

Appendix

Functional identification of C-type lectin in the diamondback moth, *Plutella xylostella* (L.) innate immunity

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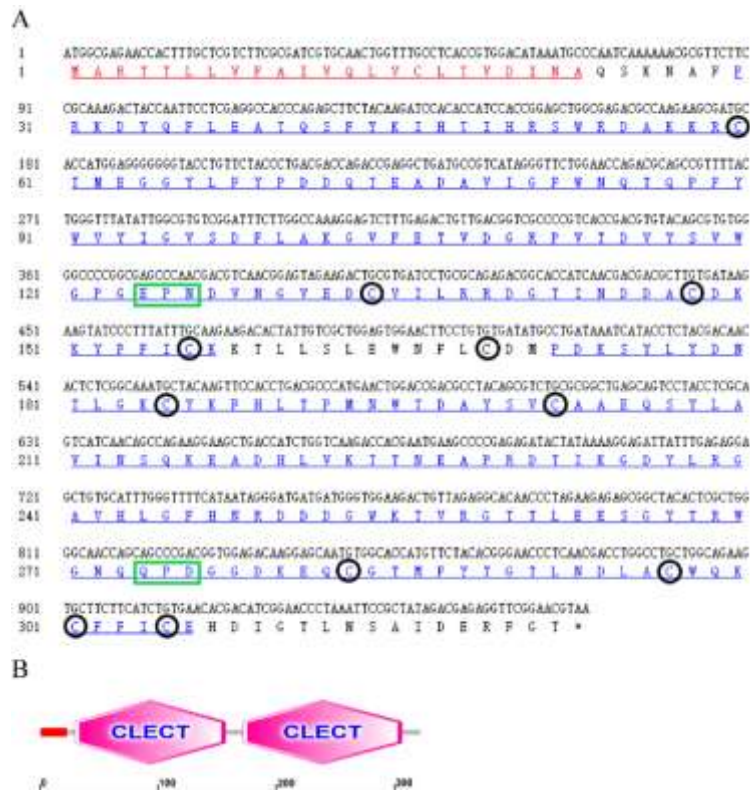
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Appendix A-E

