

SUPPLEMENTAL TABLES

Supplemental TABLE S2

*Sequence of non-modified peptides showing quantitative variations according to treatments*

Name	Aquaporin	Peptide sequence
PIP11 12 (15-29)	PIP1;1, 1;2	<sup>15</sup> FPERQPIGTSAQSDK <sub>29</sub>
PIP13 (15-29)	PIP1;3	<sup>15</sup> FPERQPIGTSAQTDK <sub>29</sub>
PIP11 (loopC)	PIP1;1	<sup>157</sup> QYQALGGGANTVAHGYTK <sub>174</sub>
PIP12 (loopC)	PIP1;2	<sup>157</sup> QYQALGGGANTIAHGYTK <sub>174</sub>
PIP13 (loopC)	PIP1;3	<sup>152</sup> GFQPNPYQTLGGGANTVAHGYTK <sub>174</sub>
PIP14 (loopC)	PIP1;4	<sup>153</sup> GFQPTPYQTLGGGANTVAHGYTK <sub>175</sub>
PIP21 (4-16)	PIP2;1	<sup>4</sup> DVEAVPEGGFQTR <sub>16</sub>
PIP21 (17-33)	PIP2;1	<sup>17</sup> DYQDPPPAPFIDGAEK <sub>33</sub>
PIP22 (4-14)	PIP2;2	<sup>4</sup> DVEGPEGFQTR <sub>14</sub>
PIP22 (15-32)	PIP2;2	<sup>15</sup> DYEDPPPTFFDADELTK <sub>32</sub>
PIP24 (4-16)	PIP2;4	<sup>4</sup> DLDVNESGPPAAR <sub>16</sub>
PIP27 (16-32)	PIP2;7	<sup>16</sup> DYVDPPPAPLLDMGELK <sub>32</sub>
PIP21 (loopC) 24 (loopC-a)	PIP2;1, 2;4	<sup>145</sup> AFQSSYYTR <sub>153</sub>
PIP22 (loopC)	PIP2;2	<sup>143</sup> AFQSSYYDR <sub>151</sub>
PIP24 (loopC)	PIP2;4	<sup>154</sup> YGGGANELADGYNK <sub>167</sub>
PIP27 (loopC)	PIP2;7	<sup>142</sup> TPYNTLGGGANTVADGYSK <sub>160</sub>
PIP11-14 (H4)	PIP1;1-1;4	<sup>173</sup> TKGSGLGAEIIGTF <sub>186</sub>
PIP1s & PIP25 (H5/loopE)	PIP1;1-1;5, 2;5	<sup>225</sup> ATIPITGTGINPARSL <sub>240</sub>
PIP11 13 14 15 25 (loopE)	PIP1;1, 1;3-1;5, 2;5	<sup>241</sup> GAAIY <sub>246</sub>
PIP21 22 (loopE)	PIP2;1, PIP2;2	<sup>232</sup> SFGAAVIYNK <sub>241</sub>
PIP12 (loopE)	PIP1;2	<sup>241</sup> GAAIIF <sub>246</sub>
PIP13 (7-27)	PIP1;3	<sup>7</sup> DVRVGANKFPERQPIGTSAQT <sub>27</sub>
PIP14 (7-28)	PIP1;4	<sup>7</sup> DVRVGANKFPERQPIGTSAQST <sub>28</sub>
PIP14 (7-30)	PIP1;4	<sup>7</sup> DVRVGANKFPERQPIGTSAQSTDK <sub>30</sub>
PIP15 (7-30)	PIP1;5	<sup>7</sup> DVNVGANKFPERQPIGTAAQTESK <sub>30</sub>
PIP15 (H4)	PIP1;5	<sup>174</sup> TKGSGLGAEIVGTF <sub>187</sub>
PIP21-25 (H1)	PIP2;1-2;5	<sup>58</sup> TVIGY <sub>62</sub>
PIP21 22 (H6)	PIP2;1-2;2	<sup>257</sup> IGAAIAAF <sub>264</sub>
P22 23 (17-24)	PIP2;2-2;3	<sup>17</sup> DPPPTPFF <sub>24</sub>
PIP24 (H6)	PIP2;4	<sup>252</sup> VGPMIGAAAAAFY <sub>264</sub>
PIP27 (loopC-H4)	PIP2;7	<sup>158</sup> SKGTALGAEIIGTF <sub>171</sub>
TIP11 (122-133)	TIP1;1	<sup>122</sup> KFATGGLAVPAF <sub>133</sub>
TIP11 (161-181)	TIP1;1	<sup>161</sup> ATAIDPKNGSLGTIPIAIGF <sub>181</sub>
TIP11 12 (165-181)	TIP1;1-1;2	<sup>165</sup> DPKNGSLGTIPIAIGF <sub>181</sub>
TIP11 12 (182-193)	TIP1;1-1;2	<sup>182</sup> IVGANILAGGAF <sub>193</sub>
TIP11 12 (194-204)	TIP1;1-1;2	<sup>194</sup> SGASMNPVAVAF <sub>204</sub>
TIP11 (238-249)	TIP1;1	<sup>238</sup> FINTTHEQLPTT <sub>249</sub>
TIP12 (2-16)	TIP1;2	<sup>2</sup> PTRNIAIGGVQEEVY <sub>16</sub>
TIP12 (37-45)	TIP1;2	<sup>37</sup> AGSGSGIAF <sub>45</sub>

TIP12 (46-64)	TIP1;2	<sup>46</sup> NKITDNGATTPSGLVAAAL <sup>64</sup>
TIP12 (162-182)	TIP1;2	<sup>162</sup> ATAVDPKNGSLGTIPIAIGF <sup>182</sup>
TIP12 (236-240)	TIP1;2	<sup>236</sup> DFVFI <sup>240</sup>

The table lists non-modified aquaporin peptides showing quantitative variations according to treatments. The first column indicates the peptide names, as referred to Fig. 4. The second and third columns indicate the corresponding aquaporin name and peptide sequence, respectively. Data were interrogated with Mascot 2.2.07 *via* ProteinScape software (Bruker Daltonics) against Tair10. The interrogation parameters were set to accept one putative missed cleavage, a 15 ppm mass range for the parent peptide, and a 0.05 Da variation for MS/MS fragment. Cystein carbamidomethylation was selected as a fixed modification.

