPLQPGESLSSIANESGLPSK--LLQ	DYNPGVDFSLGSGLVETPKDQNGS
		Transmembrane	
AtCERK1/LYK1	224	SSKQDGVGAGVIAGIVIGVIVALLLILFIVY	YA-YRKNKSKGDSFSSS--IPLSTK
OsCERK1	231	SP-GKGASAGAIAGGVVAGVV--VLA	AIFLYIIFYRRRKAKQATLLQS--
VvLYK1-1	216	SS--TGLAGGVIAGISIAAVGVLLITVCI	IYIGFYRKRKVKKEAALLPT--
VvLYK1-2	223	LS-NSGISSGVIAGISVAGIVGSLFAFFL	FARICKRKKVKVLFPAASEQQYMQ
VvLYK1-3	220	LS-ONGISVGIAGISVAGVAGSLLAFVLY	VGIYKRKMG-KAPLLPAAFEDQHM
		Serine/Threonine Kinase	
AtCERK1/LYK1	337	YYAELRGEKAAIKKMDMEASKQFLAELKVL	TRVHHVNLVRLIGYCVESLFLVYEY
OsCERK1	339	YYAELRGEKAAIKKMDMQATHEFLAELKVL	THVHHLNLVRLIGYCTESLFLVYEY
VvLYK1-1	331	YYAELRGEKAAIKKMDMQASREFLAELKVL	THVHHLNLVRLIGYCVESLFLVYEY
VvLYK1-2	341	YYAELRGEKAAIKKMDMQASKEFLAELKVL	THVHHLNLVRLIGYCVESLFLVYEY
VvLYK1-3	337	YYAELQGGKAAIKKMDMQASKEFLAELKVL	THVHHFNLVRLIGYCVTGSLETVY
AtCERK1/LYK1	397	NLQHLHGSGREPLPWTKRVOIALDSARGLE	YIHEHTVPVYVHRDIKSANILIDQ
OsCERK1	399	NLSQHLRGMGYEPLSWAARIQIALDSARGLE	YIHEHTVPVYIHRDIKSANILIDKN
VvLYK1-1	391	NLSQHLRSGRDPLQWSSRVQIALDSARGLE	YIHEHTVPVYIHRDIKSANILIDKN
VvLYK1-2	401	NLSHHLRSGKDPWPSSRVQIALDSARGLE	YIHEHTVPVYIHRDIKIPANILIDK
VvLYK1-3	397	NLSQHLRSGNDPLPWSTRVQIALDAARGLE	YIHEHTVPVYVHRDIKSANILIDKN
AtCERK1/LYK1	457	VADFGTLTKLTEVGGSSAT---RGAMGTFG	YMAPET-VYGEVSAKVDVYAFGVV
OsCERK1	459	VADFGTLTKLTEVGGTSMPTGTRVVGTFG	YMPPEYARYGDVSPKVDVYAFGVV
VvLYK1-1	451	VADFGTLTKLTEVGSSSLP--TRLVGTFG	YMPPEYAQYGDVSPKVDVYAFGVV
VvLYK1-2	461	VADFGTLTKLTEVGSASIP--TRLVGTFG	YMPPEYAQYGDVSPKIDVFAFGV
VvLYK1-3	457	VADFGTLTKLTVAGSSSLP--TRLVGTFG	YMPPEYAQFGAVTPKIDVYAFGVV

