

Table 1 Suppl. Primers used in this research.

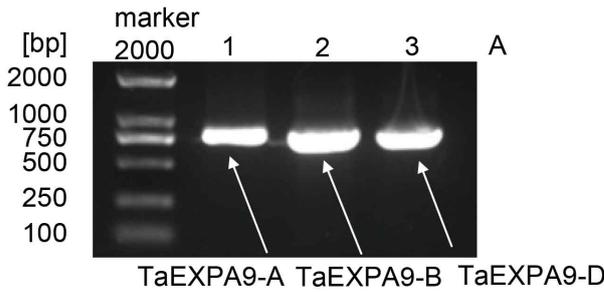
Name	Primer sequences (5'-3')
<i>TaEXPA9-A</i> (RT-qPCR)	FW: ATGGCCGCCGCAATGC RV: CGCACCTCTCCCCCTCGTT
<i>TaEXPA9-B</i> (RT-qPCR)	FW: ATGGCCGCCGCACTGC RV: CACCTCTGCCCCCTCGTTGAAC
<i>TaEXPA9-D</i> (RT-qPCR)	FW: ATGGCCGCCGCAATGC RV: GCGCTGCCCCCTCGTTGAA
<i>ACTIN</i> (wheat)	FW: TCCAATCTATGAGGGATACACGC RV: TCTTCATTAGATTATCCGTGAGGTC
<i>ACTIN</i> ( <i>Arabidopsis</i> )	FW: CCACATGCTATTCTGCGTTTGACC RV: CATCCCTTACGATTTACGCTCTGC
<i>TaEXPA9-A</i> (clone the CDS)	FW: ATGGCCGCCGCAATGC RV: CTAGACCCTGAAGTTGTTGCCCTCG
<i>TaEXPA9-B</i> (clone the CDS)	FW: ATGGCCGCCGCACTGC RV: TCAGACCCTGAAGTTCTTGCCCTC
<i>TaEXPA9-D</i> (clone the CDS)	FW: ATGGCCGCCGCAATGC RV: CTAGACCCTGAAGTTCTTGCCCTCG
<i>TaEXPA9-A</i> (addition of <i>Bg</i> III)	FW: TGACCATGGTAGATCTGATGGCCGCCGCAATGCCACCG RV: CCTTTACTAGTCAGATCGACCCTGAAGTTGTTGCCCTCG
<i>TaEXPA9-B</i> (addition of <i>Bg</i> III)	FW: TGACCATGGTAGATCTGATGGCCGCCGCACTGCCACCG RV: CCTTTACTAGTCAGATCGACCCTGAAGTTCTTGCCCTC
<i>TaEXPA9-D</i> (addition of <i>Bg</i> III)	FW: TGACCATGGTAGATCTGATGGCCGCCGCAATGCCACCG RV: CCTTTACTAGTCAGATCGACCCTGAAGTTGTTGCCCTCG
<i>TaEXPA9-A</i> (addition of <i>Xba</i> I and <i>Bam</i> HI)	FW: GAGAACACGGGGACTCTAGAATGGCCGCCGCACTGCCACCG RV: CCGGGGATCCTCTAGACTAGACCCTGAAGTTGTTGCCCTCG
<i>TaEXPA9-B</i> (addition of <i>Xba</i> I and <i>Bam</i> HI)	FW: GAGAACACGGGGACTCTAGAATGGCCGCCGCAATGCCACCG RV: CCGGGGATCCTCTAGATCAGACCCTGAAGTTCTTGCCCTCG
<i>TaEXPA9-D</i> (addition of <i>Xba</i> I and <i>Bam</i> HI)	FW: GAGAACACGGGGACTCTAGAATGGCCGCCGCAATGCCACCG RV: CCGGGGATCCTCTAGACTAGACCCTGAAGTTCTTGCCCTCG

Table 2 Suppl. The physical and chemical properties of *TaEXPA9-A/B/D* proteins predicted by *ExPASy* online site.

Protein	Formula	Molecular mass [kDa]	Isoelectric point	Global hydrophobicity (GRAVY)	Instability index	Aliphatic index
TaEXPA9-A	C <sub>1288</sub> H <sub>1947</sub> N <sub>367</sub> O <sub>358</sub> S <sub>16</sub>	28.814	9.40	-0.048	43.33	69.96
TaEXPA9-B	C <sub>1271</sub> H <sub>1926</sub> N <sub>364</sub> O <sub>358</sub> S <sub>16</sub>	28.546	9.51	-0.069	46.23	67.59
TaEXPA9-D	C <sub>1274</sub> H <sub>1935</sub> N <sub>365</sub> O <sub>357</sub> S <sub>16</sub>	28.590	9.61	-0.039	42.86	69.77

Table 3 Suppl. The number and proportion of amino acids in the TaEXPA9-A/B/D proteins predicted by *ExPASy* online site.

Amino acids	TaEXPA9-A		TaEXPA9-B		TaEXPA9-D	
	Number of amino acids	Content of amino acid [%]	Number of amino acids	Content of amino acid [%]	Number of amino acids	Content of amino acid [%]
Ala (A)	31	11.6	33	12.4	32	12.0
Gly (G)	30	11.2	30	11.3	30	11.3
Pro (P)	22	8.2	19	7.1	17	6.8
Leu (L)	21	7.8	21	7.1	21	7.5
Ser (S)	19	7.1	21	7.9	20	7.5
Val (V)	19	7.1	17	6.4	17	6.8
Arg (R)	18	6.7	18	6.8	18	6.8
Asn (N)	18	6.7	16	6.0	17	6.4
Thr (T)	13	4.9	13	4.9	14	5.3
Phe (F)	13	4.9	13	4.9	13	4.9
Trp (W)	9	3.4	9	3.4	9	3.4
Tyr (Y)	9	3.4	9	3.4	9	3.4
Cys (C)	8	3.0	8	3.0	8	3.0
Gln (Q)	8	3.0	10	3.8	10	3.8
Met (M)	8	3.0	8	3.0	8	3.0
Asp (D)	7	2.6	7	2.6	7	2.6
Ile (I)	5	1.9	6	2.3	6	2.3
Glu (E)	4	1.5	4	1.5	3	1.1
His (H)	4	1.5	3	1.1	3	1.1
Lys (K)	2	0.7	3	1.1	3	1.1
Pyl (O)	0	0.0	0	0.0	0	0.0
Sec (U)	0	0.0	0	0.0	0	0.0



