Manuscript Title:

A thaumatin-like protein of *Ocimum basilicum* confers tolerance to fungal pathogen and abiotic stress in transgenic Arabidopsis

Author List:
Rajesh Chandra Misra, Sandeep, Mohan Kamthan, Santosh Kumar and Sumit Ghosh*****

Supplementary Information

Supplementary Figure S1-S7
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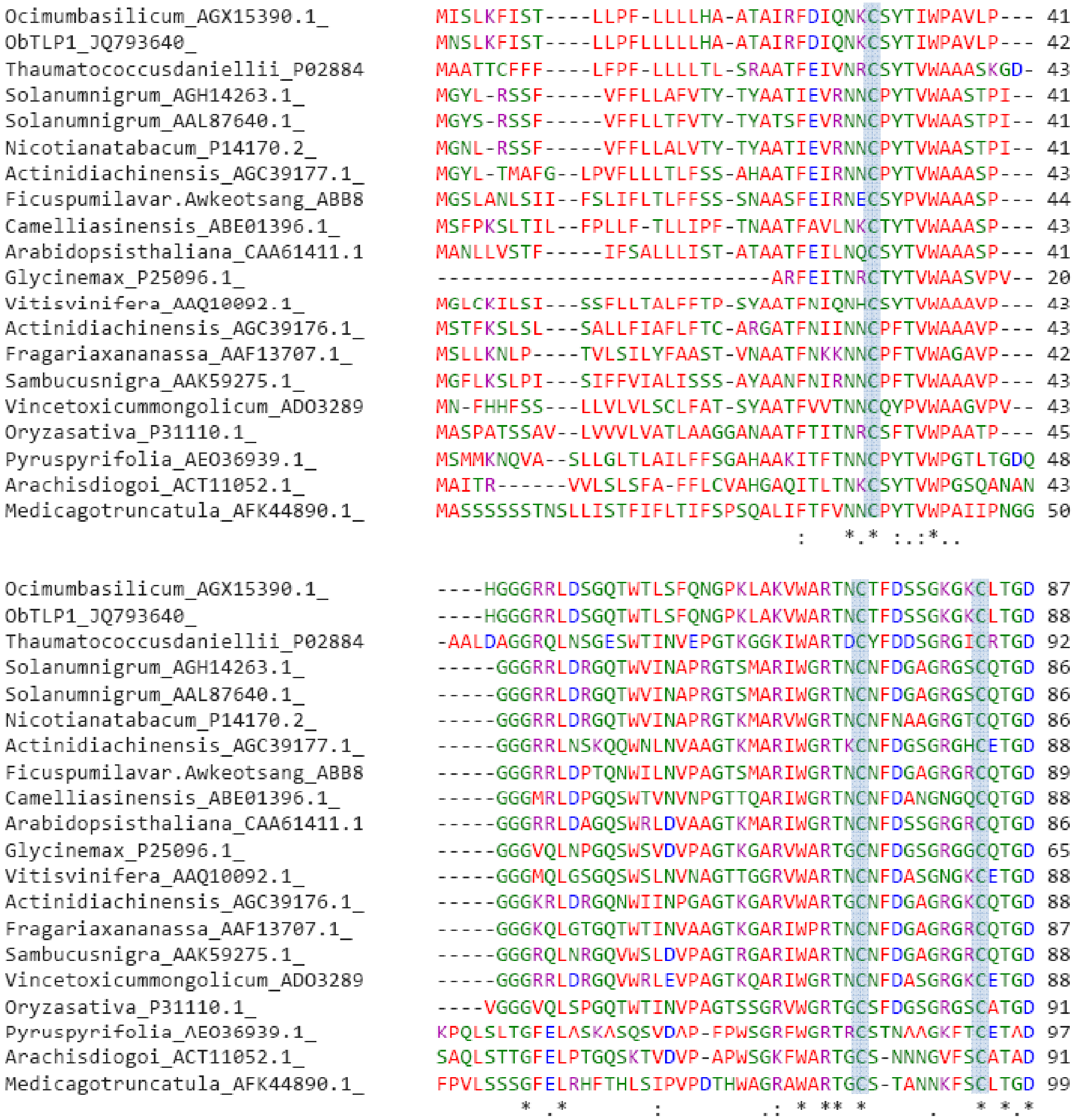

