Supplementary Materials

Supplementary materials for the article "A Novel Deoxynivalenol-Activated Wheat *Arl6ip4* Gene Encodes An Antifungal Peptide with Deoxynivalenol Affinity and Protects Plants against *Fusarium* Pathogens and Mycotoxins"

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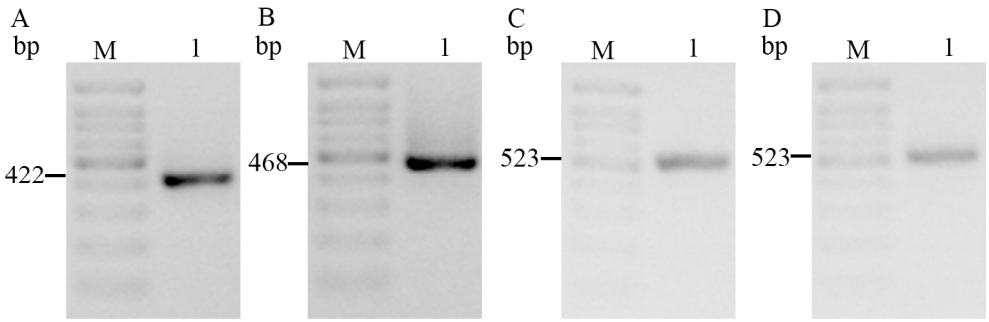

Figure S1 PCR amplification of *TaArl6ip4* using of 3′-RACE and 5′-RACE from the wheat Zhengmai9023 cDNA.

Lane M: Marker 3000 bp; A, lane 1 represents the product of *TaArl6ip4* 3′-RACE; B, lane 1 represents the product of *TaArl6ip4* 5′-RACE; C, lane 1 represents the full-length *TaArl6ip4* PCR product using cDNA as a template; D, lane 1 represents the full-length *TaArl6ip4* PCR product using genomic DNA as a template. All the PCR products were electrophoresed on 1% agarose gels.

