

Classification and genome-wide analysis of chitin-binding proteins gene family in pepper (*Capsicum annuum* L.) and transcriptional regulation to *Phytophthora capsici*, abiotic stresses and hormonal applications

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Table S1. Primers for gene sequencing and confirmation.

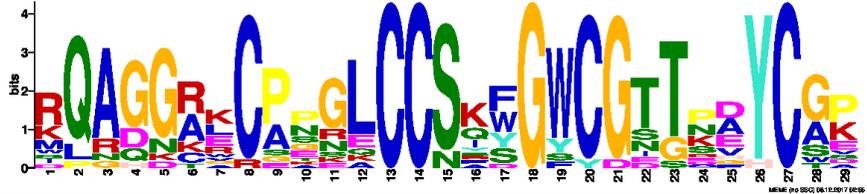
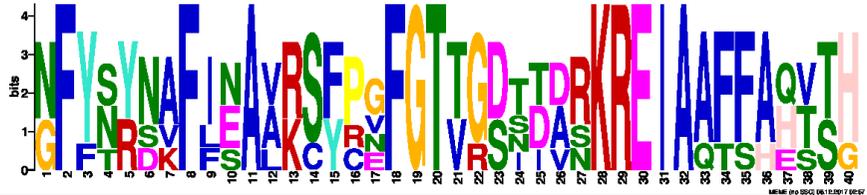
Gene Name	Accession#	Primer sequence (5'→3')
<i>CaChiI1</i>	Capana07g001653	F ATGAAGCTTTGTGAATTCACAGC
		R TTACATAGAATCGATTAAGAGTCCA
<i>CaChiI2</i>	Capana10g001143	F CACTTTGTCACTTTCTTTCCATCAC
		R CCTCGTCAAAAATATTTTCTTCCAG
<i>CaChiI3</i>	CA10g09850	F GCCACTTGTTTCCCATTACC
		R CTGATCTCAAAGTTGAACTTATC
<i>CaChiIII1</i>	Capana03g000778	F ATGATGAGAACTAGAGAGACAGC
		R CTAGAGAGCATTGAGCAGGAAG
<i>CaChiIII3</i>	CA03g30170	F CCATCCTGTCATCACAAAATGATG
		R GGAACGACCAACATCTAGCTAG
<i>CaChiIII5</i>	CA03g30190	F CGTCTTATAACAATTCACAAAATG
		R CTTGTTATACGAGGTTAGACC
<i>CaChiIV2</i>	Capana06g002084	F ATGTTGGCTCAGAATTGTGAGTG
		R CTAACAAGAGAGATTATCCCCAG
<i>CaChiVI1</i>	CA07g09480	F GTAAGAAAGAAGCATAAAGAG
		R GTATCATACGTTTCATTAGTTGG
<i>CaChiVI2</i>	Capana08g001237	F ATGGAGAAGCTAAGTACTACTGC
		R TCAGTTATCACCACAATCAAC
<i>CaChiVI3</i>	CA08g10220	F CAACCACATTATAGCAGCTCT
		R CAGACTAAACAGAGCCATG

Table S2. Chemistry and domains organization of CaChi's genes of pepper.

