

Title:

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

Jorge Pérez-Díaz¹, Willian Batista-Silva², Rubén Almada³, David B. Medeiros², Stéphanie Arrivault⁴, Francisco Correa¹, Adriana Bastías⁵, Pamela Rojas¹, María Francisca Beltrán¹, María Francisca Pozo¹, Wagner L. Araújo² and Boris Sagredo¹.

¹Instituto de Investigaciones Agropecuarias CRI Rayentué, Av. Salamanca s/n, Sector Los Choapiños, Rengo, Chile

²Max Planck Partner Group at the Departamento de Biología Vegetal, Universidade Federal de Viçosa, 36570-900, Viçosa-MG, Brazil

³Centro de Estudios Avanzados en Fruticultura, CEAF, Camino Las Parcelas 882, Sector Los Choapiños, Rengo, Chile

⁴Max Planck Institute of Molecular Plant Physiology, Am Mühlenberg 1, D-14476 Potsdam-Golm, Germany

⁵Universidad Autónoma de Chile. Av. Pedro de Valdivia 425, Providencia, Santiago, Chile

| Name | Len | cTP | mTP | SP | other | Loc |
|--|-----|--------------|--------|-----|--------|-----|
| AtHXK3 (<i>Arabidopsis thaliana</i>) | 493 | 0.992 | 0.0006 | 0.0 | 0.0016 | C |
| HXK3 (<i>P. persica</i>) | 494 | 0.973 | 0.0015 | 0.0 | 0.0238 | C |
| HXK3 M.F12/1 (<i>P. avium</i>) | 494 | 0.974 | 0.0065 | 0.0 | 0.0181 | C |
| HXK3 M.2624 (<i>P. cerasifera</i> x <i>P. munsoniana</i>) | 494 | 0.972 | 0.0017 | 0.0 | 0.0238 | C |

Table S1. Subcellular localization prediction of the HXKs proteins in *A. thaliana*, *P. persica*, and *Prunus* rootstock genotypes. The highest TargetP scores are written in bold font. cTP: chloroplast transit peptide score; mTP: mitochondrial targeting peptide score; SP: Signal peptide.