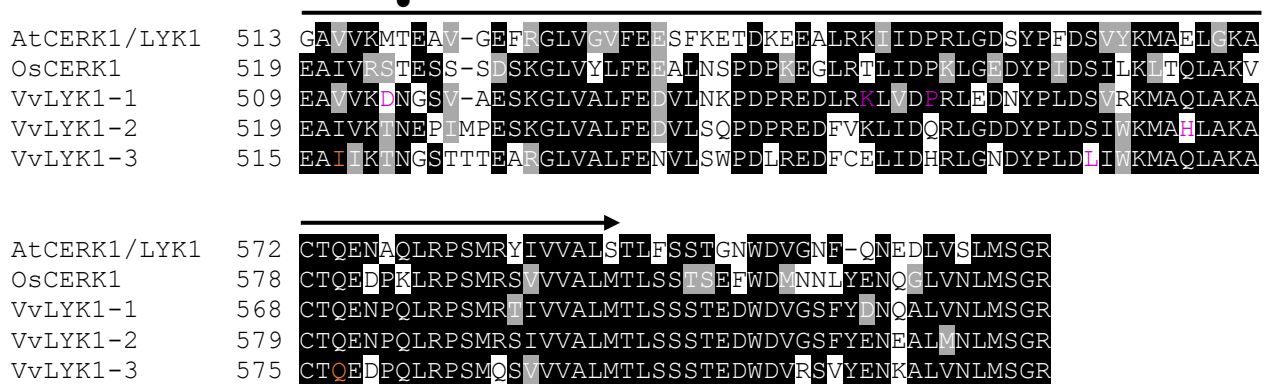


Fig. S3. Alignment of AtCERK1/LYK1, the rice OsCERK1 and its putative orthologs in grapevine (VvLYK1-1/-2/-3). Protein sequences were aligned with T-Coffee. Black and gray shading representing identical and positive amino acids, respectively, was visualized with Boxshade. The predicted signal peptide, the lysin motifs (LysM), the transmembrane region and the serine/threonine (S/T) kinase are shown. The residues of the chitin-binding site in AtCERK1/LYK1 LysM2 are indicated by asterisks and their conservation is highlighted in the grades of green. The residues E110, E114 and I141 bind to N-acetyl moieties of (GlcNAc)₅, while Q109, T112, Y113, A138, T139, N140, P142 and L143 interact with hydroxyl and hydroxymethyl groups of glucose part (Liu *et al.*, 2012). Conserved Cys residues (in red) form disulfide bridges (indicated by arrows). The RD type of kinase is highlighted in blue. SNPs between the NCBI reference sequence from *V. vinifera* cv Pinot Noir (PN40024) and cv Cabernet-Sauvignon are highlighted in purple (VvLYK1-1: Q99H, L220I, A221S, H264Q, S265T, S313T, D514E, K543R, P547A; VvLYK1-2: K67T, E73K, G95R, I186V, E216G, R279G, H574Q; VvLYK1-3: F116Y, I142L, V250A, A493E, L564F) or in orange when compared to cv Gamay (VvLYK1-1: no SNP; VvLYK1-2: no SNP; VvLYK1-3: -224A, V250A, A493E, I517V, Q577L). Residues S266, S268, S270, S274 and T519 (indicated by ●) were found to be phosphorylated in AtCERK1/LYK1 after chitin treatment (Petutschnig *et al.*, 2010). A: Ala, C:Cys, D:Asp, E:Glu, F:Phe, G:Gly, H:His, I:Ile, K:Lys, L:Leu, M:Met, N:Asn, P:Pro, Q:Gln, R:Arg, S:Ser, T:Thr, V:Val, W:Trp, Y:Tyr.

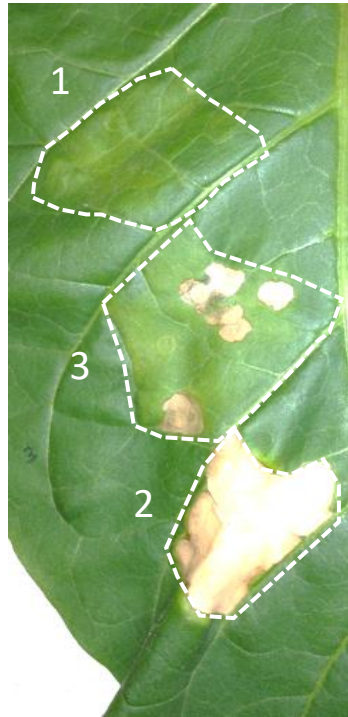


Fig. S4. Necrosis observed in response to the over-expression of VvLYK1-2 in *Nicotiana benthamiana*. Symptoms observed 2d after leaf infiltration with (1) *A. tumefaciens* GV3101 alone (negative control), (2) *A. tumefaciens* GV3101 containing the construct *RPV1^{TIR1-193}* (positive control; Williams et al. 2016), (3) *A. tumefaciens* GV3101 containing the construct *pART27-VvLYK1-2*.

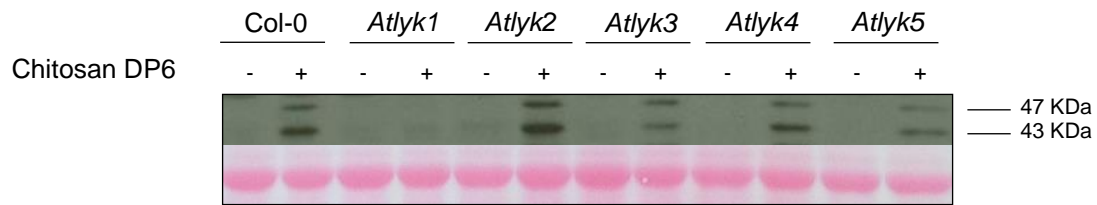


Fig. S5. Immunodetection of MAPKs in *Arabidopsis* mutants *Atlyk1-5* in response to chitosan. Plants of the different *Arabidopsis Atlyk* mutants have been treated with 1 mg.mL⁻¹ chitosan DP6 for 10 min before protein extraction. Activation of the two MAPKs was detected by immunoblot with an antibody raised against the human phosphorylated extracellular regulated protein kinase 1/2 (α -pERK1/2). Fifteen μ g of proteins were loaded in each lane. Homogeneous loading was checked by Ponceau red staining. Similar results were obtained in three independent experiments.