Genes	Domains Interval				Formula	Total number of atoms	
	Chitin_bind_1	Glyco_hydro_19	super family	chitinase_glyco_hydro_19			Barwin
<i>CaChiI1</i>	24-62	84-316		---	---	C ₁₅₅₄ H ₂₃₆₆ N ₄₅₂ O ₄₆₁ S ₂₃	4856
<i>CaChiI2</i>	24-62	84-187		---	---	C ₈₉₇ H ₁₃₇₅ N ₂₆₁ O ₂₅₉ S ₁₄	2806
<i>CaChiI3</i>	24-62	84-154		---	---	C ₇₁₅ H ₁₀₉₆ N ₂₀₀ O ₂₂₁ S ₁₃	2245
<i>CaChiIII1</i>	47-85			---	---	C ₇₃₅ H ₁₁₄₉ N ₂₀₉ O ₂₂₃ S ₁₉	2335
	97-136	---		---	---		
<i>CaChiIII2</i>	35-73			---	---	C ₉₀₈ H ₁₃₇₃ N ₂₇₁ O ₂₇₄ S ₂₆	2852
	83-119	---		---	---		
	143-181			---	---		
<i>CaChiIII3</i>	48-86			---	---	C ₁₂₅₂ H ₁₉₂₇ N ₃₄₉ O ₃₆₉ S ₃₆	3933
	102-140	---		---	---		
	156-194			---	---		
	210-248			---	---		
<i>CaChiIII4</i>	2-36			---	---	C ₇₅₆ H ₁₁₅₁ N ₂₁₃ O ₂₃₄ S ₂₆	2380
	52-90	---		---	---		
	106-143			---	---		
<i>CaChiIII5</i>	57-95			---	---	C ₉₈₅ H ₁₅₇₀ N ₂₉₀ O ₂₇₉ S ₁₆	3140
	103-119	---		---	---		
<i>CaChiIII6</i>	49-87			---	---	C ₆₉₁ H ₁₀₇₉ N ₁₈₇ O ₂₂₃ S ₁₈	2198
	96-132	---		---	---		
<i>CaChiIII7</i>	48-85			---	---	C ₈₉₄ H ₁₄₀₀ N ₂₅₀ O ₃₀₆ S ₂₆	2876
	94-132	---		---	---		
	143-181			---	---		
<i>CaChiIV1</i>	33-59	---		78-277	---	C ₁₃₂₀ H ₁₉₅₉ N ₃₅₇ O ₄₁₄ S ₁₉	4069

<i>CaChiIV2</i>	3-34	---	53-252	---	C ₁₂₂₆ H ₁₈₃₂ N ₃₃₀ O ₃₇₉ S ₂₁	3788
<i>CaChiVI1</i>	22-59	---	---	---	C ₃₇₄ H ₅₉₉ N ₁₁₇ O ₁₂₇ S ₉	1226
<i>CaChiVI2</i>	26-65	---	---	83-198	C ₉₁₃ H ₁₄₂₃ N ₂₇₃ O ₂₈₈ S ₁₅	2912
<i>CaChiVI3</i>	26-65	---	---	78-193	C ₉₅₆ H ₁₄₉₆ N ₂₈₈ O ₃₀₂ S ₁₆	3058
<i>CaChiVI4</i>	43-80	---	---	---	C ₅₁₄ H ₈₁₂ N ₁₅₀ O ₁₅₂ S ₁₃	1641

Table S3. Ten highly conserved motifs found in CaChi's proteins.

No.	Motif consensus sequence	E-value	Regular expression (RE) describing the motif.	Length (AA)
1		1.5e-156	RQAGGRKCPPGLCCSKFGWCGTTPAYCGP	29
2		6.8e-060	NFYSYNAFINAVRSFPGFTTGDTTARKRE IAAFFAQTTH	40
3		1.2e-044	RGPIQLSWNFNYGPAGKAIGFDGLNDPDI ARDPVISFKTALWYWMNNCH	50

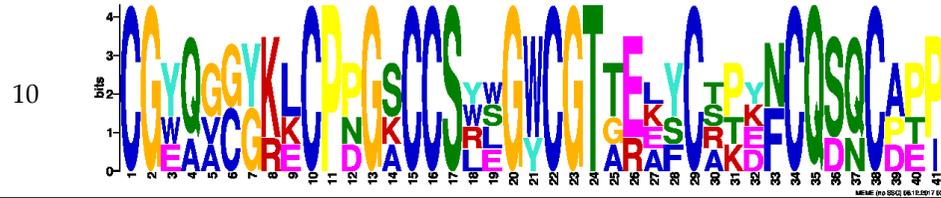
4		1.2e-039	<p>NCQSQCPSGSPKPPTPGPRPGDISGVIS NSMFDQMLKHRNDNACQ GK</p>	49
5		1.6e-034	<p>LLLALLLTVSAEQS</p>	15
6		2.2e-024	<p>PTGQCCSISGWCGATEDYCEE</p>	21
7		3.8e-023	<p>QNVRATYHLYNPQNVGWDLNAVSAYCSTWD ANKPLAWRSKYGWTAFCGPV</p>	50
8		2.2e-019	<p>AQTIVRIVDQCSNGGLDLINVFRQIDTDGV GNQRGHLIVDYQFVDCGDN</p>	50



