

## RESEARCH ARTICLE

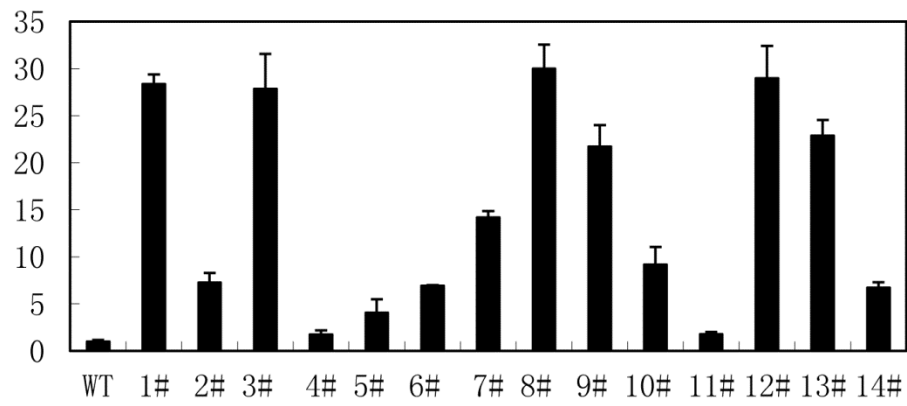
# Overexpression of annexin gene AnnSp2, enhances drought and salt tolerance through modulation of ABA synthesis and scavenging ROS in tomato

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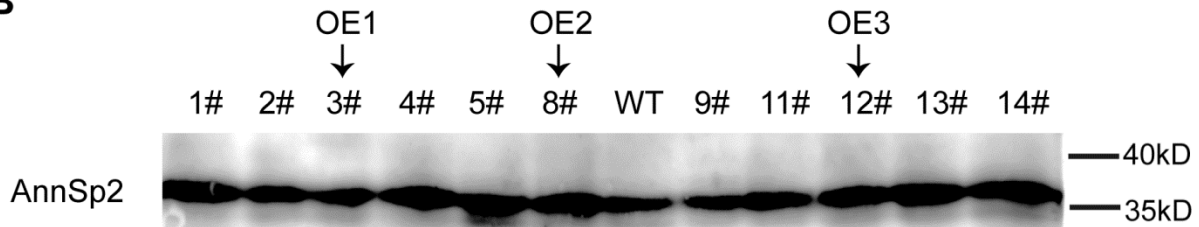
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+ TTTTGTGAAG ATCT-motif GAATGTAATC TTGAACTTTT TTTTTTTTT GTAATCTTAC AAAAAAGTA TTATTGTGTAC
- AAAAAAATTC CTTACATTAG AACTTGAAAA AAAAAAATAA CATTAGAATG TTTTTCAT AATAACAATG
+ TTTTCTTCT TCTTCTAAT CTGCAAAATA TTTAATAAAC AAAAT Box4 TAATA AATGAAACT TACCAACAT
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- AAGACCTTCT TTCTAATCCT TTTATAATAA ATGTGGGAAA TAGGTAGTTT ATCAATAATA AATATCTGCA
+ GACACAAACA CATTAGAGA AAATAAATAG AAAAAACCTG GT1-motif GTTAAATTAT TACTTGAAAA Box4 ATTAATAACC
- CTGTGTTTGT GTAATCTCT TTTATTTATC TTTTGTGGAC CAATTAATA ATGACCTTTT TAATTTTGG
+ TCTTTAAATA ATATCTTTTT TCGATTTTAA TGTGGACTAA TGAAAAAAGC ATTCGCTCTT AATAAAATAA AT1-motif
- AGAAATTTAT TATAGAAAA AGCTAAAATT ACACCTGATT ACTTTTTTCG ATAAGCAGAA TTATTTTATT
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+ ATTTTGAAAA TATATTAAT ATTGAATAGT ATTTAATAAT AATGATAAAA TTATTCTAAA ATAGTAATTT AT1-motif
- TAAAATTTT ATATAATTTA TAACCTATCA TAAATTTATTA TTACTATTTT AATAAGATTT TATCATTAAA
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- TTTTCCGGTG TACTATTATA TCATCTCAAC CACTCCAATG TCCAATGGAT TAGGAGGTT CAGGATTATG
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- AGAGCGGTA ATGTTGCGTT TTAACCCAT GTACACCGCA ATGTTACCAC ATCAGTATT TCAATACAAT
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+ ATGAAGATGC TGAGCAACTC AAAAAAGCT
- TACTTCTACG ACTCGTTGAG TTTTTTCGA
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**Fig. S1** *Cis*-acting element analysis of AnnSp2 promoter. ABRE (TACGTG), *cis*-acting element involved in abscisic acid responsiveness. ATCT-motif (AATCTAATCT), part of a conserved DNA module involved in light responsiveness. BOX 4 (ATTAAT), part of a conserved DNA module involved in light responsiveness. GCN4-motif (TGTGTCA), *cis*-acting regulatory element involved in endosperm expression. G-BOX (CACGTC/TACGTG), *cis*-acting regulatory element involved in light responsiveness. HSE (AAAAAATTTC), *cis*-acting element involved in heat stress responsiveness. SP1 (CC(G/A)CCC), light responsive element. TCA-element(GAGAAGAATA), *cis*-acting element involved in salicylic acid responsiveness. AT1-motif (ATTAATTTTACA/ AATTATTTTTTATT), part of a light responsive module. AE-box (AGAAACAT), part of a module for light response. GT1-motif (GGTTAA), light responsive element. TATC-box (TATCCCA), gibberellin-responsive element. MBS (TAACTG/CAACTG), MYB binding site involved in drought inducibility. ARE (TGGTTT), *cis*-acting regulatory element essential for the anaerobic induction.