Supplemental Tables

Table S1. The *Vitis vinifera* VvLYK family contains 15 putative genes in the grapevine genome.

NCBI Gene	NCBI mRNA	NCBI Protein	Previous annotation (Zhang et al. 2009)	Closest Arabidopsis homolog	Suggested new gene name	Gene length ¹ (bp)	Expression validated with EST data	Expression validated with RNA-Seq data
LOC100255092	XM_010658923.2	XP_010657225.1	VvLYK1	AtLYK1	VvLYK1-1	1 845	Y	Y
LOC100257218	XM_010657064.2	XP_010655366.1	VvLYK2	AtLYK1	VvLYK1-2	1 878	Y	Y
LOC100262323	XM_010657063.2	XP_010655365.1	VvLYK3	AtLYK1	VvLYK1-3	1 866	Y	Y
LOC100264694	XM_019225274.1	XP_019080819.1	VvLYK11	AtLYK2	VvLYK2	2 028	Y	Y
LOC100256626	XM_002283592.4	XP_002283628.2	VvLYK12	AtLYK3	VvLYK3-1	1 815	Y	Y
LOC100250732	XM_019219283.1	XP_019074828.1	Not described	AtLYK3	VvLYK3-2	Unknown	No ESTs	Incomplete coverage
LOC100256178	XM_002272778.4	XP_002272814.3	Not described	AtLYK3	VvLYK3-3	Unknown	2 ESTs	Incomplete coverage
LOC100242712	XM_002269372.3	XP_002269408.1	VvLYK4	AtLYK4	VvLYK4-1	2 037	1 EST	Y
LOC100264999	XM_010650900.2	XP_010649202.1	VvLYK6	AtLYK4	VvLYK4-2	1 947	1 EST	Y
LOC100258108	XM_002277295.4	XP_002277331.3	VvLYK10	AtLYK5	VvLYK5-1	2 167	Y	Y
Not annotated: tBLASTn match on emb AM480420.1	MF939897	New sequence	Not described	AtLYK5	VvLYK5-2	2 078	Y	Y
LOC100264758	XM_002280034.2	XP_002280070.1	VvLYK7	No match	VvLYK6	2 200	No ESTs	Y
LOC100259809	XM_002269436.4	XP_002269472.2	VvLYK5	No match	VvLYK7	Unknown	No ESTs	Incomplete coverage
LOC100248852	XM_002281844.3	XP_002281880.2	VvLYK8	No match	VvLYK8	Unknown	1 EST	Incomplete coverage
LOC100248488	XM_002276794.3	XP_002276830.3	VvLYK9	No match	VvLYK9	Unknown	2 ESTs	Incomplete coverage

¹ Gene length based on Cabernet Sauvignon sequence - verified by RNA Seq data. Genes in bold = putative pseudogene

Table S2. Percentage of amino acid identity or similarity between VvLYK1-1/-2/-3 and AtCERK1/LYK1 or OsCERK1.

	whole protein sequence		LysM domain		Kinase domain	
	Identity	Similarity	Identity	Similarity	Identity	Similarity
VvLYK1-1 vs AtCERK1	60%	73%	46%	68%	77%	85%
VvLYK1-2 vs AtCERK1	57%	71%	44%	65%	74%	84%
VvLYK1-3 vs AtCERK1	56%	69%	51%	68%	70%	80%
VvLYK1-1 vs OsCERK1	61%	73%	44%	58%	80%	91%
VvLYK1-2 vs OsCERK1	58%	71%	42%	57%	79%	89%
VvLYK1-3 vs OsCERK1	56%	70%	43%	61%	74%	86%

Results were obtained with the NCBI protein BLAST program on the whole protein sequence or the LysM ectodomain or the kinase domain. Protein sequences used: AtCERK1/LYK1 (NP_566689), OsCERK1 (A0A0P0XII1), VvLYK1-1 (XP_010657225), VvLYK1-2 (XP_010655366), VvLYK1-3 (XP_010655365). Lys Motifs (LysM, PF01476) and Kinase domain (PF07714) were annotated with SMART. The signal peptide and the outer juxtamembrane region were not included in each LysM domain.

