

***Bna.EPF2* Enhances Drought Tolerance by Regulating Stomatal  
Development and stomatal size in *Brassica napus***

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**Supplemental Files**

#### **Bna.EPF1**

BnaA09g43550D: MKSFLLLVLFSLFFVGSIFASRHHFTHFYPSHNNHHVG-----MKRQRREDTVOVAGSRLPDCSHACGSCSPCRLVMVSFVCASIEEAETCPMAYKCMCKNKSYPVF : 102  
BnaC08g36150D: MKSFLLLVLFSLFFVGSIFASRHHFTHFYPSHNNHHVGMMGRIMKRQRREDTVOVAGSRLPDCSHACGSCSPCRLVMVSFVCASIEEAETCPMAYKCMCKNKSYPVF : 107

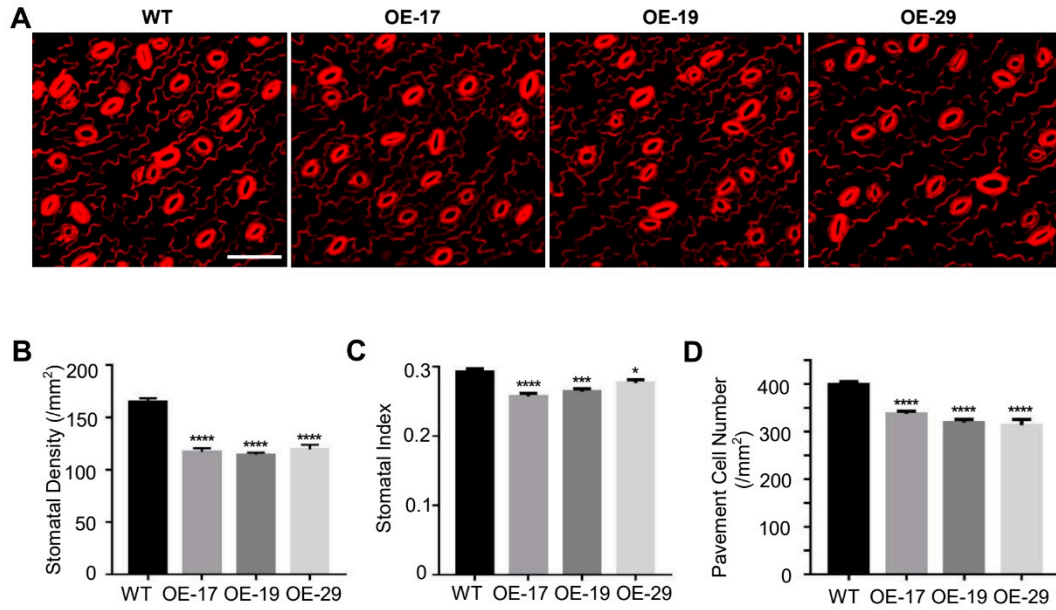
#### **Bna.EPFL6**

BnaA03g13910D: YGSGTPSSSSSHPSSEKSRKRSIFYIFLLCVVLYTLATSIFPSSTSSFYIRNNSCKLGHFYAOEEEGKSTLVIKMRIGDWTKEAELRRLKGLGSSE : 106  
BnaC03g16880D: YLRRTQQRCCHVLLARLSAYRFPASAHEDKYSNELWSVHHIWWFADRLSVFQSRFSIKL-----EEEGKSTLVIKMRIGDWSKEAELRRLKGLGSSE : 100  
BnaA03g13910D: PRCTSKGGRCTPCKPVHVPVSSGTPVTAEYYPEAWRCKCGNKLYMF : 146  
BnaC03g16880D: PRCTSKGGRCTPCKPVHVPVSSGTPVTAEYYPEAWRCKCGNKLYMF : 140

