

Title:

Prunus Hexokinase 3 genes alter primary C-metabolism and promote drought and salt stress tolerance in *Arabidopsis* transgenic plants

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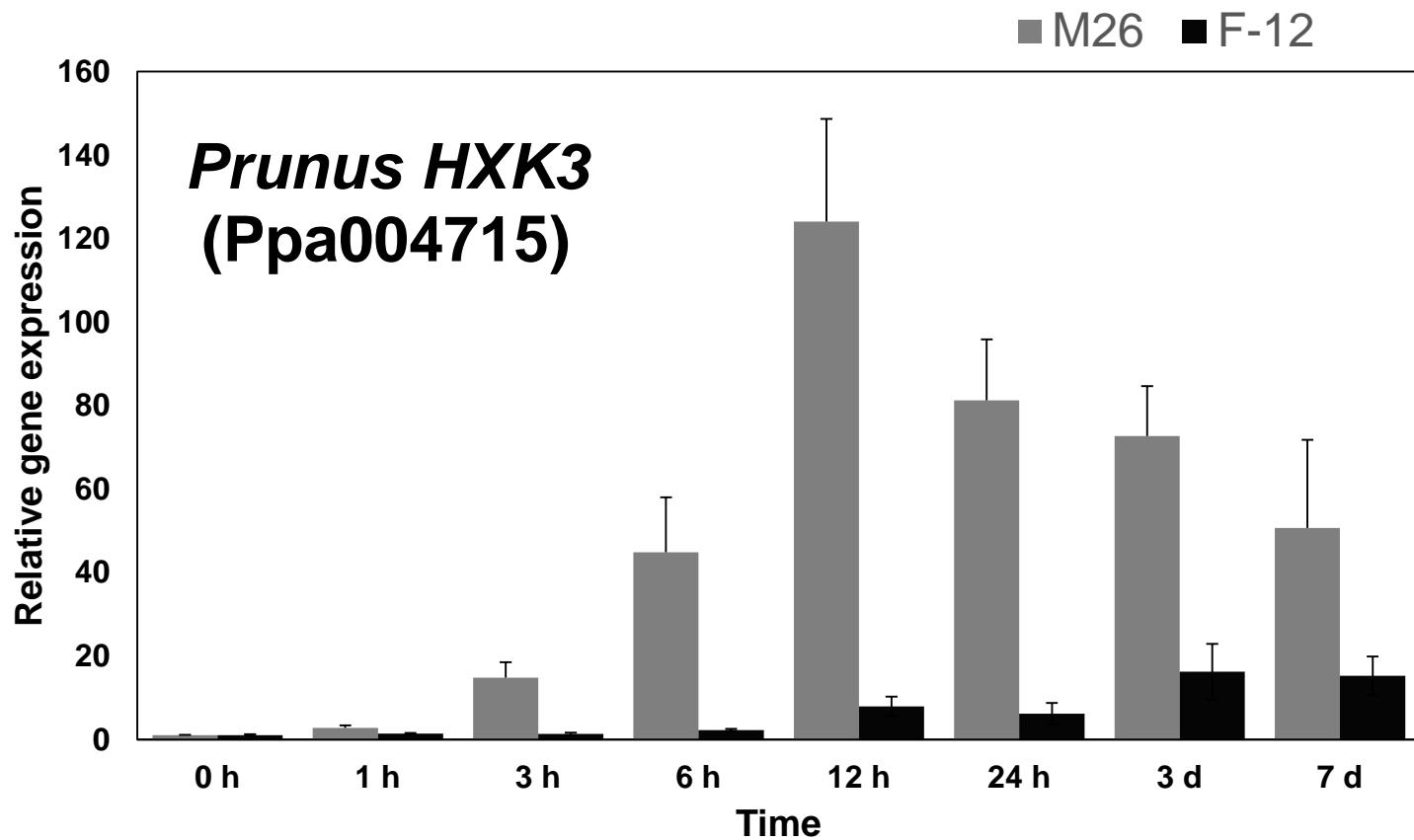


Figure S1. Expression patterns of the *HXK3* genes in *Prunus* plants subjected to hypoxia.
Gene expression was examined by qRT-PCR using total RNA from rootstock roots of Mariana 2624 and Mazzard F12/1 after treatment with hypoxia for 0, 1, 3, 6, 12, 24, 72 and 168 h. They are expressed as mean relative expression values +/- standard error (n=3), normalized with *TEF2* as a constitutively expressed gene.