7.6e-017

QGFGPTIRAINGRLECDGPNPQAVARRVEY
YTQYCEQLGVDPGDN

45



3.3e-016

CGYQGGGKLCPPGSCCSWWGWCGTTEEYCS
PEFCQSQCAPP

41

Table S4. Function of the *cis*-acting elements found in the promoter region of CaChi's.

Cis acting elements	Motif	Function	Reference
Defense-related and stress stimulative elements	TC-rich repeats	Defense and stress responsiveness	[1]
	W box	Fungal elicitor-responsive element	[2]
	WUN-motif	Wound-responsive element	[3]
	HSE	Heat stress responsiveness	[3]
	LTR	Low temperature responsiveness	[4]
	MBS	MYB binding site involved in drought-inducibility	[5]
Plant hormones responsive elements	CGTCA-motif	MeJA responsiveness	[6]
	GARE-motif	GA-responsive element	[7]
	TCA-element	Salicylic acid responsiveness	[8]
	TGA-element	Auxin-responsive element	[3]
	ERE	Ethylene-responsive element	[9]
	ABRE	ABA responsiveness	[10]
	AuxRR-core	Auxin responsiveness	[11]
	P-box	GA-responsive element	[12]

Table S5. Primers for qRT-PCR of CaChi's genes of pepper.

Gene name	RT-PCR Primer sequence (5'→3')	Product length
<i>CaChiI1</i>	F: AAACCTCCCATGAACTACCG R: GTTAAAAGGTCAACTCCGATGG	227
<i>CaChiI2</i>	F: GGACATCAGCGGTGTAATCT R: CAGCATTGATGAAGGCATTGT	116
<i>CaChiI3</i>	F: CTATTGCTCTCTTATTGCTGATGG R: CAGGACCACAATAGGCATCG	133
<i>CaChiIII1</i>	F: TCTTCCCTTTCATCTTCCAACC R: AGTTTGTTCTCCGCATCGTC	229
<i>CaChiIII2</i>	F: CTCCACCTCCACCTAATTTCC R: CCACACCAACCCCATCTAC	100
<i>CaChiIII3</i>	F: TGGTGCGGAACTACAGAAAAG R: TTCTACCATCAGCTTGCCTTC	127
<i>CaChiIII4</i>	F: GGAATGCAAGCTGATGGTAGA R: CTCTGACTTTGACACCACTCTT	110
<i>CaChiIII5</i>	F: GAAAGCAAGCTGCTGGTAGA R: TGACTTTGACAGTAGGGAGAAC	106
<i>CaChiIII6</i>	F: GTCCTAGTGAAGAGTGTGTAGTT R: CGCATAAACCTGGCTCTTCT	114
<i>CaChiIII7</i>	F: TCAGCCAAAACATCTCTTCCC	121

	R: CATCAGGGCATTCTCTACCAC	
<i>CaChiIV1</i>	F: TCTTTGCTCATGTCACCCAC R: ATCTCTTGCAACTATGTCAGGG	227
<i>CaChiIV2</i>	F: TCAAGTGCTATCCTGAATTCGG R: CTTCCAGAGACACAAGGGTAC	182
<i>CaChiVI1</i>	F: CAATACGGGTTCTGTGGTACG R: CAACATTTTCAGCTTCGCCAG	103
<i>CaChiVI2</i>	F: TGGGACTTGAATGCGGTTAG R: TCACTATCGTCTGAGCTCCTG	178
<i>CaChiVI3</i>	F: CGACATGGGATGCTAATAAGCC R: CGTTGTTTGAGCTCTGGTTCG	143
<i>CaChiVI4</i>	F: GTCTTTGTGATTTTGGCCCTG R: TTGGCGAACATGGTAGTGG	191