B

A	ATGCCGCGCCGCAATGCCACCGCCGCGCTCCCGCCCTCCTCGCGCGCTGCTGCTCCTCTGGTGGCGCTTCCGCGCCG	80
B	ATGCCGCGCCGCAATGCCACCGCCGCGCTCCCGCCCTCCTCGCGCGCTGCTGCTCCTCTGGTGGCGCTTCCGCGCCG	74
D	ATGCCGCGCCGCAATGCCACCGCCGCGCTCCCGCCCTCCTCGCGCGCTGCTGCTCCTCTGGTGGCGCTTCCGCGCCG	74
Consensus	at gggcgccgca t gccaccg cgc gc ccgcc cct cgcggcgct gct gct ct ggt g gct t cggc gc	
A	CGCGCGCGCGCTCCCGCGCGCTGACACCGCGCGCGACTGGCAGAGCGCGCAAGCCACTTCTACGCGCGCAAGCGACGCT	160
B	CGCGCGCGCGCTCCCGCGCGCTGACACCGCGCGCGACTGGCAGAGCGCGCAAGCCACTTCTACGCGCGCAAGCGACGCT	154
D	CGCGCGCGCGCTCCCGCGCGCTGACACCGCGCGCGACTGGCAGAGCGCGCAAGCCACTTCTACGCGCGCAAGCGACGCT	154
Consensus	cgcggcgcgct ccc ggcgt gt acac ggcggcgact ggagagcgcgcacgcacact t ct acggcgcgacgagcgcct	
A	CGCGCACCATGGCGCGCGCTCGCGGTACGGGAACCTGTACAGCCAGGGGTACGGGTGAACAACCGCGCGCTGACACG	240
B	CGCGCACCATGGCGCGCGCTCGCGGTACGGGAACCTGTACAGCCAGGGGTACGGGTGAACAACCGCGCGCTGACACG	234
D	CGCGCACCATGGCGCGCGCTCGCGGTACGGGAACCTGTACAGCCAGGGGTACGGGTGAACAACCGCGCGCTGACACG	234
Consensus	ccggcaccat gggcgcgcgct gcgggt acgggaacct gt acagccagggt acgggt gaacaacggcgcgct gagcacg	
A	CGCGTGTTC AACGAGCGCGAGAGTCCCGCGCTGCTTCGAGATCCGCTGCGTGAACCAAGCGGTCGGTGGCGTGGTGGCT	320
B	CGCGTGTTC AACGAGCGCGAGAGTCCCGCGCTGCTTCGAGATCCGCTGCGTGAACCAAGCGGTCGGTGGCGTGGTGGCT	314
D	CGCGTGTTC AACGAGCGCGAGAGTCCCGCGCTGCTTCGAGATCCGCTGCGTGAACCAAGCGGTCGGTGGCGTGGTGGCT	314
Consensus	gcgct gt t caacgagggg ag g tgcgg gc t gct t cgagat ccggt gcg gaaccagcc ggggt gggcgt ggt gcct	
A	CGCGCGCGCGCGCTCCATCGTGGTCAACCGCACCAACTTCTGCCCGCCAACTACCGCTCCCGTCCGACGACGGCGCT	400
B	CGCGCGCGCGCGCTCCATCGTGGTCAACCGCACCAACTTCTGCCCGCCAACTACCGCTCCCGTCCGACGACGGCGCT	394
D	CGCGCGCGCGCGCTCCATCGTGGTCAACCGCACCAACTTCTGCCCGCCAACTACCGCTCCCGTCCGACGACGGCGCT	394
Consensus	ccgggg gggcgt ccat cgt gt caccgccacacaact t ct gcccgcccaact acggcct ccct c gacgacggcggt	
A	GGTGCAACCCGCGCGCGCGCACTTCGACCTCGCCATGCCATGTTCTCCACATGGCGCAGTACCGCGCGCGCATCGTC	480
B	GGTGCAACCCGCGCGCGCGCACTTCGACCTCGCCATGCCATGTTCTCCACATGGCGCAGTACCGCGCGCGCATCGTC	474
D	GGTGCAACCCGCGCGCGCGCACTTCGACCTCGCCATGCCATGTTCTCCACATGGCGCAGTACCGCGCGCGCATCGTC	474
Consensus	ggt gcaaccgccccgccccact t cgacct cgcgat gccat gt t cct ccacat ggc cagt acccgccggcat cgt c	
A	CGCGTCTCCTACCGCGCGGTGGCGTCCCGGAAGTCCGCGCGGTCCGCTTCAACATCAACCGGTTCAAGTACTTCAACCT	560
B	CGCGTCTCCTACCGCGCGGTGGCGTCCCGGAAGTCCGCGCGGTCCGCTTCAACATCAACCGGTTCAAGTACTTCAACCT	554
D	CGCGTCTCCTACCGCGCGGTGGCGTCCCGGAAGTCCGCGCGGTCCGCTTCAACATCAACCGGTTCAAGTACTTCAACCT	554
Consensus	cccgt ct cct accgcccgggt gggt gccggaagt cggcggggt gcggt t cac at caacgggt t caggt act t caacct	
A	GGTGCTCATCACCAACGTGGCGCGCGCGCGGACCTGTCGGCGCGACCGTGAAGGGTCCAGCACCGGTTGATGCCA	640
B	GGTGCTCATCACCAACGTGGCGCGCGCGCGGACCTGTCGGCGCGACCGTGAAGGGTCCAGCACCGGTTGATGCCA	634
D	GGTGCTCATCACCAACGTGGCGCGCGCGCGGACCTGTCGGCGCGACCGTGAAGGGTCCAGCACCGGTTGATGCCA	634
Consensus	ggt gct at cac aacgt gcc ggggc ggggacct ggt gcg gc agcgt gaaggggt ccagcaccgggt ggat gccca	
A	TGTCCGCAACTGGCGCGCAACTGCCAGTCCAAGTCCGTCCTCGTCCGCGAGCGCTCTCCTTCCGCGTCAACCGCACG	720
B	TGTCCGCAACTGGCGCGCAACTGCCAGTCCAAGTCCGTCCTCGTCCGCGAGCGCTCTCCTTCCGCGTCAACCGCACG	714
D	TGTCCGCAACTGGCGCGCAACTGCCAGTCCAAGTCCGTCCTCGTCCGCGAGCGCTCTCCTTCCGCGTCAACCGCACG	714
Consensus	t gt c cgcaact ggggcagaaact ggcagt ccaac cc t cct cgt cggcag c ct ct cct t ccggt caccgccagc	
A	GACCGCGCACCTCCACCTCCTGGAACCGCGCGCGCGCAACTGGCGCTTCCGCGAGACCTTCCAGGGCAAACACTTCAAG	800
B	GACCGCGCACCTCCACCTCCTGGAACCGCGCGCGCGCAACTGGCGCTTCCGCGAGACCTTCCAGGGCAAACACTTCAAG	794
D	GACCGCGCACCTCCACCTCCTGGAACCGCGCGCGCGCAACTGGCGCTTCCGCGAGACCTTCCAGGGCAAACACTTCAAG	794
Consensus	gaccgcccacct ccacct cct ggaacgcgcg cc ca a ct ggcgt t cgg cagacct t cgagggcaa aact t cag	
A	GGTCTA	806
B	GGTCTG	800
D	GGTCTA	800
Consensus	ggt ct	