>***Prunus persica* | Prupe.1G366000 HXK3 promoter**

AAAAAGTAAAATAAAATCAAATAATAGTCATACTTTATTAAACATAACTATCCATTATTTCTTAATTCAAGAAAAAAATCAATTAGCACATA
CGTAGTATGGCCAAGGGGCTGTGGTACAGAAAGAGCAATGGTATGTGGCATACCGTTCTATCAAGTCCAAAGTAATCAATACAGAAAATGCCAATCCAAC
TATGGTAGGGTAGGATGCCACGACTAAAAGTTGATGGAAGTCTCAGAATTGCAAACCAAAATCATTAAAAGGATTAAGGATAAGAAGTATCCATCTCATCAA
TCATTATCACATTAACAACAAACATCTAACCTAGCCCAGCCCTGAAGCCAGCTTAATTATCGTATTACAAAGATTGCTGCTGCTGCTGGCG
CTCCAAGCATATGAATATTTACTTGTTATTGAAACTATTGATATACTTAAACGACTGAAAACAACAATTATGGATATACTTAAACGACTGGA
AAAACAACAACATAGTCGAGCTACAACACCACAAAAGCACAATCTCGCGTTGTGCTCCATCTACAAACCCCCATCGTATTGTCGCCAATCCAACCTTGT
CCAAACCTCAGCTTGTACCCAATCAAAAACAATAAAAGCAAAACAACGGGAAGGTAACGAACAGTTAATTCTGTTAGCTGTTAATTGCTT
TCATGTCAGCTACCCAAAACCACGTCGTTGCTTCTGAGCATTTCGACTTTTATTATAAAATTATAAAACACAACTAAACAAATCACAGTTG
GAACCTGCCAGTTGGTGCATCGACAGAAATAAGACCAACAATAGTACCACTATGCTCCCTCTGCGCCTGTTCACTATTTCCCTCTTCTTTTCA
AGATCTGCCTTAATACAAC**ATG**

>**M.F12/1 | denovo HXK3 promoter**

GGTGGGTGGCAGCAGTGGTCACTCTCTTCTATTGTTCTCGTCTCGGACTGGGCAAAAGAGAGGGACTCTCGCCAATATCCAACACCAACCAATTCC
AATTACAATTTCTATAATGTTGGTCTCTCTGACTCTGCTTTGGTCAAATCCAACCCAGACTCAAATCCAGCCCACAACTGGCCTCAAACCTGGGCTGGAC
CGAAACTCACAAATCGGGCTATAATGCACATGACCGTGGCCTACCAACTACACATCTATCCATGTAGAGAGAGAGAGAGAGAGAGAGAGGGTAGAATAGG
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ACAACAAATAACATCTAACAAACCTAGCCCAGCCCTGAACCCAGCTTAATTACCATCGTATTAAAACGAATGGAATACAACAATGTTGCAATCAATTGAG
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CAATCAAAAACAATAAAAAGCAAAATACAAAACAACGGGAAGGTAACGATGACTAATGAACGGCGCATAAAAAAATGATGAGTTAATTCCAGTTAGCTGTT
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GGAACCTGCCAGTTGGTGCATCGACAGAAATAAGACCAACAATAGTACCGCTATGCTCCCTCTGTCCTGTTCACTATTTCCCTCTTCTTTTCA
AGATCTGCCTTAATACAAC**ATG**

>**M.2624 | denovo HXK3 promoter**

CTTATGGTCAAATCTAACCCAGACTCAAATCCAGCCCACAAACTTGGGCTGGGCCGAAACTCACAAATCAGACTCGTGGGCCCTCCAACACTACACATCCATCGTAGG
GAAAGAGAGAGGGAGAGAGCAACCACAGCACATCGCATGGACAGAAAGAGCAATGGTATGTGGCATGCCCTTCTATCAAGTCCAAAGTAATCAATACAGAAA
ATGCCAATCCAACATGGTAGGATGGGACAACACTTAAAGTTGATGAAAGTCTCAGAATTAAACCAAGAATCATTAAAAGGATTAAGGATAAGAATTATC
CATCTTCATCATCAATCATTACACATTAACAACAAATAACATCTTAATGACCTAGCCCAGCCCTATAGATCGATGATTGTATAAAAGGAATTGCCGCTGCTGCT
GGCGCTCCAAGCATATGAATATTACTTGTTTATTGGTAACTATTGATATACTTAAACGAATAGAAAAACAACGACATGTTGCAATCAGTTGAG
CTATAACAACACAAAAGCAAAACCACAGATCTCTCTTGTGCTCCATCGTATTGTCGCCAATCCAACCTTGTCCAACCCCTCAGCTTGTACCGAGTCGAAA
AACAAATAACACAAAACAATGGCACTGGTAACAATGACTAATCAACGGCGCATAAAAACCGATGACTAACGAACAGTTAATTCCAGTTAGCTGTTAATG
CTGTCGTCAGCTACCCAAAACCACGTCGTTGCTTCTGAGCATTTCGACTTTTATTATAAAATTAAATGCAAGAACCTAAACAAATCATAGT
TGGGAACCTTGCCAATTGGTGCATCCACAGAAATAAGACCAACAATAGTACCACTATGCTCCCTCTGTCCTGTTCACTATTTCCCTCTTCTTTTAAT
AGATCTGCCTTAATACAAC**ATG**