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                                           * .*       :        .: * ** *     .   * *.*
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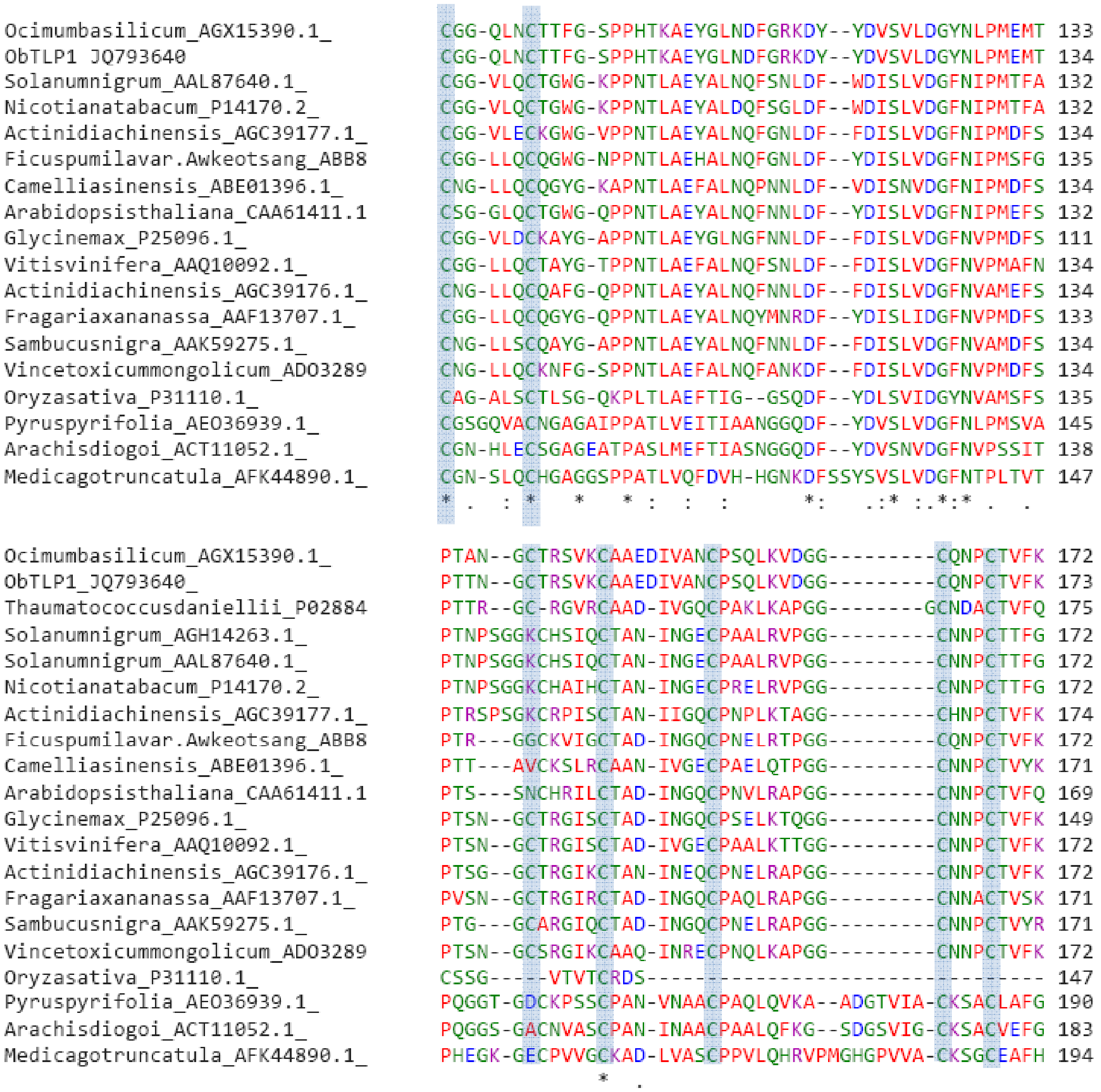
Supplementary Figure S1. Amino acid sequence comparison of *O. basilicum* ObTLP1 with PR5 members of other plant species. Multiple sequence alignment was carried out by using ClustalW2 program (http://www.ebi.ac.uk/Tools/msa/clustalw2/). Identical amino acid residues are depicted as *, while conserved and semi-conserved substitutions are marked as : and ., respectively. Sixteen cysteine resides that are conserved in TLPs are highlighted.



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Ficuspumilavar.Awkeotsang_ABB8      CGG-LLQCQGWG-NPPNTLAEHALNQFGNLDF--YDISLVDGFNIPMSFG 135
Camelliasinensis_ABE01396.1_        CNG-LLQCQGYG-KAPNTLAEFALNQPNNLDF--VDISNVDGFNIPMDFS 134
Arabidopsisthaliana_CAA61411.1      CSG-GLQCTGWG-QPPNTLAEYALNQFNNLDF--YDISLVDGFNIPMEFS 132
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Vitisvinifera_AAQ10092.1_           CGG-LLQCTAYG-TPPNTLAEFALNQFSNLDF--FDISLVDGFNVPMAFN 134
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Fragariaxananassa_AAF13707.1_       CGG-LLQCQGYG-QPPNTLAEYALNQYMNRDF--YDISLIDGFNVPMDFS 133
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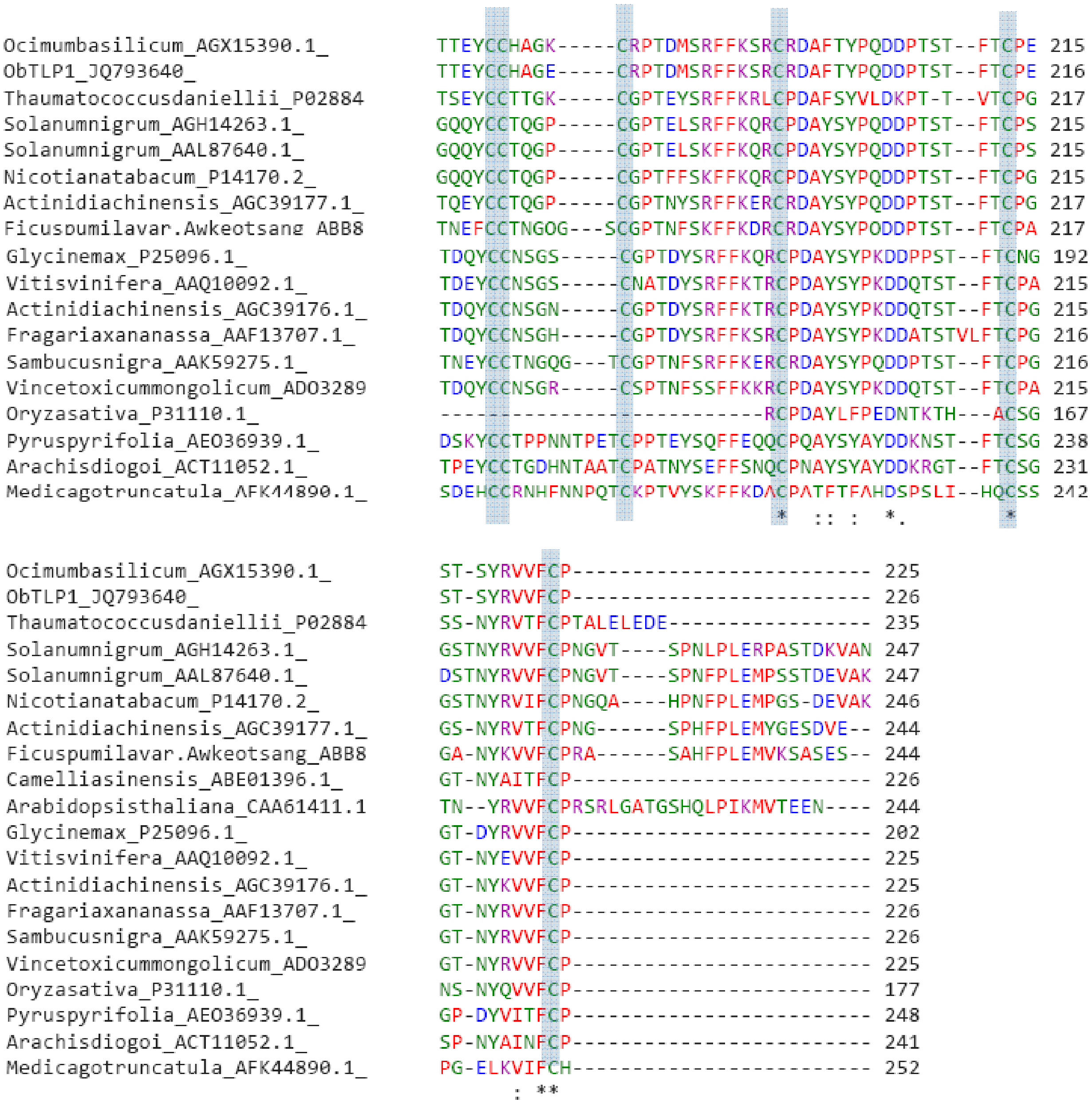
Supplementary Figure S1. Continued.