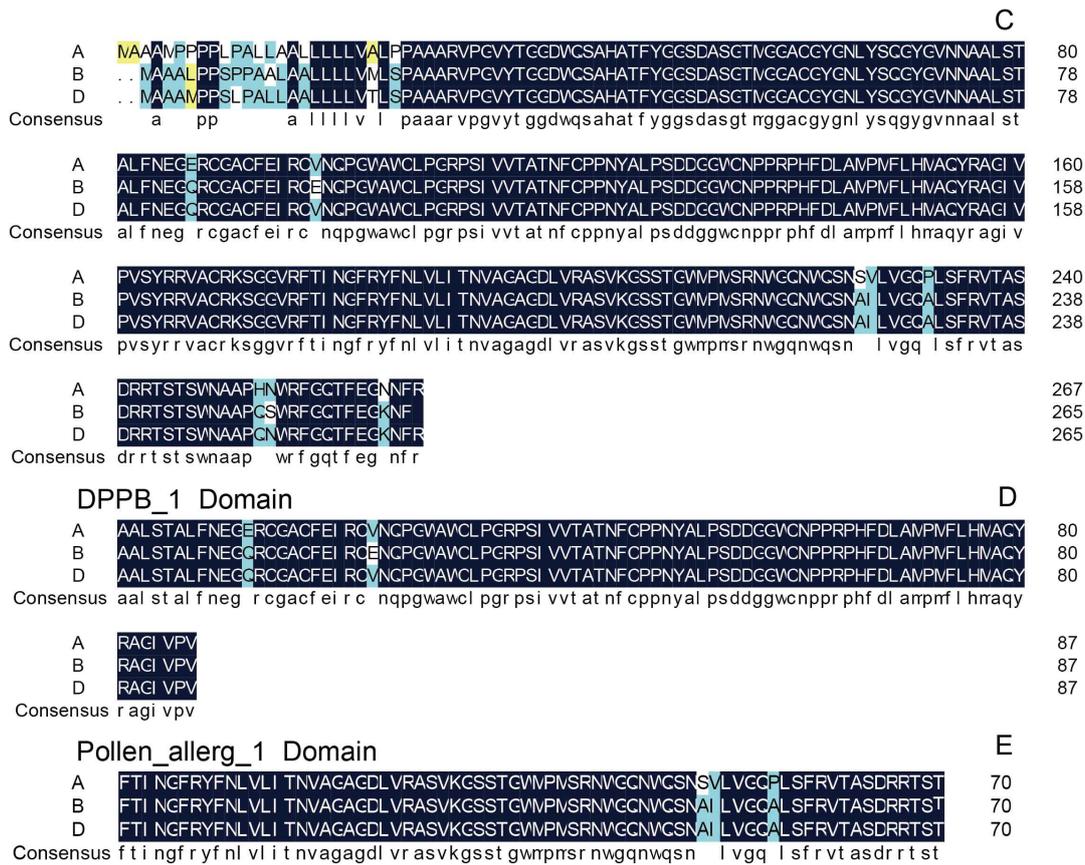
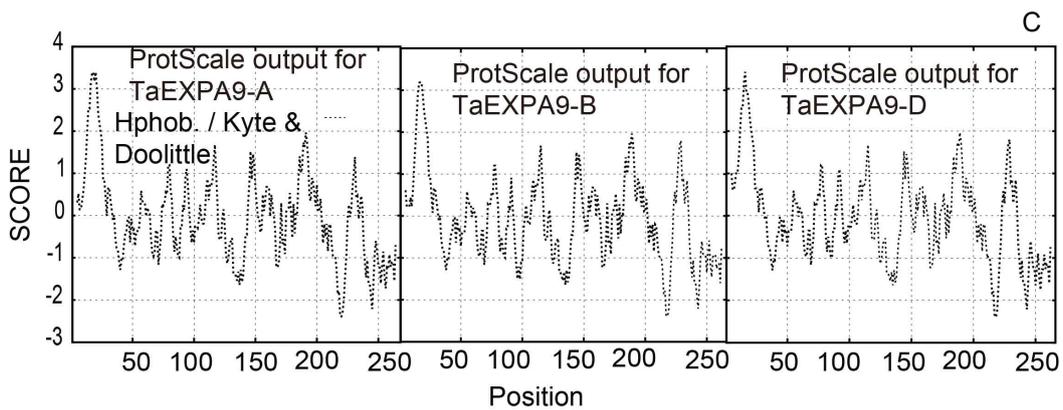
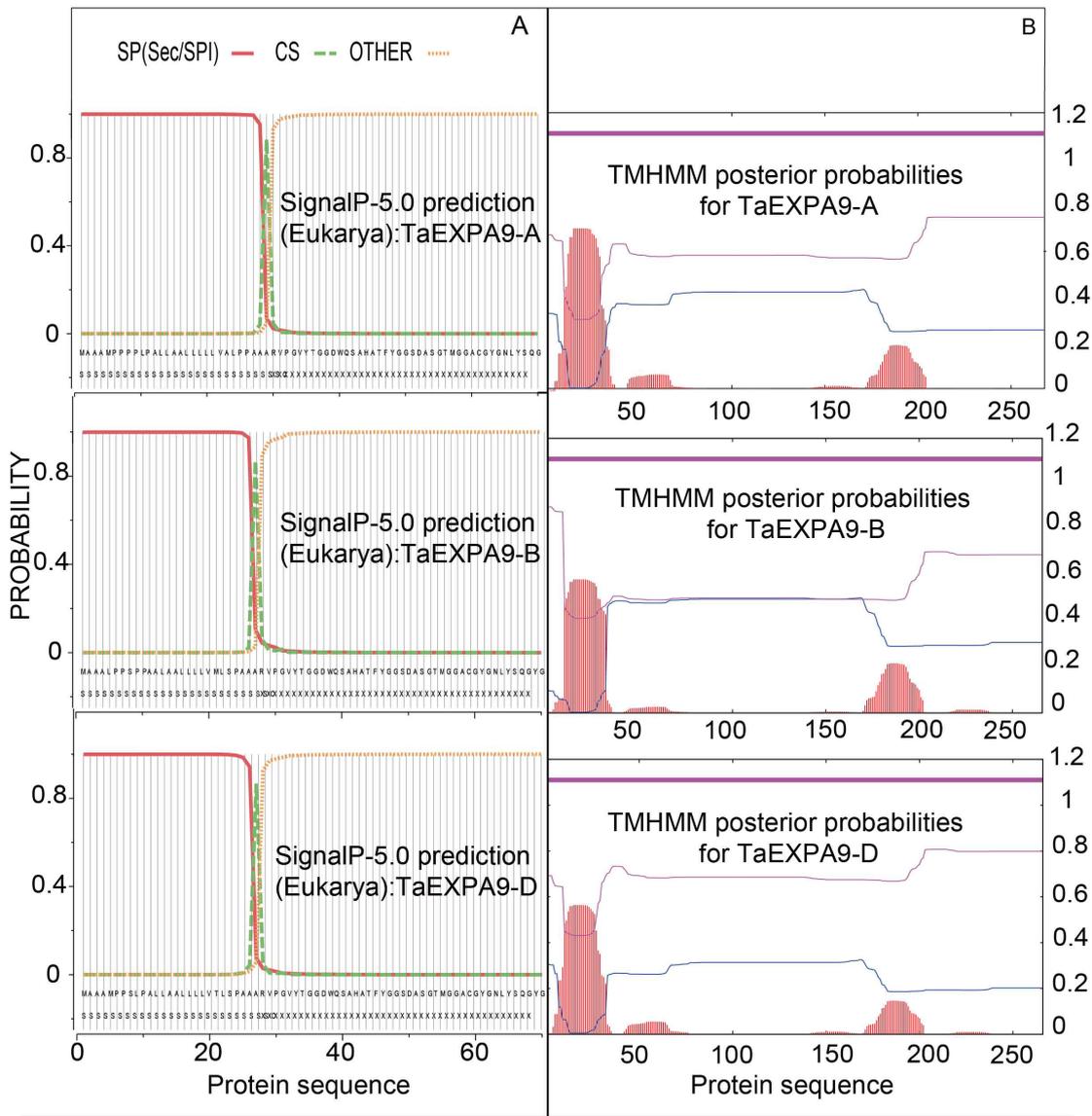