| Metabolites | WT | C4 | C7 | M6 | M7 |
|-----------------------------|-------------|--------------------|--------------------|--------------------|--------------------|
| <i>Alanine</i> | 1.00 ± 0.14 | 0.76 ± 0.37 | 0.67 ± 0.21 | 1.14 ± 0.46 | 1.83 ± 0.11 |
| <i>Asparagine</i> | 1.00 ± 0.35 | 1.99 ± 0.17 | 0.98 ± 0.43 | 0.97 ± 0.71 | 0.80 ± 0.38 |
| <i>Aspartate</i> | 1.00 ± 0.18 | 0.80 ± 0.19 | 0.93 ± 0.14 | 1.01 ± 0.28 | 1.13 ± 0.24 |
| <i>Glutamate</i> | 1.00 ± 0.15 | 0.86 ± 0.37 | 0.91 ± 0.24 | 1.12 ± 0.42 | 0.88 ± 0.24 |
| <i>Glutamine</i> | 1.00 ± 0.13 | 1.46 ± 0.61 | 0.56 ± 0.09 | 1.34 ± 0.16 | 0.91 ± 0.29 |
| <i>Glycine</i> | 1.00 ± 0.23 | 0.96 ± 0.99 | 0.60 ± 0.25 | 0.94 ± 0.82 | 0.90 ± 0.46 |
| <i>Isoleucine</i> | 1.00 ± 0.12 | 1.22 ± 0.12 | 1.56 ± 0.07 | 1.15 ± 0.11 | 1.24 ± 0.22 |
| <i>Methionine</i> | 1.00 ± 0.13 | 0.91 ± 0.13 | 0.75 ± 0.18 | 1.10 ± 0.50 | 0.87 ± 0.17 |
| <i>Ornithine</i> | 1.00 ± 0.15 | 1.21 ± 0.28 | 1.09 ± 0.12 | 1.09 ± 0.28 | 0.59 ± 0.02 |
| <i>Proline</i> | 1.00 ± 0.14 | 0.80 ± 0.26 | 0.69 ± 0.07 | 0.97 ± 0.72 | 0.74 ± 0.27 |
| <i>Pyroglutamate</i> | 1.00 ± 0.24 | 0.96 ± 0.93 | 0.68 ± 0.35 | 0.88 ± 0.49 | 0.97 ± 0.27 |
| <i>Serine</i> | 1.00 ± 0.23 | 0.77 ± 0.22 | 0.83 ± 0.25 | 1.07 ± 0.22 | 0.78 ± 0.29 |
| <i>Threonine</i> | 1.00 ± 0.19 | 0.80 ± 0.10 | 0.89 ± 0.14 | 1.20 ± 0.34 | 0.88 ± 0.28 |
| <i>Valine</i> | 1.00 ± 0.17 | 0.85 ± 0.20 | 0.87 ± 0.21 | 1.27 ± 0.24 | 0.80 ± 0.23 |
| <i>Ascorbate</i> | 1.00 ± 0.10 | 0.98 ± 0.20 | 1.10 ± 0.34 | 1.46 ± 0.18 | 0.80 ± 0.34 |
| <i>Citrate</i> | 1.00 ± 0.23 | 2.48 ± 0.20 | 2.40 ± 0.28 | 2.74 ± 0.23 | 2.28 ± 0.38 |
| <i>Dehydroascorbate</i> | 1.00 ± 0.32 | 0.77 ± 0.20 | 0.95 ± 0.29 | 1.16 ± 0.25 | 0.66 ± 0.23 |
| <i>Fumarate</i> | 1.00 ± 0.11 | 1.82 ± 0.18 | 1.76 ± 0.02 | 1.35 ± 0.24 | 1.18 ± 0.21 |
| <i>Glycerate</i> | 1.00 ± 0.14 | 0.64 ± 0.26 | 0.70 ± 0.08 | 0.92 ± 0.34 | 0.76 ± 0.27 |
| <i>Isocitrate</i> | 1.00 ± 0.23 | 1.03 ± 0.80 | 0.69 ± 0.24 | 1.40 ± 0.57 | 0.73 ± 0.12 |
| <i>Lactate</i> | 1.00 ± 0.17 | 0.81 ± 0.04 | 0.74 ± 0.12 | 1.37 ± 0.22 | 1.33 ± 0.46 |
| <i>Malate</i> | 1.00 ± 0.09 | 1.44 ± 0.51 | 1.36 ± 0.50 | 1.74 ± 0.15 | 1.62 ± 0.15 |
| <i>Succinate</i> | 1.00 ± 0.06 | 1.17 ± 0.23 | 1.19 ± 0.44 | 1.94 ± 0.15 | 1.17 ± 0.27 |
| <i>Threonate</i> | 1.00 ± 0.10 | 0.95 ± 0.11 | 1.15 ± 0.32 | 1.48 ± 0.55 | 1.02 ± 0.30 |
| <i>Allose</i> | 1.00 ± 0.07 | 1.06 ± 0.17 | 1.07 ± 0.25 | 1.25 ± 0.20 | 1.35 ± 0.18 |
| <i>Fructose</i> | 1.00 ± 0.31 | 1.85 ± 0.38 | 1.69 ± 0.23 | 0.65 ± 0.51 | 0.40 ± 0.14 |
| <i>Fructose-6-phosphate</i> | 1.00 ± 0.14 | 1.10 ± 0.14 | 1.31 ± 0.28 | 1.61 ± 0.23 | 1.59 ± 0.10 |
| <i>Galactinol</i> | 1.00 ± 0.09 | 3.32 ± 1.48 | 2.66 ± 1.23 | 5.23 ± 4.95 | 1.35 ± 1.16 |
| <i>Galactose</i> | 1.00 ± 0.21 | 0.30 ± 0.39 | 0.39 ± 0.04 | 0.73 ± 0.15 | 0.27 ± 0.11 |
| <i>Glucose</i> | 1.00 ± 0.39 | 0.30 ± 0.04 | 0.39 ± 0.15 | 0.43 ± 0.12 | 0.27 ± 0.16 |
| <i>Lyxose</i> | 1.00 ± 0.16 | 0.78 ± 0.20 | 0.85 ± 0.22 | 1.07 ± 0.40 | 0.53 ± 0.08 |
| <i>Mannose</i> | 1.00 ± 0.07 | 0.96 ± 0.08 | 1.06 ± 0.25 | 1.24 ± 0.20 | 3.45 ± 4.15 |
| <i>Psicose</i> | 1.00 ± 0.31 | 0.85 ± 0.38 | 0.69 ± 0.23 | 1.32 ± 0.51 | 0.40 ± 0.34 |
| <i>Ribose</i> | 1.00 ± 0.06 | 0.81 ± 0.12 | 0.80 ± 0.10 | 1.36 ± 0.25 | 1.03 ± 0.09 |
| <i>Ribulose-5-phosphate</i> | 1.00 ± 0.24 | 0.82 ± 0.17 | 0.87 ± 0.21 | 1.25 ± 0.39 | 0.86 ± 0.14 |
| <i>Sorbitol</i> | 1.00 ± 0.25 | 1.02 ± 0.45 | 0.89 ± 0.41 | 1.39 ± 0.35 | 0.42 ± 0.31 |
| <i>Sucrose</i> | 1.00 ± 0.16 | 0.98 ± 0.28 | 0.85 ± 0.21 | 0.73 ± 0.29 | 0.76 ± 0.34 |
| <i>Talose</i> | 1.00 ± 0.24 | 1.01 ± 0.43 | 0.88 ± 0.39 | 1.39 ± 0.22 | 0.43 ± 0.31 |
| <i>Xylulose</i> | 1.00 ± 0.11 | 0.77 ± 0.18 | 0.82 ± 0.21 | 1.16 ± 0.31 | 1.04 ± 0.21 |
| <i>Glycerol</i> | 1.00 ± 0.13 | 0.64 ± 0.03 | 0.51 ± 0.17 | 1.03 ± 0.23 | 0.67 ± 0.17 |
| <i>Guanidine</i> | 1.00 ± 0.17 | 1.02 ± 0.57 | 0.74 ± 0.25 | 1.66 ± 0.38 | 0.77 ± 0.23 |
| <i>Putrescine</i> | 1.00 ± 0.17 | 0.59 ± 0.10 | 0.43 ± 0.12 | 0.65 ± 0.20 | 0.61 ± 0.14 |

Table S2. Relative metabolite content in leaves (4-week-old plants) of WT and *HXK3* transgenic lines. Data are normalized with respect to the mean relative amount calculated for WT (to allow statistical assessment, individual plants from this set were normalized in the same way). Data are presented as means ± SE (n = 5). Values set in bold in *HXK3* transgenic lines plants were determined by the Student's t test to be significantly different (P < 0.05) from WT.