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Ficuspumilavar.Awkeotsang ABB8      TNEFCCTNGQG---SCGPTNFSKFFKDRCRDAYSYPQDDPTST--FTCPA 217
Glycinemax_P25096.1_               TDQYCCNSGS-----CGPTDYSRFFKQRCPDAYSYPKDDPPST--FTCNG 192
Vitisvinifera_AAQ10092.1_          TDEYCCNSGS-----CNATDYSRFFKTRCPDAYSYPKDDQTST--FTCPA 215
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Fragariaxananassa_AAF13707.1_      TDQYCCNSGH-----CGPTDYSRFFKSRCPDAYSYPKDDATSTVLFTCPG 216
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Vincetoxicummongolicum_ADO3289     TDQYCCNSGR-----CSPTNFSSFFKKRCPDAYSYPKDDQTST--FTCPA 215
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Medicagotruncatula_AFK44890.1_     SDEHCCRNHFNNPQTCKPTVYSKFFKDACPATFTFAHDSPSLI--HQCSS 242
                                                               *  :: :  *.        *

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Solanumnigrum_AGH14263.1_          GSTNYRVVFCPNGVT----SPNLPLERPASTDKVAN 247
Solanumnigrum_AAL87640.1_          DSTNYRVVFCPNGVT----SPNFPLEMPSSTDEVAK 247
Nicotianatabacum_P14170.2_         GSTNYRVIFCPNGQA----HPNFPLEMPGS-DEVAK 246
Actinidiachinensis_AGC39177.1_     GS-NYRVTFCPNG-------SPHFPLEMYGESDVE-- 244