Fig. 1 Suppl. Electrophoretic gel diagram and sequence contrast of *TaEXPA9-A/B/D*. *A* - Electrophoresis gel showing the PCR-amplified *TaEXPA9* gene. Lane 1 shows the *TaEXPA9-A* gene; Lane 2 shows the *TaEXPA9-B* gene; and Lane 3 shows the *TaEXPA9-D* gene. *B* - Sequence analysis of *TaEXPA9-A/B/D*. Alignment of the CDS regions of *TaEXPA9-A/B/D*. *C* - Alignment of *TaEXPA9-A/B/D* proteins. *D* - Alignment of the DPPB\_1 domain of *TaEXPA9-A/B/D*. *E* - Alignment of the Pollen\_allerg\_1 domain of *TaEXPA9-A/B/D*.



		D		
Analysis Report		SeqID: <i>TaEXPA9-A</i>	<i>TaEXPA9-B</i>	<i>TaEXPA9-D</i>
SVM	LOCALIZATION	RELIABILITY	RELIABILITY	RELIABILITY
Amino Acid Comp.	Extracellular	0.398	0.450	0.471
N-peptide Comp	Extracellular	0.519	0.607	0.642
Partitioned seq.Comp.	Extracellular	0.708	0.751	0.747
Physico-chemical Comp.	PlasmaMembrane	0.680	0.540	0.646
Neighboring seq.Comp	Extracellular	0.871	0.847	0.890
CELLO Prediction	<b>Extracellular</b>	<b>2.685*</b>	<b>3.194*</b>	<b>3.396*</b>
	PlasmaMembrane	1.296	0.980	0.826
	Vacuole	0.354	0.257	0.266
	Lysosomal	0.265	0.160	0.163
	Nuclear	0.128	0.118	0.101
	Mitochondrial	0.089	0.092	0.080
	Chloroplast	0.068	0.090	0.073
	Cytoplasmic	0.041	0.044	0.037
	ER	0.036	0.029	0.027
	Golgi	0.016	0.013	0.012
	Peroxisomal	0.013	0.012	0.011
	Cytoskeletal	0.010	0.010	0.009

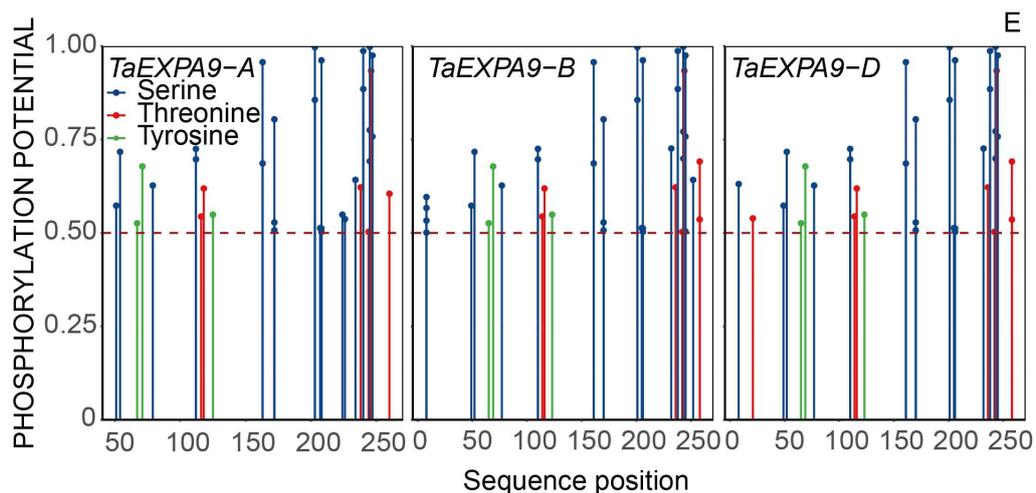
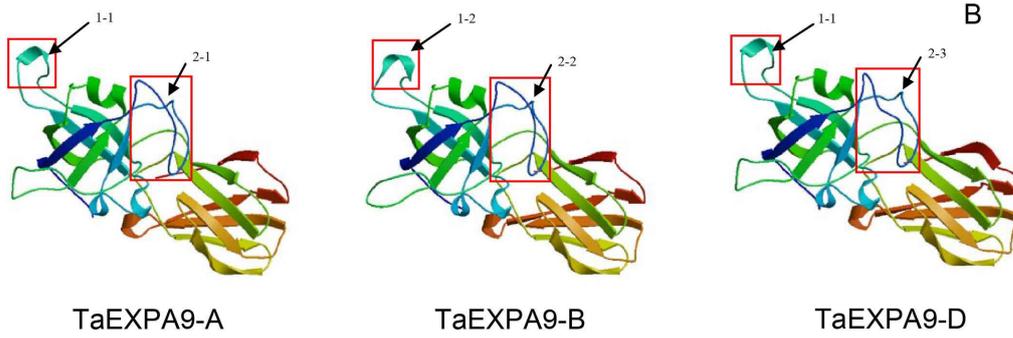
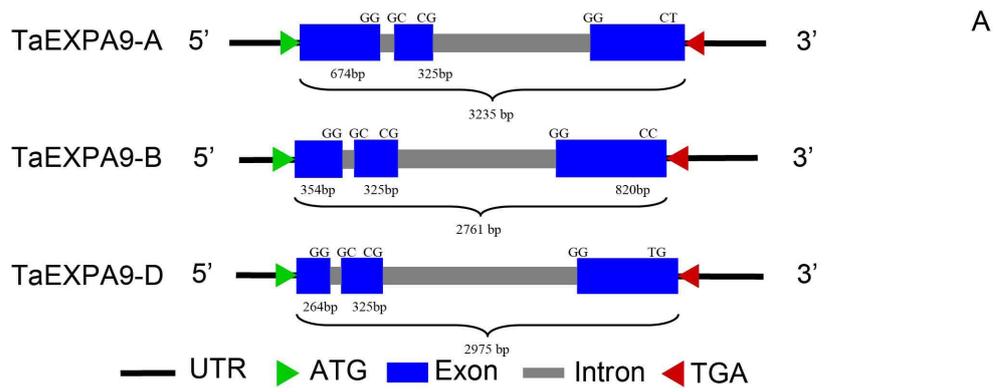


Fig. 2 Suppl. Five bioinformatics analysis of *TaEXPA9-A/B/D*. *A* - The signal peptide sites of *TaEXPA9-A/B/D* proteins predicted by *SignalP 4.1* server. *B* - The transmembrane construction analysis of *TaEXPA9-A/B/D* proteins predicted by *TMHMM* server v.2.0. *C* - The hydrophobicity analysis of *TaEXPA9-A/B/D* proteins predicted by *ExPASy-Prot* scale. *D* - The subcellular localization analysis of *TaEXPA9-A/B/D* proteins predicted by *CELLO* v 2.5. *E* - The phosphorylation sites analysis of *TaEXPA9-A/B/D* proteins predicted by *NetPhos*.