Figure S2. Nucleotide sequences of *Prunus HXK3* promoters. 992 bp upstream regions of the transcription starting sites of the *HXK3* genes.

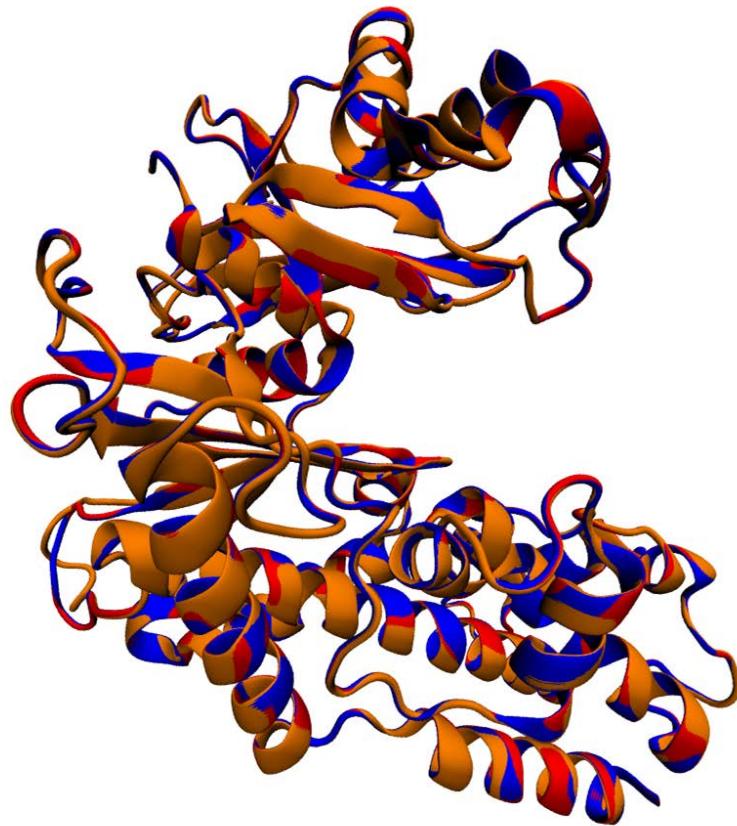


Figure S3. Protein modeling of HXK3 proteins. Comparison through a Richardson diagram of the structural models of the HXK3 proteins of the Mariana 2624 (red), Mazzard F12/1 (orange) and *Prunus persica* (blue).