SUPPLEMENTAL TABLE S4

*Aquaporin quantitative behavior according to treatments*

treatment	Quantitative aquaporin behavior
NaCl <sub>tmax</sub>	↓PIP1, ↓PIP2, ↓PIP <sub>p</sub> , ↓TIP
man <sub>tmax</sub>	↑PIP1, ↑PIP <sub>p</sub>
H <sub>2</sub> O <sub>2</sub> <sub>tmax</sub>	↑PIP1, ↑PIP <sub>p</sub>
NO <sub>tmax</sub>	↓PIP1, ↓PIP <sub>p</sub>
dark	↑PIP <sub>p</sub>
dark <sub>suc</sub>	↑PIP <sub>p</sub>
dark <sub>man</sub>	↑PIP <sub>p</sub>
P <sub>starv</sub>	↓PIP <sub>p</sub>
P <sub>resup</sub>	↓PIP1, ↓PIP <sub>p</sub> , ↑TIP
N <sub>starv</sub>	↓TIP, ↓PIP2*

“↑” and “↓” refer to increased and decreased abundance, respectively, as described in Figs.

4 and 5.

“PIP<sub>p</sub>”: phosphorylated form of PIP2 and PIP1

\*: observed in ELISA

## LEGENDS TO SUPPLEMENTAL FIGURES

Supplemental FIG. S1. **Time-, dose-dependency and specificity of DEA-NO effects on root water transport.** *A*, Time-dependent variations of pressure-induced sap flow,  $J_v$  (expressed as a percentage of the mean value in the 10 min preceding treatment with DEA-NO) in roots bathed in a solution in the absence (control) or in the presence of 50  $\mu\text{M}$ , 100  $\mu\text{M}$ , 250  $\mu\text{M}$  or 500  $\mu\text{M}$  DEA-NO. *B*, typical long-term kinetic recording of  $J_v$  after addition of 100  $\mu\text{M}$  DEA-NO in the root medium (arrow), showing a spontaneous recovery of  $J_v$  after 25 min.  $J_v$  is expressed as a percentage of the initial  $J_v$  ( $9.2 \cdot 10^{-2} \mu\text{l}\cdot\text{sec}^{-1}$ ). *C*, specificity of DEA-NO effects. Plants were treated for 20 min with 100  $\mu\text{M}$  DEA-NO, for 10 min with 100  $\mu\text{M}$  cPTIO, or pretreated for 10 min with 100  $\mu\text{M}$  cPTIO prior to incubation for 20 min with 100  $\mu\text{M}$  DEA-NO. Data represent the average percentage of  $J_v$  inhibition ( $\pm\text{SE}$ ) in  $n = 3-4$  plants from 3 independent cultures.

Supplemental FIG. S2. **Root and shoot dry weights and hydraulic conductance ( $L_0$ ) in phosphate (A, B, C) or nitrate (D, E, F) starved plants.** Plants were cultivated in control conditions (control) or in the absence of phosphate or nitrate for 6 days (starvation). Data represent the average value ( $\pm\text{SE}$ ) of  $n \geq 11$  plants from 3 independent cultures.

Supplemental FIG. S3. **MS/MS spectra of modified peptides.** The peptide name refers to the name in Table 1.

Supplemental FIG. S4. **Extension of the isotopic MS profile of deamidated parent ions.** The peptide name (which refers to the name in Table 1),  $m/z$ , the charge and the score are indicated. The arrow indicates the monoisotopic mass.

Supplemental FIG. S5. **Abundance of PIP and TIP peptides according to treatments.** *A, B*, Heat maps according to treatments of the abundance of non-modifiable PIP1(*A*) and PIP2 (*B*) peptides. Peptides were quantified and clustered as described in Fig. 4. Corresponding peptide sequences are described in supplemental Table S1. *C*, ELISA assays using anti-PIP1 and anti-PIP2 antibodies on microsomal proteins extracted from roots of control and treated plants ( $\text{NO}_{\text{tmax}}$ ,  $\text{P}_{\text{starv}}$ ,  $\text{N}_{\text{starv}}$ ). Values for each treated sample were compared to, and expressed as a percentage of the control value. Data represent the average value ( $\pm$ SE) of  $n = 5$  plants from 3 independent cultures. Letters and asterisks above bars indicate statistically significant ( $p < 0.05$ ) values between points. *D*, Heat map according to treatments of the abundance of non-modifiable TIP peptides. Same procedures and convention as in *A*.

Supplemental FIG. S6. **Sequence alignment of the C-terminal part of five *At*PIP2s (*A*) and of the loopC of all 13 *At*PIPs (*B*).** Bold red characters indicate two conserved phosphorylatable Ser residues (*A*) and a conserved Asn residue (*B*).

Supplemental FIG. S7. **Modelling of *At*PIP2;7 structure showing the vicinity of deamidated Asn152 with pore-forming residue Arg224.** Modeling was performed with SWISS-MODEL (<http://swissmodel.expasy.org/>) a modeling server, using the structure *So*PIP2; 1 in closed conformation (PDB code: 1Z98) (56).