Ficuspumilavar.Awkeotsang_ABB8     GA-NYKVVFCPRA------SAHFPLEMVKSASES-- 244
Camelliasinensis_ABE01396.1_       GT-NYAITFCP------------------------- 226
Arabidopsisthaliana_CAA61411.1     TN--YRVVFCPRSRLGATGSHQLPIKMVTEEN---- 244
Glycinemax_P25096.1_               GT-DYRVVFCP------------------------- 202
Vitisvinifera_AAQ10092.1_          GT-NYEVVFCP------------------------- 225
Actinidiachinensis_AGC39176.1_     GT-NYKVVFCP------------------------- 225
Fragariaxananassa_AAF13707.1_      GT-NYRVVFCP------------------------- 226
Sambucusnigra_AAK59275.1_          GT-NYRVVFCP------------------------- 226
Vincetoxicummongolicum_ADO3289     GT-NYRVVFCP------------------------- 225
Oryzasativa_P31110.1_              NS-NYQVVFCP------------------------- 177
Pyruspyrifolia_AEO36939.1_         GP-DYVITFCP------------------------- 248
Arachisdiogoi_ACT11052.1_          SP-NYAINFCP------------------------- 241
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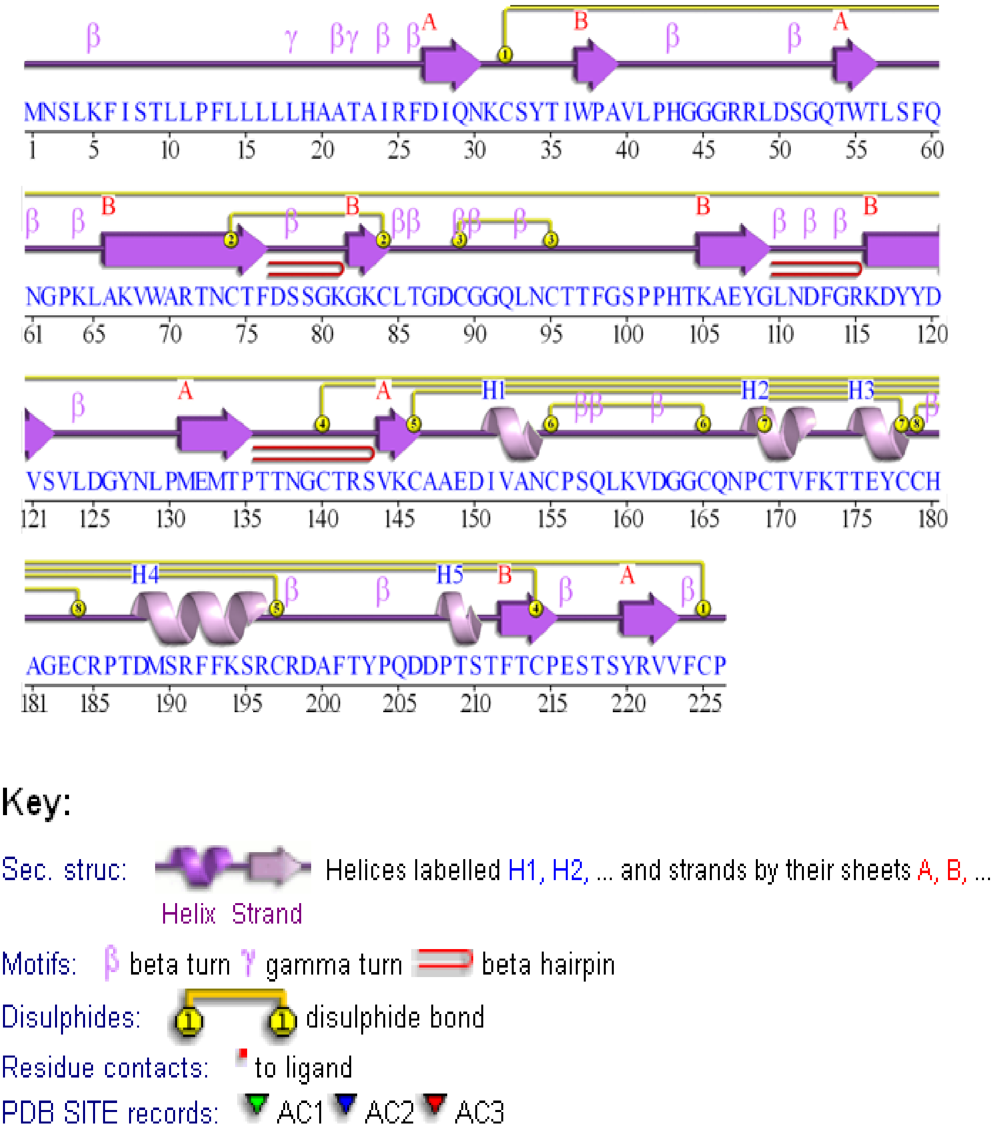
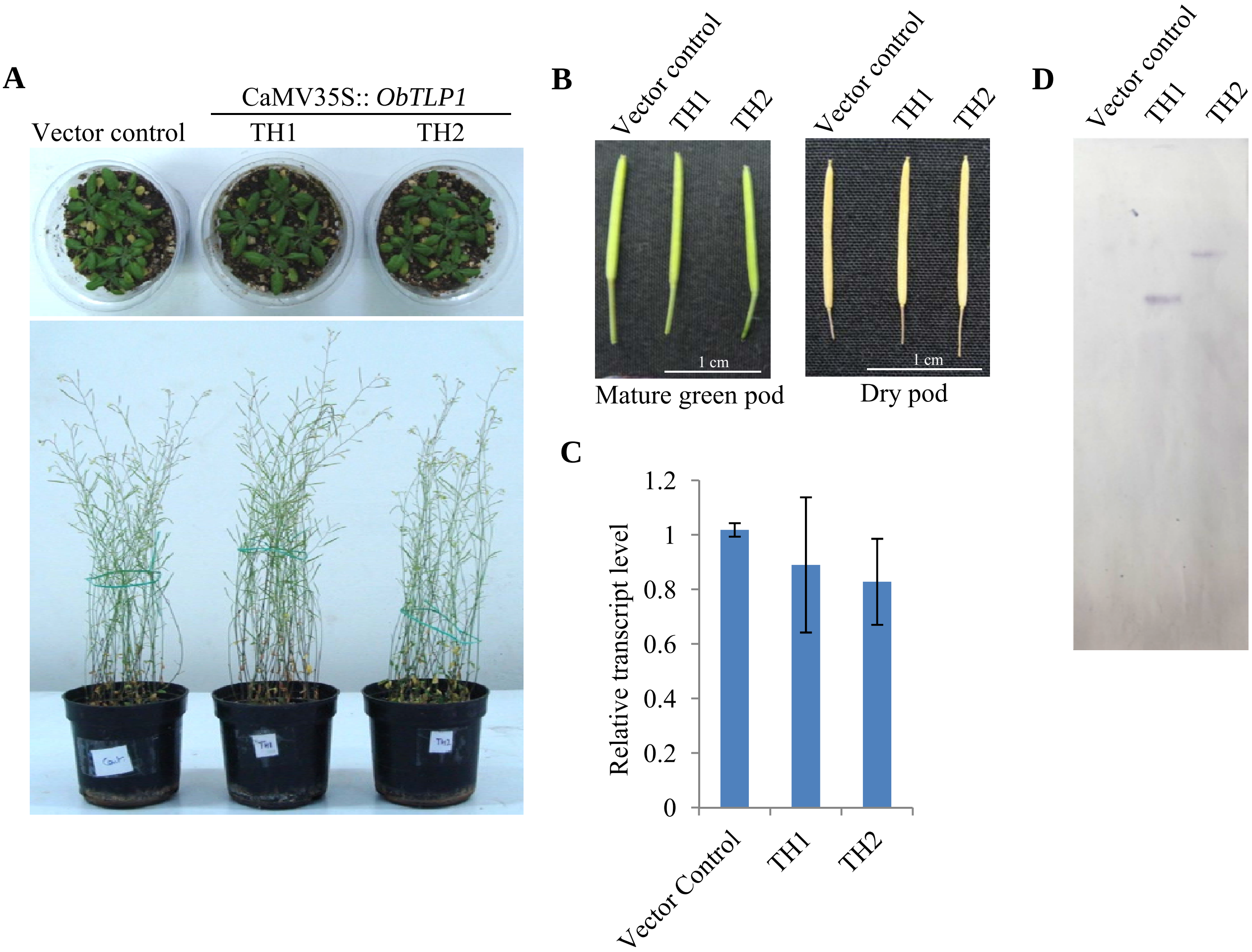
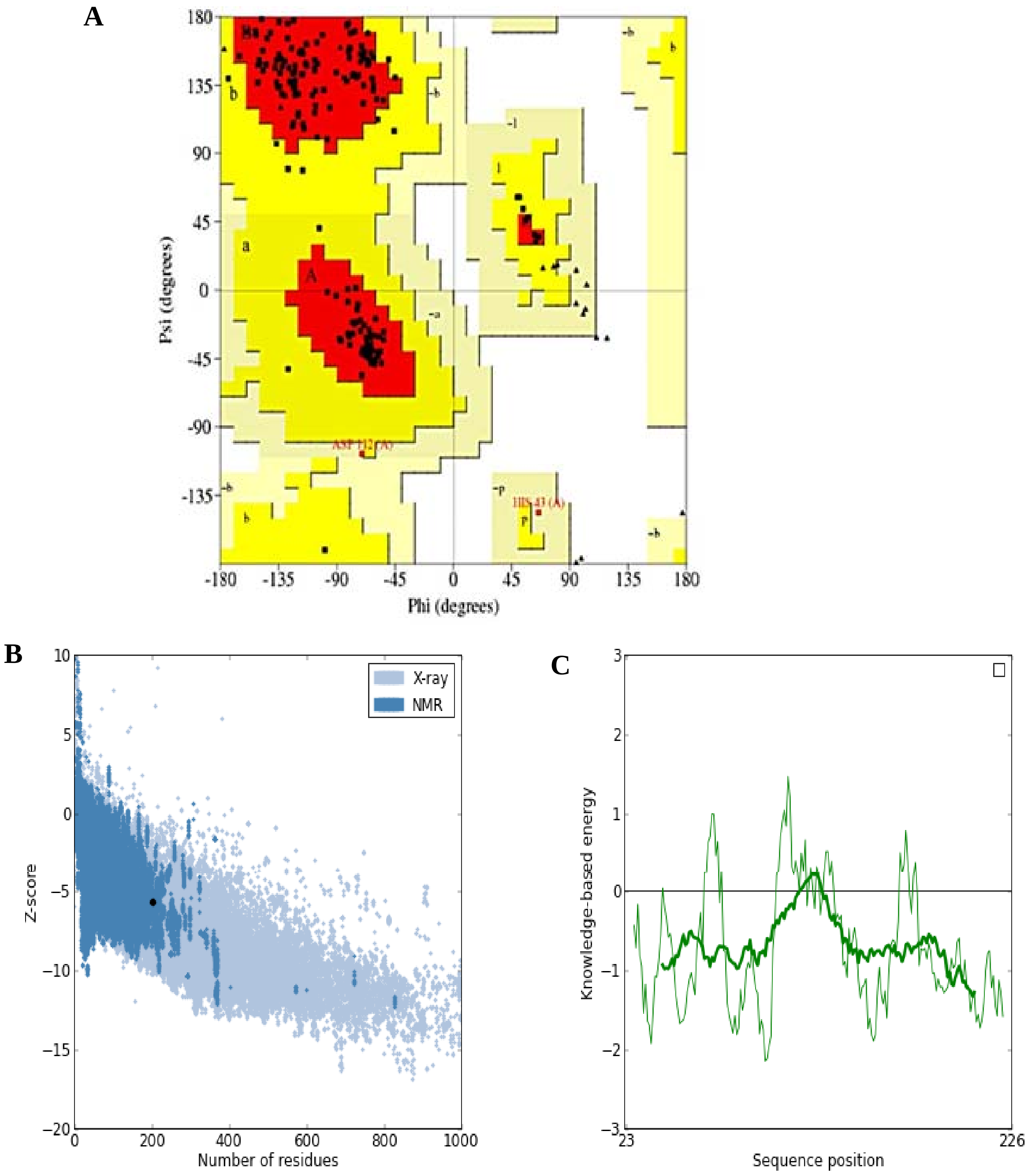
Supplementary Figure S1. Continued.