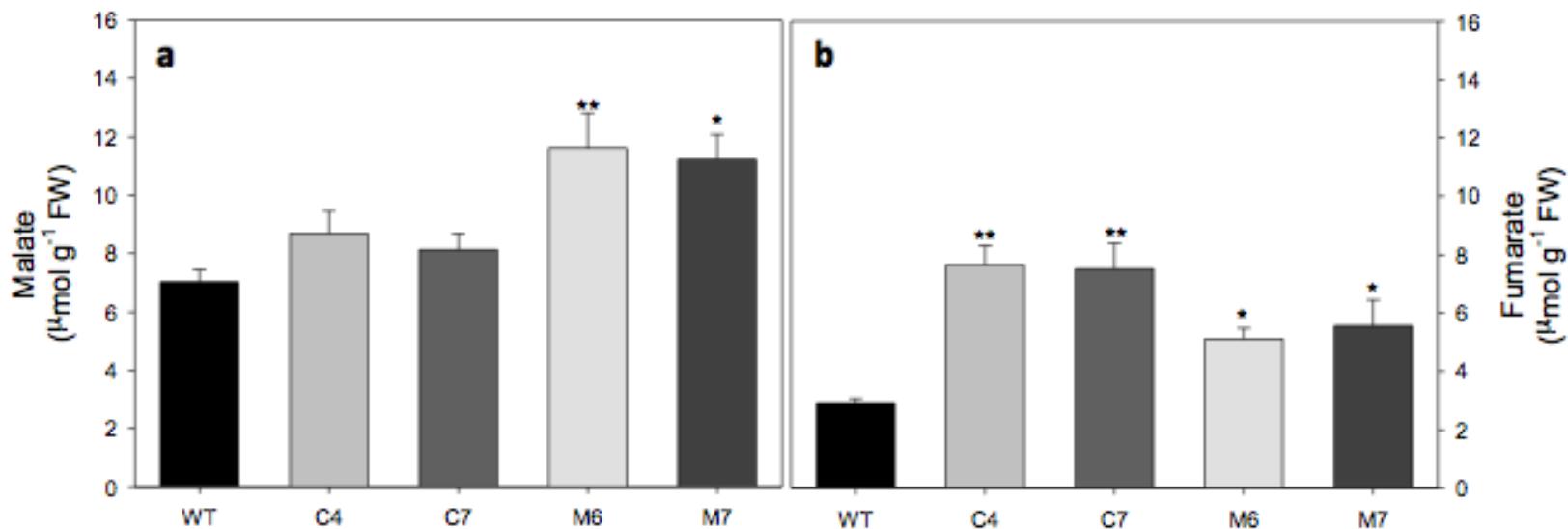


Figure S4. Organic acids content in *HXK3* transgenic lines of *Arabidopsis thaliana* plant growing under optimal condition. a Malate and **b** Fumarate content in *A. thaliana* leaves. Values are presented as means \pm SE (n = 5). Asterisks indicate that the values from lines were determined by Student's t test to be significantly different (** (P<0.01) and * (P<0.05)) from the wild-type .

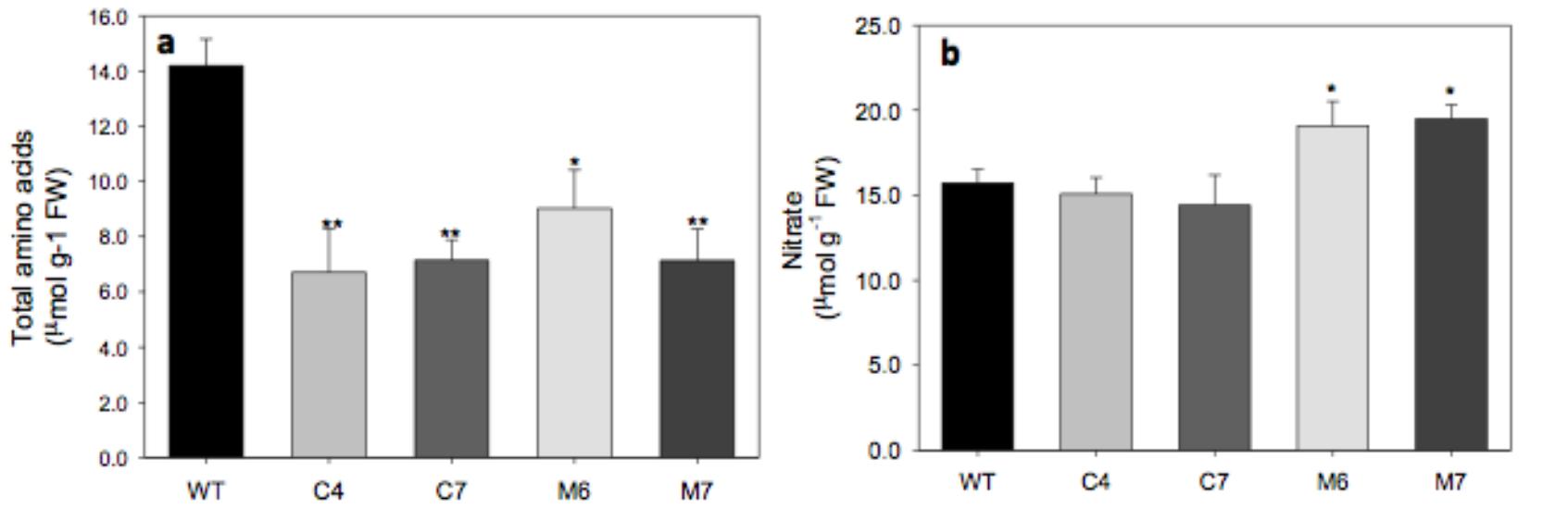


Figure S5. Nitrogen compounds in *HXK3* transgenic lines of *Arabidopsis thaliana* plant growing under optimal condition. a Total amino acids and **b** nitrate content in *A. thaliana* leaves. Values are presented as means \pm SE ($n = 5$). Asterisks indicate that the values from lines were determined by Student's t test to be significantly different (** ($P < 0.01$) and * ($P < 0.05$)) from the wild-type.