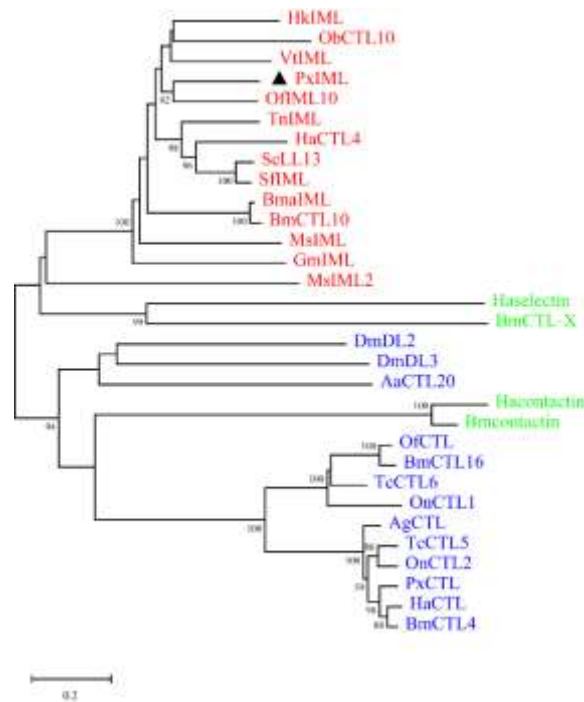
Appendix A. Primers used in this study

Primer name	Sequence(5'-3')	Function
P1(forward)	5'-ATGGCGAGAACCACTTTGCTCGTCT-3'	Cloning
P2(reverse)	5'-TTACGTTCCGAACCTCTCGTC-3'	
P3(forward)	5'-AGTCATCAACAGCCAGAAGG-3'	qRT-PCR
P4(reverse)	5'-TGCACAGCTCCTCTCAAATAA-3'	
P5(PxRPL32-F)*	5'-CAATCAGGCCAATTTACCGC-3'	
P6(PxRPL32-R)*	5'-CTGGGTTTACGCCAGTTACG-3'	
P7(NcoI-F)	5'-CATGCCATGGCCCAATCAA-3'	Protein expression
P8(Not I-R)	5'-ATTTGCGGCCCGCCTCCGAA-3'	
P9(forward)	5'-AGCGATGCACAATGGAGGGGGGGTA-3'	
P10(reverse)	5'-TACCCCCCTCCATTGTGCATCGCT-3'	
M13F	5'-TGTA AACGACGGCCAGT-3'	Sequencing
M13R	5'-CAGGAAACAGCTATGACC-3'	
T7	5'-TAATACGACTCACTATAGGG-3'	
T7ter	5'-TGCTAGTTATTGCTCAGCGG-3'	

*, Ribosomal protein L32 (GenBank accession number AB180441) (Chen *et al.* 2020).



Appendix B. Sequence and the predicted structure of PxIML. A, nucleotide sequence of PxIML ORF and the deduced amino acid sequence. Nucleotides and amino acids are numbered on the left of the sequences. The TAA stop codon is marked with an asterisk. The predicted signal peptide and CRD were underlined in red and blue, respectively. The EPN and QPD motifs are boxed. The cysteine residues were marked with black circle. B, the domain architecture of the PxIML.

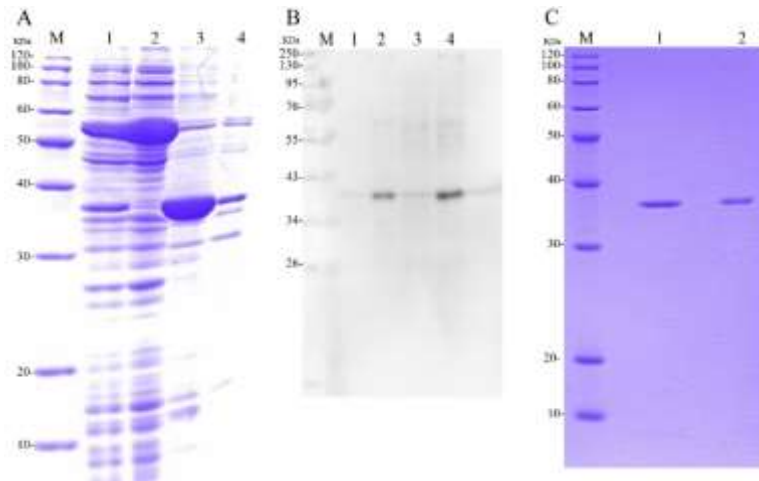


Appendix C. Phylogenetic analysis of PxIML and other insect CTLs. The Neighbor-joining phylogenetic tree was constructed by MEGA v.10.0.5 software with 1000 replications. PxIML was marked with black triangle. The immulectin, CTL-X, CTL-S group were marked by red, green and blue, respectively. Frequency values less than 50% is not displayed. HaCTL4, *Helicoverpa armigera* CTL4 (AFI47449.1); SeLL13, *Spodoptera exigua* LL13 (AQX37248.1); TnIML, *Trichoplusia ni* immulectin (XP_026744357.1); GmIML, *Galleria mellonella* immulectin (XP_026764354.1); MslIML, *Manduca sexta* immulectin (XP_030038662.1); BmaIML, *Bombyx mandarina* immulectin (XP_028037911.1); BmCTL10, *Bombyx mori* CTL10 (NP_001091784.1); PxIML, *Plutella xylostella* immulectin (MT232645). ObCTL10, *Operophtera brumata* CTL10 (KOB73959.1); OfIML10, *Ostrinia furnacalis* IML-10 (MN453202.1); HkIML, *Hyposmocoma kahamanao* immulectin (XP_026329709.1); MslIML2, *M. sexta* IML2 (AAF91316.3); Haselectin, *H. armigera* selectin (XP_021185772.1); BmCTL-X, *B. mori* CTL-X (XP_004932809.1); Hacontactin, *H. armigera* contactin (XP_021200542.1); Bmcontactin, *B. mori* contactin (XP_004931162.1); OfCTL, *O. furnacalis* CTL (XP_028164489.1); BmCTL16, *B. mori* CTL16 (NP_001091747.1); PxCTL, *P. xylostella* CTL (XP_011568909.1); HaCTL, *H. armigera* CTL (XP_021194745.1); BmCTL4, *B. mori* CTL4 (NP_001165397.1); SfIML, *Spodoptera frugiperda* immulectin (XP_035436562.1); VtIML, *Vanessa tameamea* immulectin (XP_026494963.1); TcCTL5, *Tribolium castaneum* CTL5 (NP_001164139.1); TcCTL6, *T. castaneum* CTL6 (NP_001164143.1); AgCTL, *Anopheles gambiae* CTL (XP_319374.4); AaCTL20, *Aedes aegypti* CTL20 (XP_001661644.2); OnCTL1, *Octodonta nipae* CTL1 (QEL09433.1); OnCTL2, *O. nipae* CTL2 (QEL09434.1); DmDL2, *Drosophila melanogaster* DmDL2 (NP_001014489.1); DmDL3, *D. melanogaster* DmDL3 (NP_001014490.1).