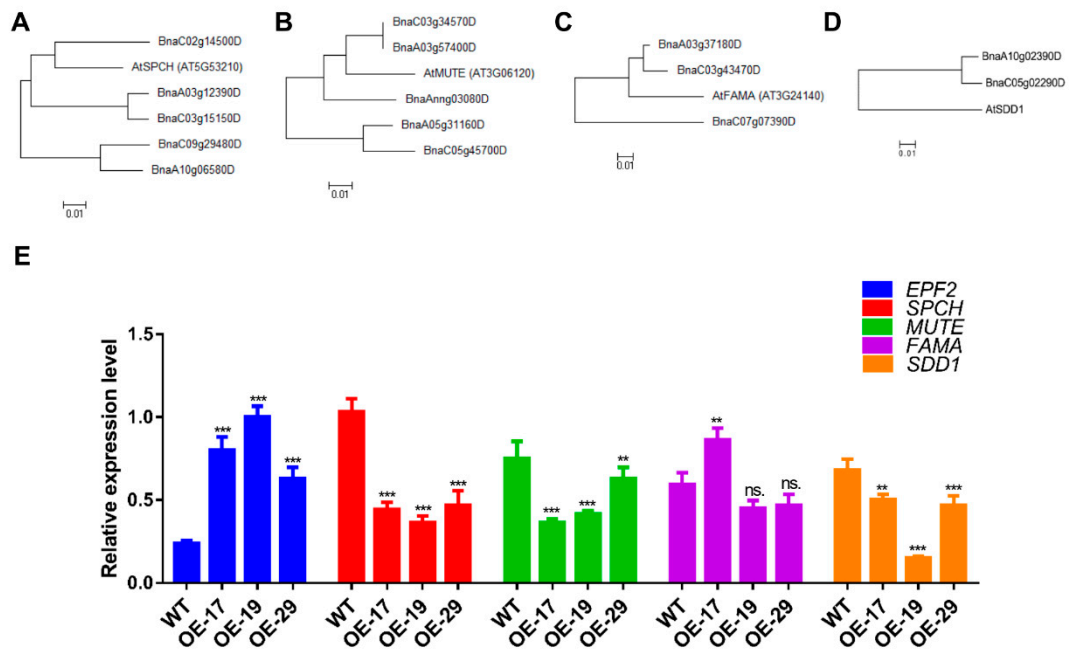
#### **Bna.EPFL9**

BnaC08g05340D: MKHEMNNMKLRCISFFFLLPGLLLGNFIVEASKARSIDDTLSLPROVHLPSRRHMIGSTAPTCTYNECRGCRYKCAEQVPVEGNDPINSAYHYRCVCHR : 101  
BnaA08g04900D: MKHEMNNMKLRCISFFFLLPGLLLGNFIVEASKARSIDDTLSLPROVHLPSRRHMIGSTAPTCTYNECRGCRYKCAEQVPVEGNDPINSAYHYRCVCHR : 101

**Supplemental Figure S1. The sequence similarity of *EPF1*, *EPFL6*, and *EPFL9* homologs in *Brassica napus*.** Alignment was performed with ClustalX (version 1.83) in multiple alignment mode and edited with GeneDoc1.0 software ([www.psc.edu/biomed/genedoc](http://www.psc.edu/biomed/genedoc)). Black shaded indicated high sequence similarity, light gray indicated sequence similarity was low.



**Supplemental Figure S2. The stomatal density in cotyledons of *Bna.EPF2* overexpression was lower compared to WT. (A)** Imaging of epidermis of 15 day-after-germination cotyledons of WT and *Bna.EPF2* overexpression lines. Cotyledon epidermal layers were stained by propidium iodide (PI) for 5 min and the images were taken by a confocal microscopy. Bar = 100  $\mu$ m. **(B-D)** Statistical analyses of stomatal density **(B)**, stomata index **(C)** and pavement cell density **(D)** in 15 day-after-germination cotyledons of WT and *Bna.EPF2* overexpression lines. Values are means  $\pm$  SE (each with at least 20 leaves). \*,  $P < 0.05$ ; \*\*\*,  $P < 0.001$ ; \*\*\*\*,  $P < 0.0001$ ; Student's t-test.



**Supplemental Figure S3. Expression levels of homologous genes involved in stomatal development pathway in *Bna.EPF2* overexpression lines. (A-D)** The phylogenetic tree of the homologous genes from *Arabidopsis thaliana* and *Brassica napus* were constructed using the MEGA6.06 software. **(E)** Gene expression levels of these homologs in WT, and *Bna.EPF2* overexpression lines. Values are means  $\pm$  SE, three biological replicates; No significantly difference (ns.),  $P \geq 0.05$ ; \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$ ; Student's t-test.

**Table S1. Primers used in this research**

Primer name	Primer sequence (5'-3')
<i>NPTII</i> -F	GGAAAACGATTCCGAAGCCCAA
<i>NPTII</i> -R	ACAACAGACAATCGGCTGCTCT
<i>epf2</i> -L	TAAAACCTCTGCCTCAACCAG
<i>epf2</i> -R	TTACCGGTATGATGGAGATGG
LBb 1.3	ATTTTGCCGATTTTCGGAAC
<i>Bna.EPF2</i> -F	TGCTCTAGAATGAAGAAGTTTCTCCGCACG
<i>Bna.EPF2</i> -R	TCCCCCGGGTCAAGCCCTAGATGGCACGTG
<i>Bna.EPF2</i> -pF	GCCCAAGCTTCCTAATCTGAATTACGTTTAAGCAA
<i>Bna.EPF2</i> -pR	GCGCGGATCCGTTTTATTTTATTTTGTCTCAACAAG
35S::JW771-F	ACGTTCCAACCACGTCTTC
<i>Bna.EPF2</i> -qL	CCCACCACAAGGAAATAAAG
<i>Bna.EPF2</i> -qR	GGAACAATCAGGTAAACTCG
<i>Bna.SDD1</i> -qL	GGAAGCAGAGGAGAGAAGGTG
<i>Bna.SDD1</i> -qR	ACTCCTAACTCGCTGCATCAC
<i>Bna.FAMA</i> -qL	GACTATCGCTGCTTTGGAGG
<i>Bna.FAMA</i> -qR	CACGTGTTTATACTTACTTGCCCTTG
<i>Bna.MUTE</i> -qL	TCTCGAGTCCAAGAAACGCC
<i>Bna.MUTE</i> -qR	GTGGCTGCGGCTAATATGGA
<i>Bna.SPCH</i> -qL	CCCTCGTCCTTCACCAAATGT
<i>Bna.SPCH</i> -qR	GAGGGGTTCGAGGGCTTATG
RT- <i>Bna.actin</i> -F	CCCTGGAATTGCTGACCGTA
RT- <i>Bna.actin</i> -R	TGGAAAGTGCTGAGGGATGC