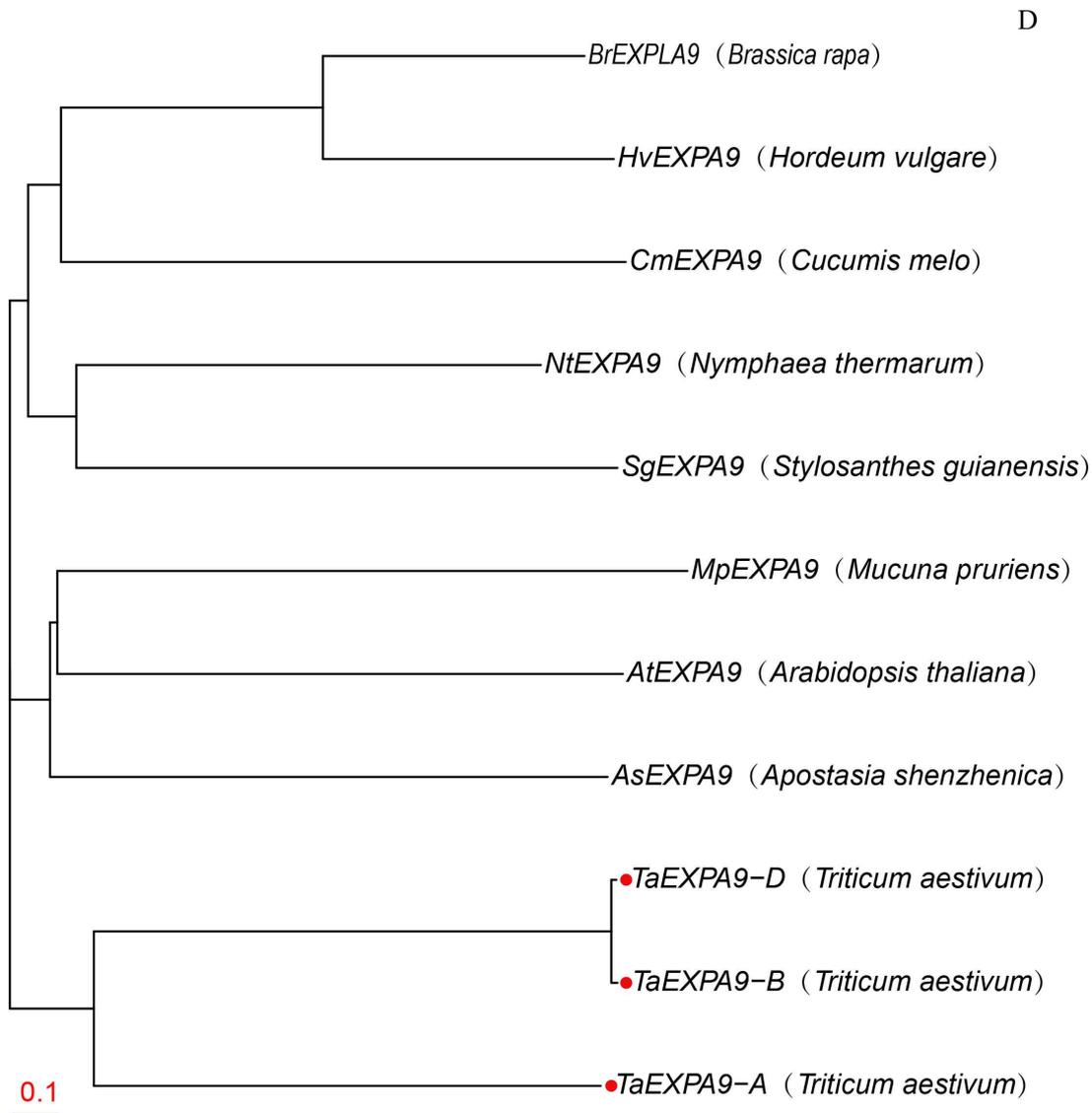
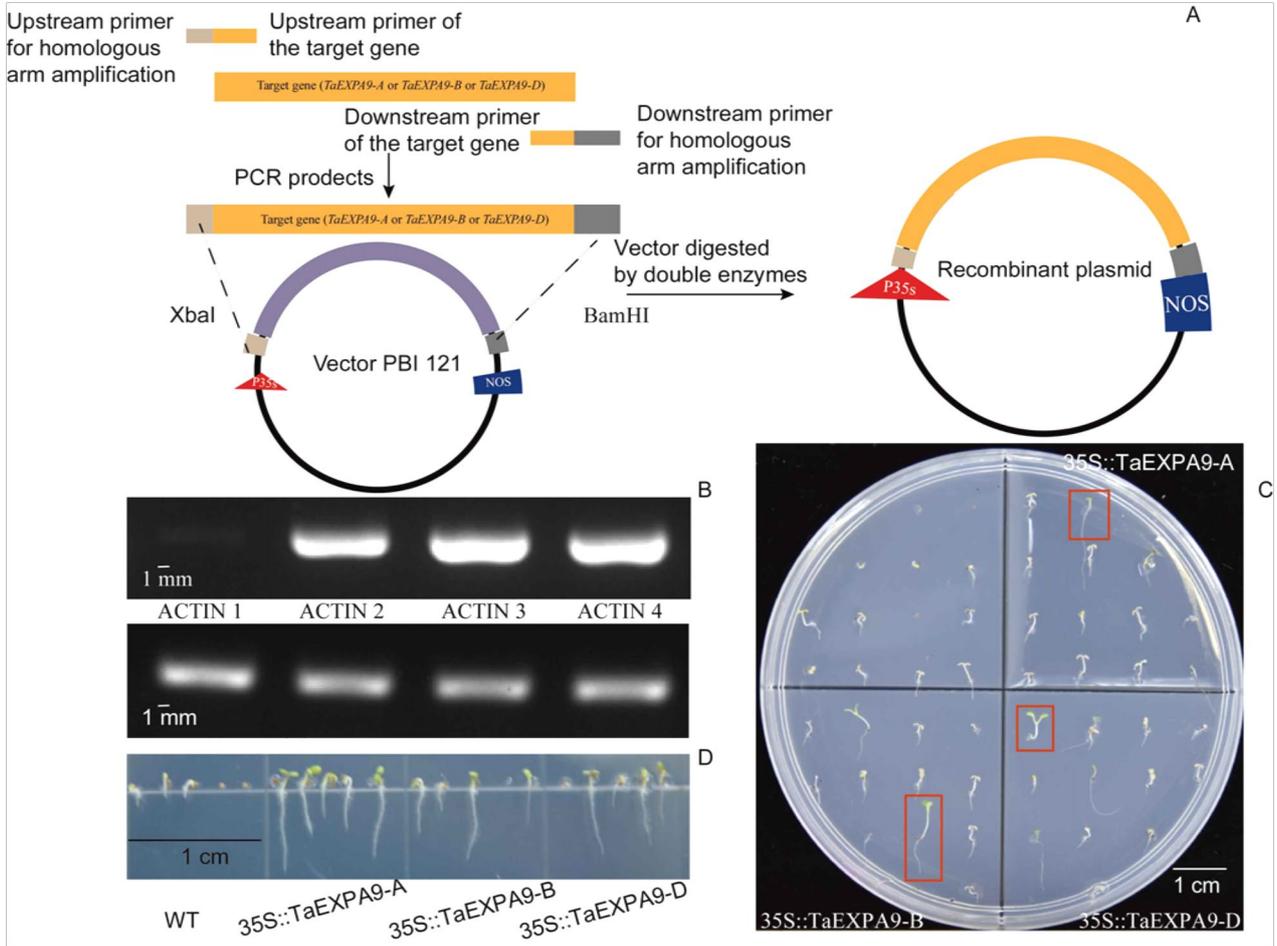