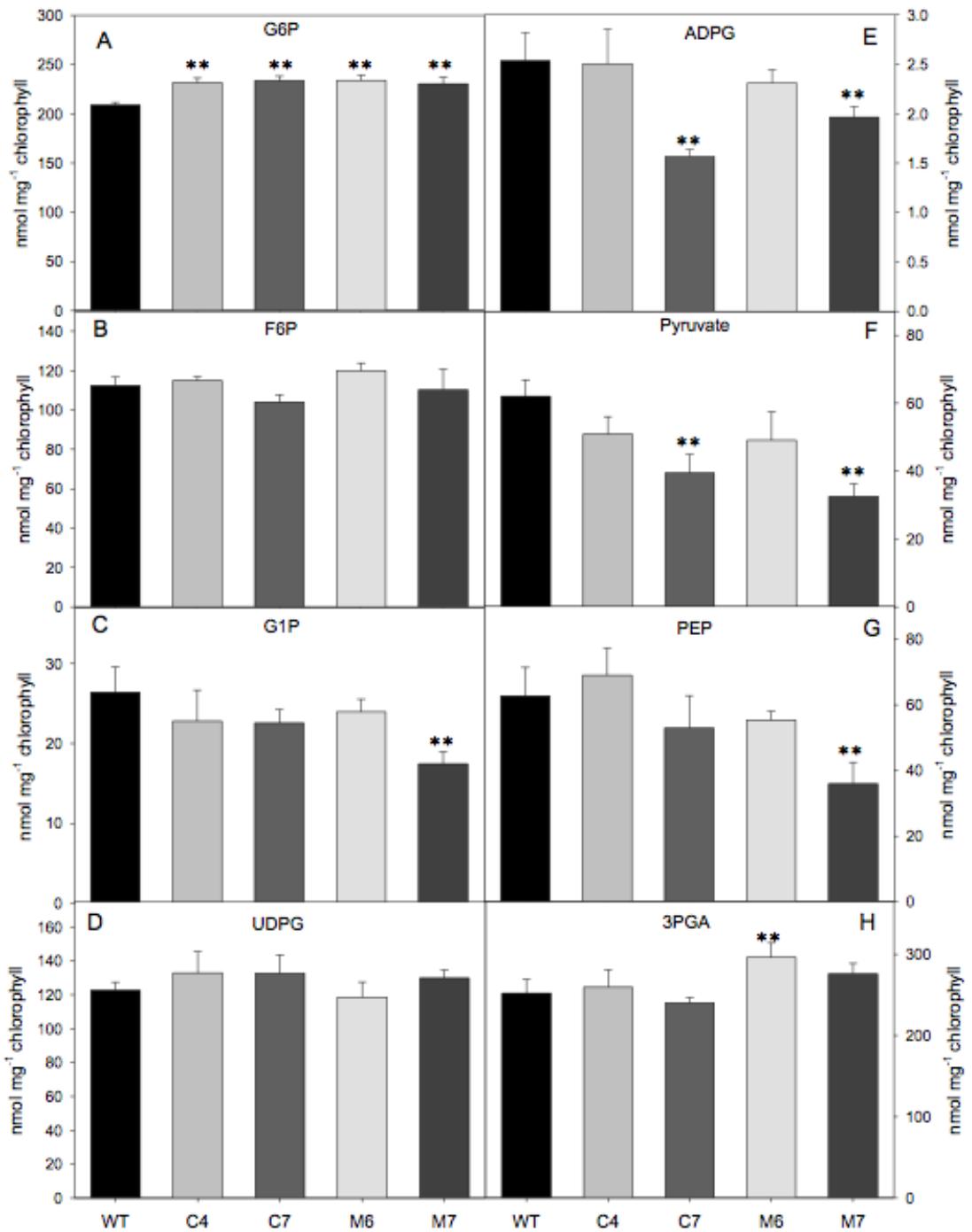


Figure S6. Phosphorylated metabolites and glycolytic intermediates content in *HXK3* transgenic lines of *Arabidopsis thaliana* plant growing under optimal condition. **a** Glucose-6-phosphate, **b** Fructose-6-phosphate, **c** Glucose-1-phosphate, **d** UDP-glucose, **e** ADP-glucose, **f** Pyruvate, **g** Phosphoenolpyruvate and **h** 3-phosphoglycerate content in *A. thaliana* leaves. Values are presented as means \pm SE ($n = 5$). Asterisks indicate that the values from lines were determined by Student's t test to be significantly different (** ($P < 0.01$) and * ($P < 0.05$)) from the wild-type