References

1. Diaz-De-Leon, F.; Klotz, K. L.; Lagrimini, L. M. Nucleotide sequence of the tobacco (*Nicotiana tabacum*) anionic peroxidase gene. *Plant Physiol.* **1993**, *101*, 1117–1118, doi:10.1104/pp.101.3.1117.
2. Rushton, P. J.; Torres, J. T.; Parniske, M.; Wernert, P.; Hahlbrock, K.; Somssich, I. E. Interaction of elicitor-induced DNA-binding proteins with elicitor response elements in the promoters of parsley PR1 genes. *EMBO J.* **1996**, *15*, 5690–5700, doi:10.1002/J.1460-2075.1996.TB00953.X.
3. Pastuglia, M.; Roby, D.; Dumas, C.; Cock, J. M. Rapid induction by wounding and bacterial infection of an S gene family receptor-like kinase gene in *Brassica oleracea*. *Plant Cell* **1997**, *9*, 49–60.
4. White, A. J.; Dunn, M. A.; Brown, K.; Hughes, M. A. Comparative analysis of genomic sequence and expression of a lipid transfer protein gene family in winter barley. **1994**, *45*, 1885–1892.
5. Nash, J.; Luehrsen, K. R.; Walbot, V. Bronze-2 gene of maize: reconstruction of a wild-type allele and analysis of transcription and splicing. *Plant Cell* **1990**, *2*, 1039–1049, doi:10.1105/tpc.2.11.1039.
6. Rouster, J.; Leah, R.; Mundy, J.; Cameron-Mills, V. Identification of a methyl jasmonate-responsive region in the promoter of a lipoxygenase 1 gene expressed in barley grain. *Plant J.* **1997**, *11*, 513–523, doi:10.1046/j.1365-313X.1997.11030513.x.
7. Skriver, K.; Olsen, F. L.; Rogers, J. C.; Mundy, J. cis-acting DNA elements responsive to gibberellin and its antagonist abscisic acid. *Proc. Natl. Acad. Sci.* **1991**, *88*, 7266–7270, doi:10.1073/pnas.88.16.7266.
8. Merkouropoulos, G.; Barnett, D. C.; Shirsat, A. H. The Arabidopsis extensin gene is developmentally regulated, is induced by wounding, methyl jasmonate, abscisic and salicylic acid, and codes for a protein with unusual motifs. *Planta* **1999**, *208*, 212–219, doi:10.1007/s004250050552.

9. Itzhaki, H.; Woodson, W. R. Characterization of an ethylene-responsive glutathione S-transferase gene cluster in carnation. *Plant Mol. Biol.* **1993**, *22*, 43–58, doi:10.1007/BF00038994.
10. Baker, S. S.; Wilhelm, K. S.; Thomashow, M. F. The 5'-region of *Arabidopsis thaliana* cor15a has cis-acting elements that confer cold-, drought- and ABA-regulated gene expression. *Plant Mol. Biol.* **1994**, *24*, 701–713, doi:10.1007/BF00029852.
11. Sakai, T.; Takahashi, Y.; Nagata, T. Analysis of the promoter of the auxin-inducible gene, parC, of tobacco. *Plant Cell Physiol.* **1996**, *37*, 906–913, doi:10.1093/oxfordjournals.pcp.a029038.
12. Kim, J. K.; Cao, J.; Wu, R. Regulation and interaction of multiple protein factors with the proximal promoter regions of a rice high pl α -amylase gene. *MGG Mol. Gen. Genet.* **1992**, *232*, 383–393, doi:10.1007/BF00266241.