Table S3. Primers used in this study. For each primer, gene family, name used (ID), sequence (5'→ 3') and usage are reported. Underlined cacc is used for Gateway directional TOPO cloning. Letters in bold indicate restriction sites added to the 5' end of gene-specific primer sequences to facilitate cloning.

Gene Family	ID	Sequence (5'-3')	Usage
VvLYK1-1	VvLYK1-1_RT_F	tacatgccaccagaatacgc	Semi-quantitative RT-PCR
	VvLYK1-1_RT_R	aaggtcctctctggtgatcag	Semi-quantitative RT-PCR
	VvLYK1-1_R2	taaacagatccaaagccacc	Genotyping
	VvLYK1-1_F2	gtgggctgtttacattcctac	Genotyping
	VvLYK1-1_qF	tggctttgttcgaggatgtg	qPCR
	VvLYK1-1_qR	cgagtgggtagtattcttcaagc	qPCR
	VvLYK1-1_Xho_F	cg ctcga gcaaatgaaacagaaggtggg	Vector construction
	VvLYK1-1_Xba_R	cg ctcga aagaaaaatggtgaagtattg	Vector construction
	VvLYK1-1_GW_F	<u>cacc</u> atgaaacagaaggtgggttaggg	Gateway construction
	VvLYK1-1_GW_R	ggcccttcagacattagattgacgagg	Gateway construction
VvLYK1-2	VvLYK1-2_RT_F	cgcttctgtgggtacatttgg	Semi-quantitative RT-PCR
	VvLYK1-2_RT_R	tcctcaaacaaagcaacgag	Semi-quantitative RT-PCR
	VvLYK1-2_F2	cccgaattgtaaaaggaag	Genotyping
	VvLYK1-2_R2	cacaggaactgtatgctcatg	Genotyping
	VvLYK1-2_qF	gaccagaggcttgagatga	qPCR
	VvLYK1-2_qR	catccaatcttcggtgatga	qPCR
	VvLYK1-2_Xba_R	cg ctcga tgctaccttctgacattag	Vector construction
	VvLYK1-2_Xho_F	gg ctcga aaacatggtcatttcaaac	Vector construction
	VvLYK1-2_GW_F	<u>cacc</u> atggtcatttcaaacagcaggaacgc	Gateway construction
	VvLYK1-2_GW_R	ggcccttctgacattagattcatcagagcc	Gateway construction
VvLYK1-3	VvLYK1-3_RT_F	ttcttcattgccactcgtc	Semi-quantitative RT-PCR
	VvLYK1-3_RT_R	caagtcctcttctcagtg	Semi-quantitative RT-PCR
	VvLYK1-3_R2	atatcctatcaggcgaaccag	Genotyping
	VvLYK1-3_F2	gttgattcagccttgtagtg	Genotyping
	VvLYK1-3_qF	ggcaatgactaccacttgac	qPCR
	VvLYK1-3_qR	ttagtgcgacaactactgactg	qPCR
	VvLYK1-3_Xba_R	cg ctcga cacactatcttctgacatc	Vector construction
	VvLYK1-3_Xho_F	g ctcga gccatcttctgtagggttc	Vector construction
	VvLYK1-3_GW_F	<u>cacc</u> atgttggttttagaatcgaagg	Gateway construction
	VvLYK1-3_GW_R	ggcttctctgacatcagattcactagagc	Gateway construction
Housekeeping genes	AtEF1_F	tgagcagcgtcttctgttca	Semi-quantitative RT-PCR
	AtEF1_R	ggtggtggcatccatctgttaca	Semi-quantitative RT-PCR
	AtOLI_qF	gagctgaagtggcttccatga	qPCR
	AtOLI_qR	cgccgacatacccatgatcc	qPCR
	VvEF1 α _qF	gaactgggtgcttgatagcc	qPCR
	VvEF1 α _qR	aaccaaaatatccggagtaaaaga	qPCR
Defense genes	AtFRK1_qF	tgaaggaagcggtcagattt	qPCR
	AtFRK1_qR	ctgactcatcgttggcctct	qPCR
	VvCHIT4C_qF	gcaaccgatgtgacatatca	qPCR
	VvCHIT4C_qR	cgctgccctagcaagtgag	qPCR
	VvSTS1.2_qF	aggaagcagcattgaagctc	qPCR
	VvSTS1.2_qR	tgaccaggcatttctacacc	qPCR
	VvPAL_qF	agtctccatggacaacaccg	qPCR
	VvPAL_qR	tgctcagcacttctgacatgg	qPCR
	VvRBOHD_qF	caccaccatgctcagtcctccat	qPCR
	VvRBOHD_qR	agcgatcttctgaagacttctgccc	qPCR