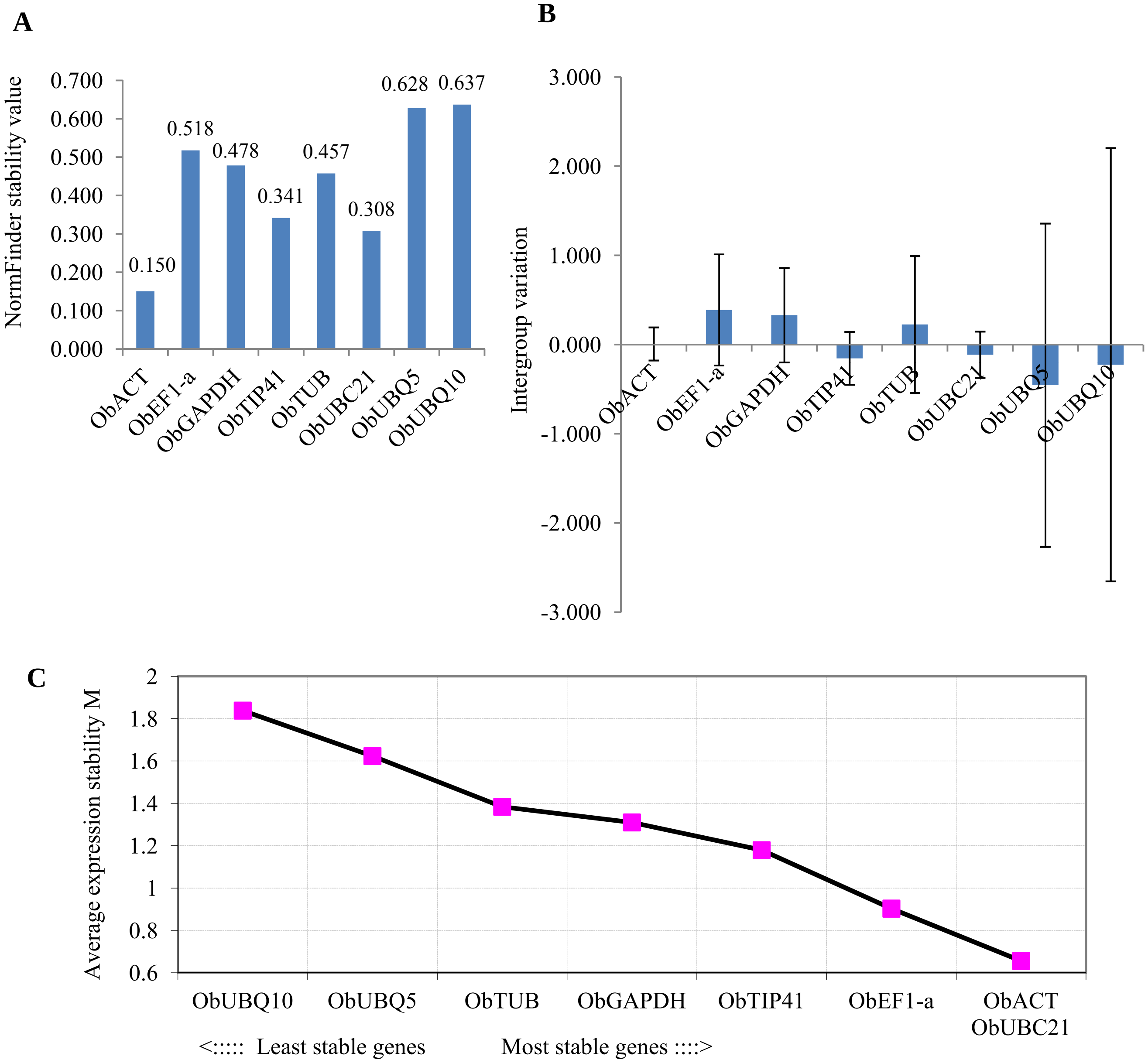
Supplementary Figure S2. A schematic presentation of the secondary structural elements in ObTLP1. α-helices are labeled with the letter 'H', and β-strands are lettered in uppercase. β, γ, and hairpin turns are also labeled. Eight disulphide bonds are mentioned with numbers 1 to 8. The secondary structure of *O. basilicum* ObTLP1 was determined using the PDBsum tool.