**A**



**B**



**Fig. S2 A** Identification of transgenic plants and expression of AnnSp2. (A) q-PCR analysis of AnnSp2 expression in wild-type (WT) and T2 OE plants. (B) **Protein accumulation** in AnnSp2. Western blot analysis of AnnSp2 transgenic plants and in wild-type (WT) plants.

**Table S1** Details of the primers used in this study

<b>Primer name</b>	<b>Forward primer sequence</b>	<b>Reverse primer sequence</b>	<b>Purpose</b>
AnnSp2	TTCCCACGTATATTCTTGAGATTG	CACACCATACATCATAAAAAGCAAA	Gene amplification
QAnnSp2	GATTCTTATGCTGCTGCTTATGGA	CAGTCAGACGTTTGGTAGCCTCA	Quantitative real-time-PCR primer
AnnSP2	CCGCTCGAGATGTCTAGTCTTAAAGTTCCA	TGCTCTAGATCAAGCATCTCCGTGCCCAATC	Subcellular localization primer
CaMV 35S	TTGAATCCTGTTGCCGGTCT	GGAAGGGTCTTGCGAAGGATAG	Transgenic plants confirmation
AREB	TTGCTGGTGGAAATGTAAGTGC	GGAATGTAACATCCTTTGAGTATCG	qRT-PCR
SPERD	AGGCATCAAGTCATCACTCTCTGGT	GAGTAATGTGAGTAAGAACCAACG	qRT-PCR
DREB	AGTTGGGGAAAATGGGTGTC	CGAGGCAATGAGTCAATTAGGT	qRT-PCR
NCED	AGTTTCCCGATTTGGTATTC	TGGAGTCTGGTGGTGTCA	qRT-PCR
P5CS	GCGATTCCTGGAAGTGTGG	AGCATGGCCAAGAACAGGAA	qRT-PCR
$\beta$ -actin	ACCTTCAATGTCCCTGCTATG	CTCCACCTTCAGAAACGCAAC	$\beta$ -actin

**Table S2** The genetic segregation analysis of over expression AnnSp2 plants in T1 generation by spraying kanamycin test

Number	Gene Name	T1 segregation		Single locus with 3:1 segregation*
		Green	yellow	
1	AnnSp2 -6	18	3	No
2	AnnSp2 -14	32	7	No
3	AnnSp2 -18	29	10	Yes
4	AnnSp2 -20	33	16	No
5	AnnSp2 -26	44	12	No
6	AnnSp2 -27	21	5	No
7	AnnSp2 -29	27	13	No
8	AnnSp2 -31	36	12	Yes
9	AnnSp2 -36	15	6	Yes
10	AnnSp2 -38	15	9	No
11	AnnSp2 -42	14	2	No
12	AnnSp2 -44	12	4	Yes
13	AnnSp2 -50	23	16	No
14	AnnSp2 -56	17	4	No

\* The analysis based on chi square test