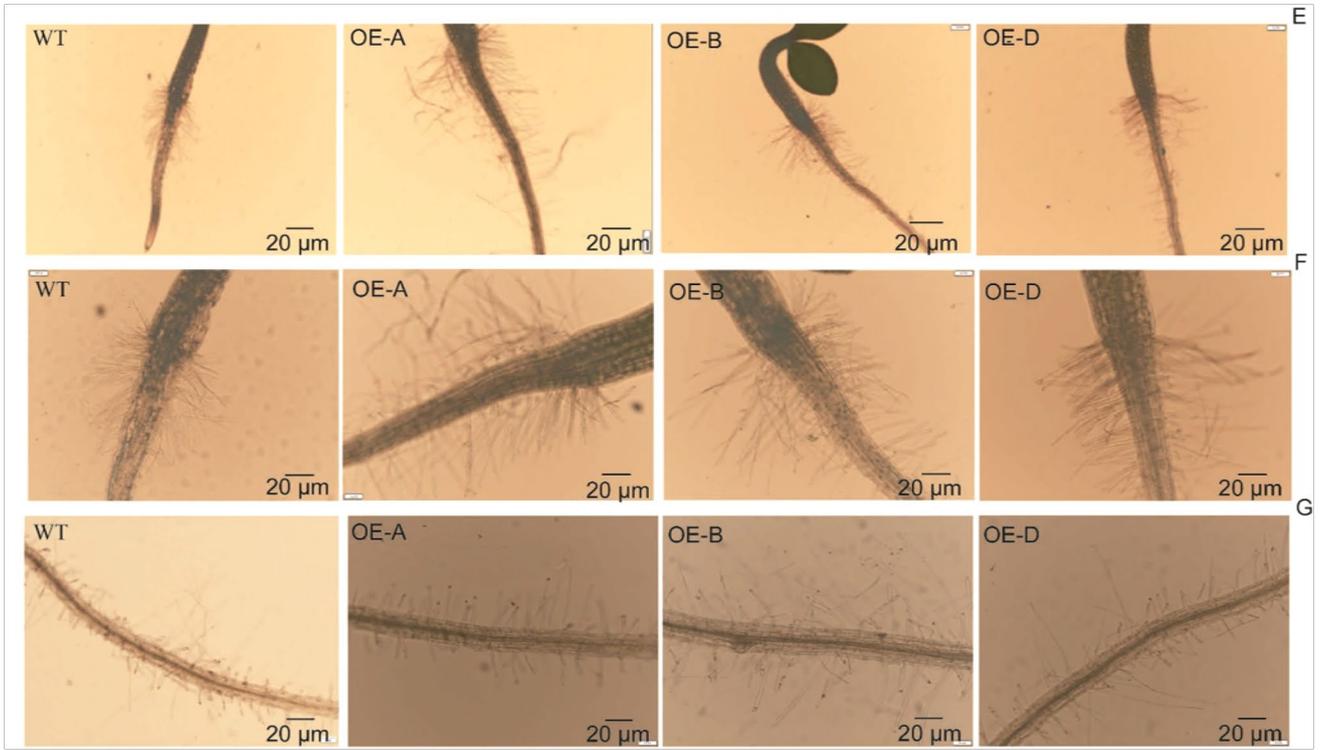