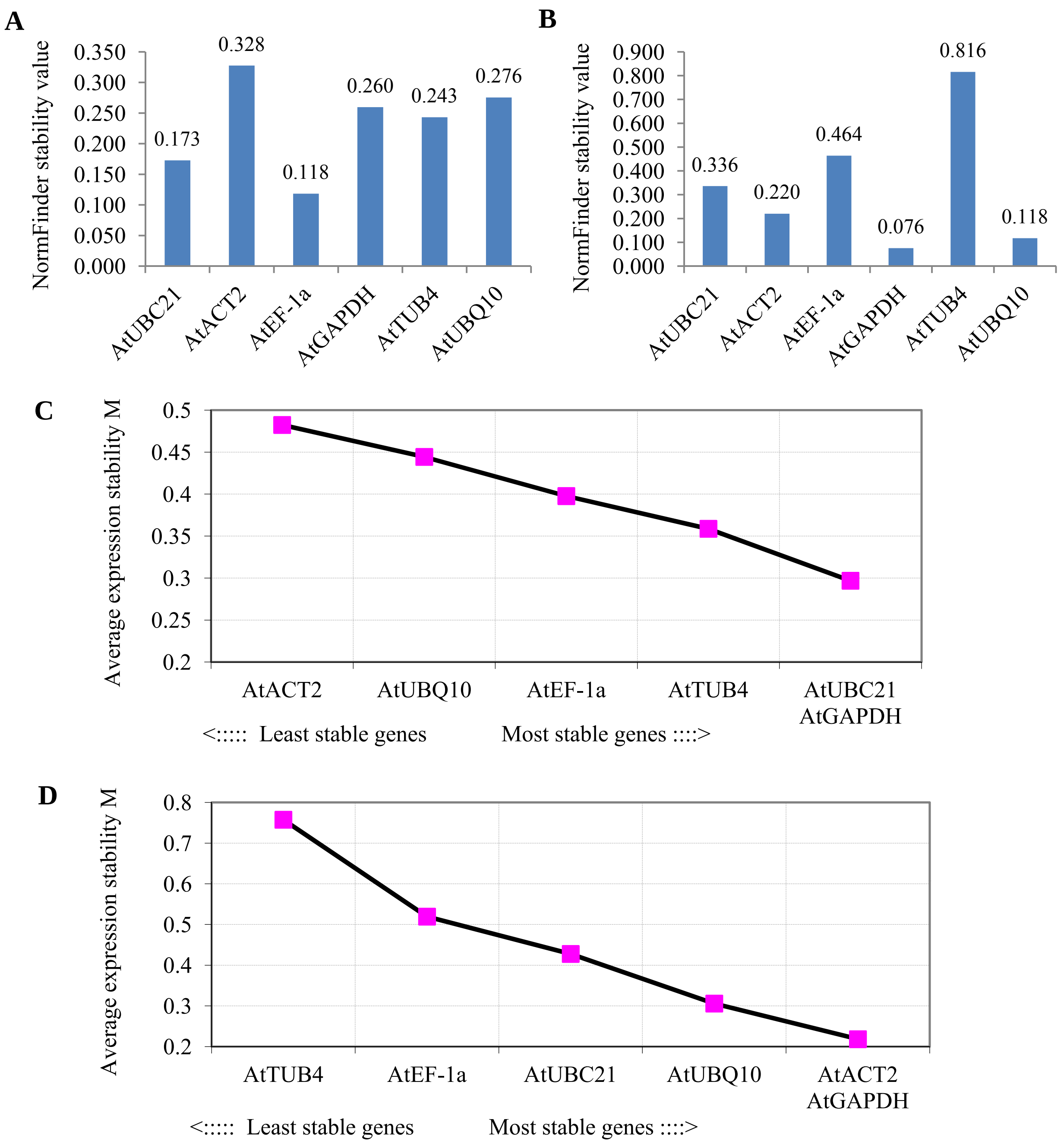
Supplementary Figure S3. Phenotypic and molecular characterization of *ObTLP1*-expressing Arabidopsis transgenic lines. (A) Photographs of vector control and *ObTLP1*-expressing Arabidopsis transgenic lines, taken at different growth stages. (B) Photographs of mature green and dry pods are shown. (C) Relative transcript level of Arabidopsis *AtOSM34* (AT4G11650), an ortholog of *ObTLP1,* was determined by qRT-PCR analysis. Data are mean ± s.d. from three biological replicates. (D) Southern blotting to determine transgene copy number in *ObTLP1*-expressing Arabidopsis transgenic lines. Genomic DNA (15 µg) was digested with XbaI and BamHI, precipitated with Ethanol/Sodium Acetate and electrophoresed on 0.8% Agarose gel. After depurination and denaturation, resolved DNAs were transferred onto Hybond N+ (GE Healthcare) following capillary method, and UV cross-linked. *ObTLP1* cDNA (725 bp) was DIG labelled and hybridized to membrane (DIG-System; Roche Diagnostics). Prehybridisation and hybridisation were performed at 65°C with standard buffer (5X SSC, 0.1% N-lauroylsarcosine, 0.02%SDS, 1% blocking reagent-Roche-11096176001). Detection was performed using an alkaline phosphatase-conjugated anti-digoxigenin antibody at 1:5000 dilutions (Roche-11093274910). After washing, blot was developed following colorimetric detection method using NBT and BCIP in detection buffer (0.1M Tris-HCl, pH-9.5 and 0.1M NaCl).