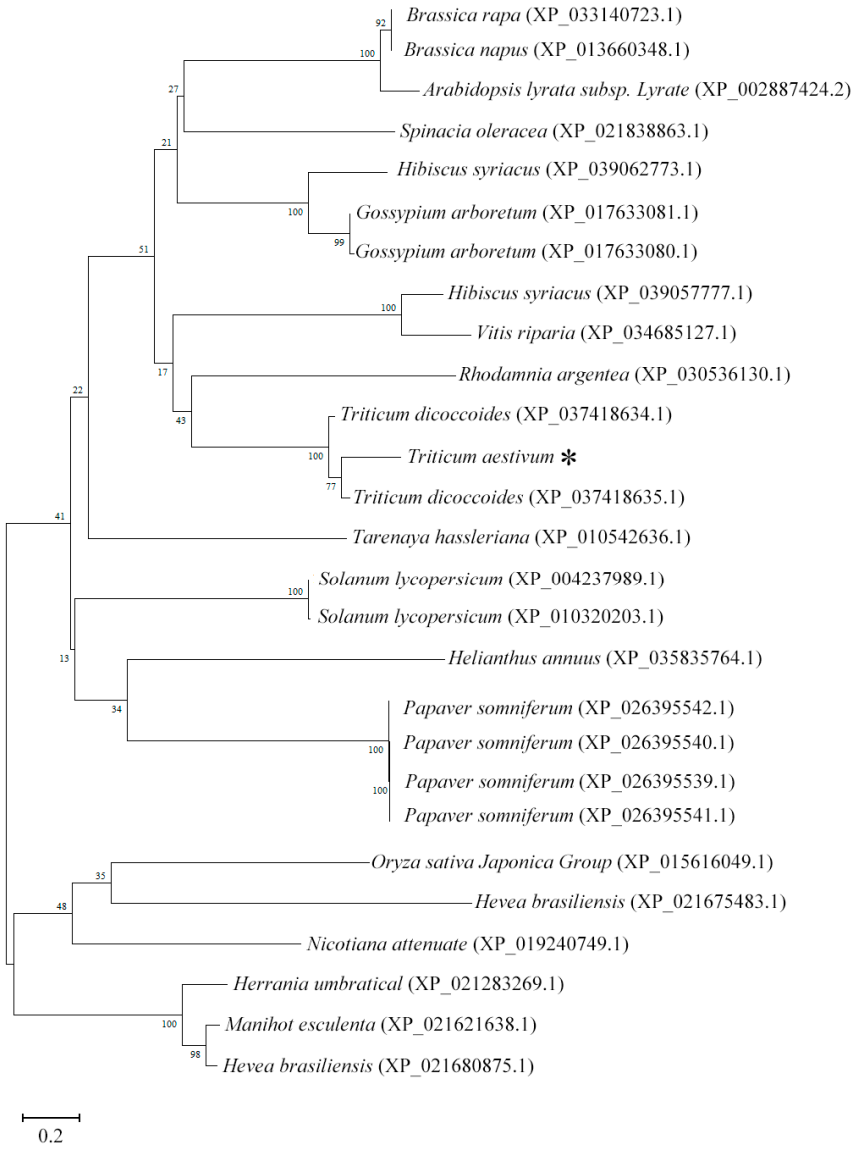

Figure S2 Phylogenetic tree analysis of deduced TaARL6IP4 amino acid sequence together with ARL6IP4 proteins from 19 plant species. *Arabidopsis* (Accession: XP_002887424.2), *Oryza sativa Japonica* Group (XP_015616049.1), *Triticum dicoccoides* (XP_037418635.1, XP_037418634.1), *Brassica rapa* (XP_033140723.1), *Brassica napus* (XP_013660348.1), *Hibiscus syriacus* (XP_039062773.1, XP_039057777.1), *Manihot esculenta* (XP_021621638.1), *Helianthus annuus* (XP_035835764.1), *Vitis riparia* (XP_034685127.1), *Rhodamnia argentea* (XP_030536130.1), *Solanum lycopersicum* (XP_004237989.1), *Spinacia oleracea* (XP_021838863.1), *Hevea brasiliensis* (XP_021680875.1, XP_021675483.1), *Herrania umbratical* (XP_021283269.1), *Tarenaya hassleriana* (XP_010542636.1), *Gossypium arboretum* (XP_017633080.1, XP_017633081.1), *Nicotiana attenuate* (XP_019240749.1), *Papaver somniferum* (XP_026395539.1, XP_026395540.1, XP_026395541.1, XP_026395542.1) and *Solanum lycopersicum* (XP_010320203.1). Amino acid sequences of ARL6IP4 proteins from different plant species were used to generate the phylogenetic tree using MEGA 11 with the neighbor-joining method. The '✻' represents amino acid sequence derived from coding sequence of *Triticum aestivum TaArl6ip4* (Accession: OK345034) in the current study.

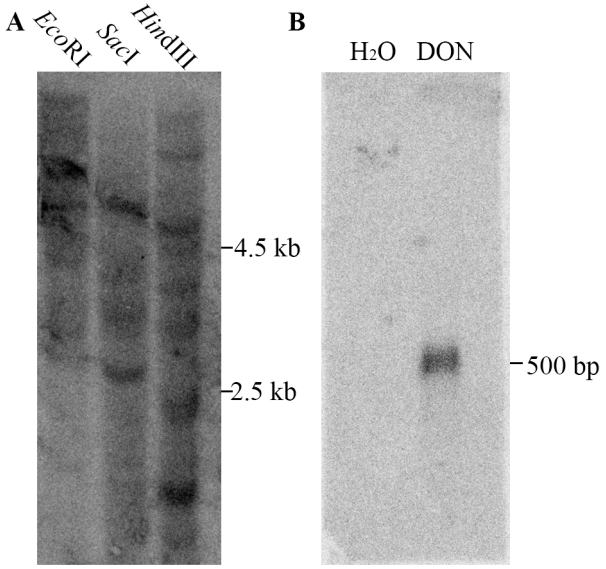

Figure. S3 Southern blotting and Northern blotting analysis of the *TaArl6ip4* gene in wheat.

A, Southern blotting analysis of the *TaArl6ip4* gene expression in wheat. The Zhengmai9023 genomic DNA was digested with *Eco*RI, *Sac*I and *Hind*III, electrophoresis was performed on 0.8% agarose gel and transferred onto nylon membranes and hybridized with a *TaArl6ip4* probe. B, Northern blotting analysis of the *TaArl6ip4* gene expression in wheat. Total RNAs were extracted from water-treated (control), and 50 μg ml^{-1} DON-treated wheat spikelets at 24 hpi, 20 μg RNAs were run on 1.2% agarose/formaldehyde gel and transferred onto nylon membranes and hybridized with a *TaArl6ip4* probe.