Fig. 3. Suppl. Gene structure and tertiary structure model of *TaEXPA9-A/B/D*. Comparative analysis and phylogenetic construction of *EXPA9* and *EXPLA9* proteins. *A* - Structure of *TaEXPA9-A/B/D*. *B* - The tertiary structure model of *TaEXPA9-A/B/D* proteins predicted by *ExpASY-SWISS-MODEL*. The dark blue region is the signal peptide region, the green region is the catalytic region, the yellow and red areas were cellulose binding areas, the red boxes represent the difference area. *C* - Alignment analysis of the amino acid sequences of *TaEXPA9-A/B/D* with *EXPA9* and *EXPLA9* proteins in eight other plant species. *AtEXPA9* (*Arabidopsis thaliana*), *HvEXPA9* (*Hordeum vulgare*), *MpEXPA9* (*Mucuna pruriens*), *SgEXPA9* (*Stylosanthes guianensis*), *CmEXPA9* (*Cucumis melo*), *AsEXPA9* (*Apostasia shenzhenica*), *NtEXPA9* (*Nymphaea thermarum*), *BrEXPLA9* (*Brassica rapa*). The dark blue represents 100 % identity, the pink represents 75 % identity, and the blue-green represents 50 % identity, as defined by *ClustalX*. The *black arrows* indicate two conserved domains: DPDD\_1 domain. (★) indicates eight C (cysteine) residues. (▲) indicates six W (tryptophan) residues in the C-terminal region. The *red box* denotes the HFD (His-Phe-Asp) motif in the central region of the sequence (for interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article). *D* - The genetic relationship of expansin protein sequences in different species was analyzed. Phylogenetic tree of *TaEXPA9-A/B/D* with the above ten proteins.