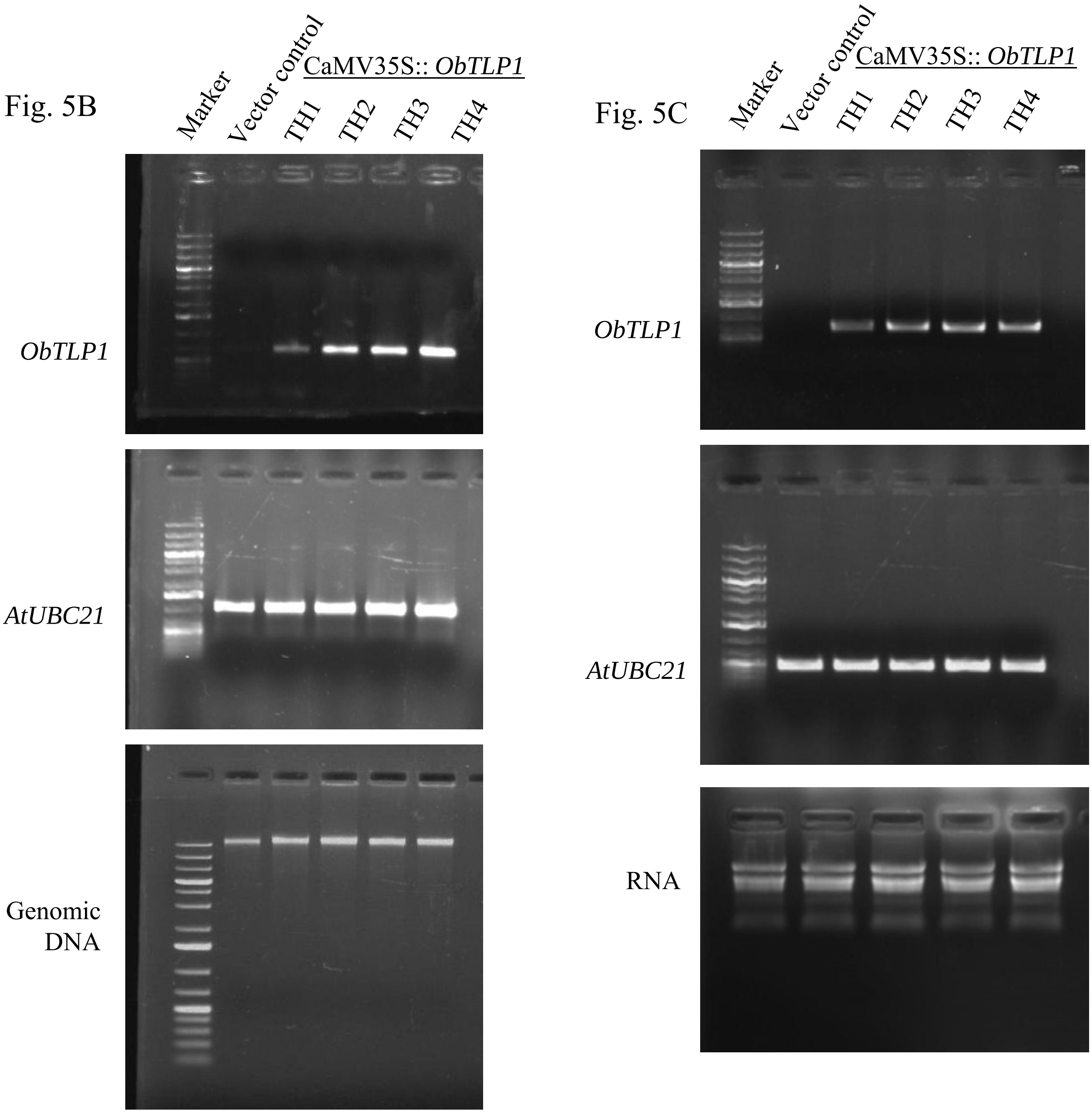
Supplementary Figure S4. Validation of the 3D structural model of ObTLP1. **(A)** Ramachandran plot was generated using PROCHECK program of Structure Analysis and Verification Server (SAVES) for the modeled ObTLP1. 94.1% of residues were found in the most favoured regions, 5.4% of residues were in the most allowed regions, and 0.5% of residues were found in the outlier regions. ProSA results showed that 3D model of ObTLP1 is very close to native structure (2VHK) determined by X-ray crystallography. **(B)** Z-score plot showing the quality of the predicted model in NMR region (dark blue). **(C)** Energy plot showing all residues of predicted model at very stable position (dark green).



Supplementary Figure S5. Identification of stably expressed reference genes, following NormFinder and geNorm programs, for qRT-PCR analysis in *O. basilicum*. (A) Most stably expressed gene has lowest NormFinder stability value. (B) Inter-group variances for the reference genes obtained from NormFinder analysis. Error bars present the average of intra-group variance. An ideal reference gene has close to zero inter-group variation and low intra-group variation. cDNAs were prepared from total of sixteen samples comprising of two groups (normal and treated tissues) with eight samples each. (C) Expression stability (M) of the reference genes determined following geNorm program. Low M value means most stable reference gene.