Appendix D. Structural features of PxIML

Domain	Motif	Ca ²⁺	Coordinators	C-score	Sugar	Coordinators	C-score
CRD1	EPN (<u>101,102,103</u>)	CA1	(46,48,52,134)	0.10	MAN	(<u>101,109</u> ,115,121,122)	0.21
		CA2	(75,79,82, 104,109 ,110)	0.05	GQ4	(27,64,67,68, <u>101,103,104,109</u> ,113,123,127,129)	0.01
CRD2	QPD (<u>251,252,253</u>)	0	0	0	RAF	(218, <u>251,253</u> ,254,270,271,272)	0.11

MAN, Alpha-D-Mannose; GQ4, Alpha-D-Glucopyranosyl; RAF, Raffinose. C-score [0-1] is a confidence score representing the quality of the generated models (Yang *et al.* 2015). The residues involved in both motif and sugar interactions are underlined. The residues involved in both Ca²⁺ and sugar interactions are in bold. The residues involved in the interaction between the motif and sugar are underlined.



Appendix E. SDS-PAGE and western blotting analyses of rPxIML. A, SDS-PAGE analyses of rPxIML. Lane M, protein molecular standard (kDa); lane 1, the lysate supernatant from IPTG induced *E. coli* BL21 (DE3) pG-Tf2 containing pET-28b-PxIML; lane 2, the lysate supernatant from IPTG induced *E. coli* BL21 (DE3) pG-Tf2 containing pET-28b; lane 3, the precipitation after lysate from IPTG induced *E. coli* BL21 (DE3) pG-Tf2 containing pET-28b-PxIML; lane 4, the precipitation after lysate from IPTG induced *E. coli* BL21 (DE3) pG-Tf2 containing pET-28b. There is strong band (between 50-60 KDa) in lane 1 and lane 2, this band is the non-target protein, and have been removed in the later purification. B, western blotting analysis of rPxIML. Lane 1, the lysate supernatant from IPTG induced *E. coli* BL21 (DE3) pG-Tf2 containing pET-28b; lane 2, the lysate supernatant from IPTG induced *E. coli* BL21 (DE3) pG-Tf2 containing pET-28b-PxIML; lane 3, the precipitation after lysate from IPTG induced *E. coli* BL21 (DE3) pG-Tf2 containing pET-28b; lane 4, the precipitation after lysate from IPTG induced *E. coli* BL21 (DE3) pG-Tf2 containing pET-28b-PxIML. C, SDS-PAGE analyses of the purified rPxIML. Lane 1 and 2, the purified protein from the lysate supernatant.

References

- Chen W, Dong Y, Saqib H S A, Vasseur L, Zhou W, Zheng L, Lai Y, Ma X, Lin L, Xu X, Bai J, He W, You M. 2020. Functions of duplicated glucosinolate sulfatases in the development and host adaptation of *Plutella xylostella*. *Insect Biochemistry and Molecular Biology*, **119**, 103316.
- Yang J, Yan R, Roy A, Xu D, Poisson J, Zhang Y. 2015. The I-TASSER Suite: protein structure and function prediction. *Nature Methods*, **12**, 7-8.