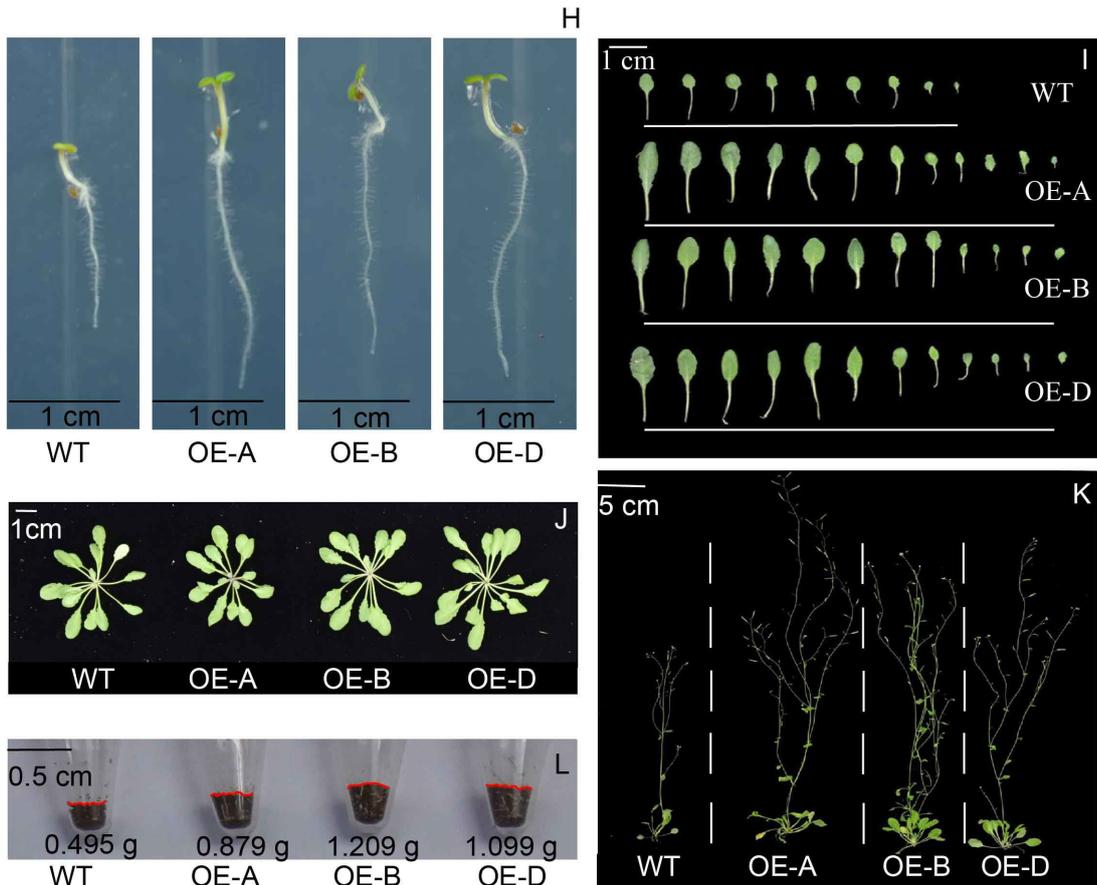


Fig. 4. Suppl. Identification and phenotypic observation results of *A. thaliana* overexpressing *TaEXPA9-A*, *B*, and *D*. WT, OE-A, OE-B, and OE-D represent wild-type and overexpressed *TaEXPA9-A/B/D* *A. thaliana*, respectively. *A* - Structure of the expression vector. *B* - The expressions of *TaEXPA9-A, B*, and *D* in transgenic lines as determined by RT-PCR. *C* - Screening of resistant transgenic plants. The green plants selected in the red box are overexpressed plants showing resistance, which can be used for subsequent phenotypic observation tests. Scale bars 1 cm. *D* - Observation on hypocotyl of *A. thaliana*, scale bars 1 cm. Observation on lateral root of *A. thaliana*. Magnification 10×10 and 10×40, scale bars 20 μm (*E, F*). *G* - Observation on root hair length of *A. thaliana*. Scale bars 20 μm. *H* - Observation on root length of *A. thaliana*. Magnification, 10×10, scale bars 1 cm. Observation on leaf number and rosette diameter of *A. thaliana*. Scale bars 1 cm (*I, J*). *K* - *A. thaliana* plants height. Scale bars 5 cm. *L* - *A. thaliana* seed yield (per plant). Scale bars 0.5 cm.