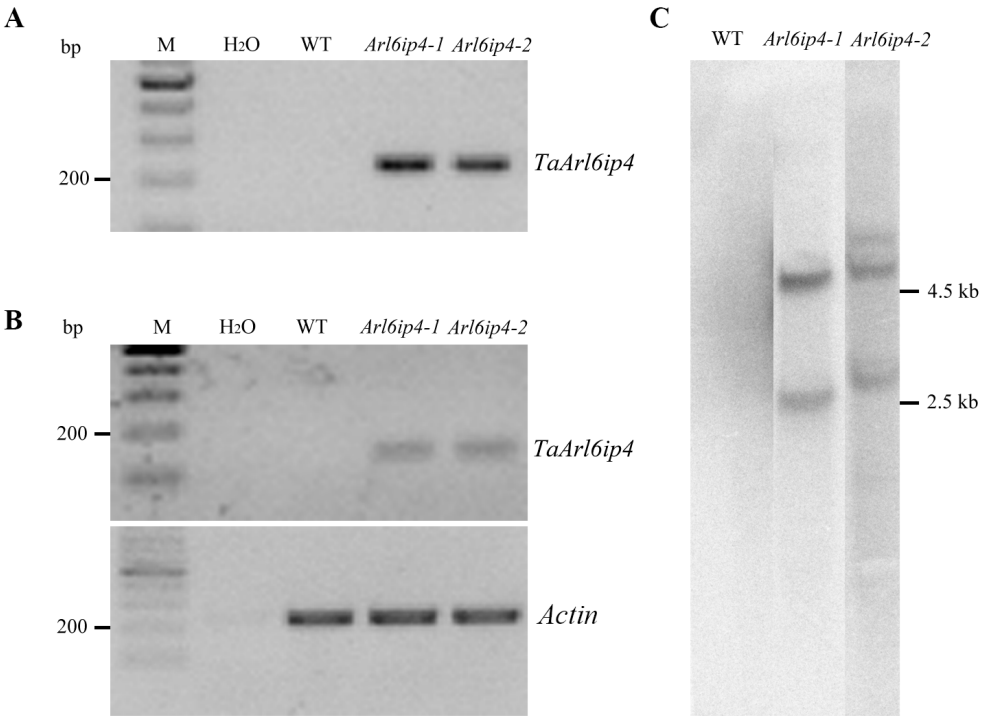

Figure S4 Molecular characterization of *TaArl6ip4* T_2 generation transgenic lines in *Arabidopsis* plants

A, PCR analysis of the transgenic plants *TaArl6ip4-1* and *TaArl6ip4-2* with gene specific primers (UTR/*TaArl6ip4*-R4-b); B, RT-PCR analysis of the transgenic plants *TaArl6ip4-1* and *TaArl6ip4-2* with primers (*TaArl6ip4*-F5/ *TaArl6ip4*-R5), the constitutively expressed *β-actin* gene (*Actin*-F/ *Actin*-R) was used as a reference gene; C, Southern blotting analysis of the transgenic plants *TaArl6ip4-1* and *TaArl6ip4-2*.

Table S1 Primer sequences used in this study

Primers	Sequences (5'-3')
5'RACE-F1	CTAATACGACTCACTATAGGGCAAGCAGTGGTATCAACGCAGAGT
5'-RACE R1	TCTTCAGCGGAACAACCGAGGCAAA
5'RACE-F2	AAGCAGTGGTATCAACGCAGAGT
5'-RACE R2	CCGGGCGTAAGCTAATAAGAGAAC
3'-RACE F1	AGGGACGGGGAAATTTTAGTGGTGTA
3'RACE R1	GCTGTCAACGATACGCTACGTAACG
3'-RACE F2	ATGGGGTTTTGCTGTGGGTGTTC
3' RACE-R2	CGCTACGTAACGGCATGACAGTG
TaArl6ip4-F3	ACATGGGGACAGAAACACATCAAGCA
TaArl6ip4-R3	GGATCAACCGACCGTCCATGAATAA
UTR	CGCGAATTCACAACACAAATCAGATTTATAGAGAGATTTATAAAAAAA AAAAA
TaArl6ip4-F4	TTATAAAAAAAAAAAAATGGGGTTTTGCTGTG
TaArl6ip4-R4a	AGCGGATCCCATTAGGCCGGGTCGA
TaArl6ip4-R4b	ATGCGGATCCTTACATTAGGCCGGGTCGAGAA
TaArl6ip4-F5	GCTGTGGGTGTTCGGATGTC
TaArl6ip4-R5	TTTCCCTTCCTTCTTTACGG
Actin-F	CAGCAATGTATGTCGCAATC
Actin-R	TAGCATGAGGAAGCGTGTAT
TaArl6ip4-F6	TCCAGGGGCCCCTGGGATCCATGGGG TTTTGCTGTGGGTGTT
TaArl6ip4-R6	CTCGAGTCGACCCGGGAATTCTTACAT TAGGCCGGGTCGAGAATG