Supplementary Figure S6. Identification of stably expressed reference genes of Arabidopsis for qRT-PCR analysis. (A-B) NormFinder stability values of the reference genes for normal (A) and *S. sclerotiorum*-infected (B) leaf tissues. (C-D) Expression stability (M) of the reference genes determined following geNorm program for normal (C) and *S. sclerotiorum*-infected (D) leaf tissues. Twelve and six cDNA samples were used for normal (A, C) and *S. sclerotiorum*-infected (B, D) tissues, respectively.



Supplementary Figure S7. Full-length gels of main figures Fig. 5B and 5C .

Supplementary Table S1. Phenotypic evaluation of *ObTLP1*-expressing Arabidopsis plants

Parameter	**Vector control**	**TH1**	**TH2**
Pods/plant	17.14 ± 2.38	17.79 ± 2.97	18.29 ± 2.61
Weight of mature green pod (mg)	3.13± 0.04	3.14 ± 0.04	3.12 ± 0.02
Weight of dry pod (mg)	1.12 ± 0.05	1.14 ± 0.04	1.13 ± 0.04
Seeds/pod	37.79 ± 3.72	39.5 ± 3.48	38.71 ± 3.34
100 seeds weight (mg)	2.03 ± 0.09	2.08 ± 0.13	2.06 ± 0.09

*Data are the mean ± s.d. (n=14).

Supplementary Table S2. List of the primers used in this study.

Sl. No.	**Targets**		**Primer sequences (5′ to 3′)**
			Semi-quantitative RT-PCR
1	*ObTLP1* (JQ793640)	F	ATGAATTCTCTCAAATTCA
		R	TTATGGGCAGAAGACTACC
2	*ObACT* (DY329457)	F	GCACTTCCTGTGAACAATAG
		R	AGAGGATACATGTTCACCAC
3	*AtUBC21* (AT5G25760)	F	GGCATCAAGAGCGCGACTGT
		R	GGCGAGGCGTGTATACATTT
			Quantitative RT-PCR
4	*ObTLP1 (JQ793640)*	F	AGATGACGCCGACGACTAAC
		R	CGCCGCGCACTTGAC
5	*ObEF-1α*	F	CCAATCTGACCAGGGTGGTT
		R	GACATGCGTCAGACGGTTGT
6	*ObGAPDH*	F	GGATGGCATTCCGAGTTCCT
		R	GCCCTCAGACTCCTCCTTGAT
7	*ObTIP41*	F	CGGATTCCTCATTCGTGGTT
		R	CGGCAAATGGGTTGTCTGA
8	*ObTUB*	F	CGAGCACGGAGTTGATCCA
		R	GCCGGAAGCCTCATTGAA
9	*ObUBC21*	F	CCGGTGGCCCCTTTATAAGA
		R	CGACGGCGAGGCTTTTTAA
10	*ObUBQ5*	F	GCGAAGATGAGACGCTGTTG
		R	CCTAGAGGTTGAGGCCAGTGA
11	*ObUBQ10*	F	TGGGTCTCAGTTGTCTGTTGGT
		R	CGGCAGGTGAGTTGTTACACA
12	*ObACT* (DY329457)	F	GGGCTGTTATTTCCTTGCTCAT
		R	CGTGCTCAGTGGTGGATCAA
13	*AtUBC21* (AT5G25760)	F	CTGAGCCGGACAGTCCTCTT
		R	TAGCGGCGAGGCGTGTATAC
14	*AtACT2* (AT3G18780)	F	CCTTTGTTGCTGTTGACTACGA
		R	CTGAATCTCTCAGCACCAA
15	*AtEF-1α (*AT5G60390)	F	CCCCTTCGTCTTCCACTTCA
		R	CCCTGTGGGAGCAAAGGTAA
16	*AtGAPDH (*AT1G13440)	F	CAAGGCTGCTGCTCACTTGA
		R	CGAACATGGGCGCATCTT
17	*AtTUB4 (*AT5G44340)	F	TCGGCGATTCTCCGTTACAG
		R	CAGCGCGAGGAACGTACTTT
18	*AtUBQ10 (*AT4G05320)	F	GGAGGTGGAGAGTTCTGAC
		R	GCGAAGATCAATCTCTGCT
19	*S. sclerotiorum* ITS genomic DNA (KC748491)	F	GGTGAACCTGCGGAAGGAT
		R	AAGAGCAGCTCGCCAAAGC
20	*B. cinerea Actin* genomic DNA (NW_001814525)	F	CCGTCTGGATTGGTGGTTCT
		R	CACTTGCGGTGGACAATGG
21	*AtVSP1* (AT5G24780)	F	GGGAACGAAGCCGAACTCTT
		R	GGGCACCGTGTCGAAGTTTA
22	*AtPR1* (AT2G14610)	F	CTCGGAGCTACGCAGAACAAC
		R	CCGCTACCCCAGGCTAAGTT
23	*AtPDF1* (AT5G44420)	F	CGCTGCTCTTGTTCTCTTTGC
		R	CCCTGACCATGTCCCACTTG
24	*AtPAL1* (AT2G37040)	F	CCCCTCCGTGGTACAATCAC
		R	CTTCACCGTTGGGACCAGTAG
25	*AtBGL2* (AT3G57260)	F	CCTCGACGTTCCCAGTTCAG
		R	ACTTGTCGGCCTCCGTTTG
26	*Arabidopsis Rubisco Large Subunit* (Atcg00490)	F	TGTTCTGCCTGTGGCTTCAG
		R	CCCAAGGGTGGCCTAAAGTT

			pET pET-28a(+) cloning
27	*ObTLP1* (JQ793640)	F	CTGGATCCATGAATTCTCTCAAATTCA
		R	AACTCGAGTTATGGGCAGAAGACTACC
			pBI 121 cloning
28	*ObTLP1* (JQ793640)	F	GCTCTAGAATGAATTCTCTCAAATTCATCTCC
		R	GGGAAATTTATGGGCAGAAGACTACC