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1 **Short title:** OsK5.2 outward Shaker channel in rice stomata

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6
7 **A dual role for the OsK5.2 ion channel in stomatal movements and K⁺ loading into xylem**
8 **sap**

9
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18
19 **One sentence summary:** The rice K⁺ channel OsK5.2 is necessary for rapid stomatal closure
20 and contributes to shoot K⁺ supply from the root, thus highlighting the differing functional
21 distribution of Shaker channels compared to Arabidopsis

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25 **Footnotes:**

26 **List of author contributions:** A.-A.V., H.S. and T.H.N. conceived the original research plans;
27 A.-A.V, E.G. and M.R.G.R. supervised the experiments; D.M., M.R.G.R., S.H. and T.H.N.
28 performed most of the experiments; C.C. and R.M. provided technical assistance to T.H.N.;
29 T.H.N. designed the experiments and analyzed the data; A.-A.V., H.S. and T.H.N. conceived the
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54 **ABSTRACT**

55 The roles of potassium channels from the Shaker family in stomatal movements have been
56 investigated by reverse genetics analyses in Arabidopsis, but corresponding information is
57 lacking outside this model species. Rice and other cereals possess stomata that are more complex
58 than those of Arabidopsis. We examined the role of the outward Shaker K⁺ channel gene *OsK5.2*.
59 Expression of the *OsK5.2* gene (*GUS* reporter strategy) was observed in the whole stomatal
60 complex (guard cells and subsidiary cells), in the root vasculature and root cortex. In stomata,
61 loss of *OsK5.2* functional expression resulted in lack of time-dependent outward potassium
62 currents in guard cells, higher rates of water loss through transpiration, and severe slowdown of
63 stomatal closure. In line with the expression of *OsK5.2* in the plant vasculature, mutant plants
64 displayed a reduced K⁺ translocation from the root system towards the leaves via the xylem. The
65 comparison between rice and Arabidopsis show that despite the strong conservation of Shaker
66 family in plants, substantial differences can exist between the physiological roles of seemingly
67 orthologous genes, as xylem loading depends on SKOR and stomatal closure on GORK in
68 Arabidopsis, whereas both functions are executed by the single *OsK5.2* Shaker in rice.

69

70 **INTRODUCTION**

71 Since a waxy cuticle covers outer leaf tissues, water vapor diffusion into the atmosphere
72 occurs mainly through the stomatal pores at the leaf surface. The size of the stomatal aperture is
73 tightly regulated to optimize gas exchanges between the leaf inner tissues and the atmosphere,
74 including CO₂ intake for photosynthesis and water loss by transpiration (Lawson and Blatt,
75 2014). This is achieved by fine tuning of the turgor pressure of the two guard cells which
76 surround the stomatal pore, and involves a complex coordinated activity of transport systems at
77 the guard cell plasma membrane and vacuolar membrane (Hedrich, 2012; Chen et al., 2012; Hills
78 et al., 2012; Kollist et al., 2014). This control also affects long distance transport of mineral

79 nutrients from the roots, which take up these nutrients, to the aerial parts, to support plant growth
80 (Marschner et al., 1996).

81 Potassium ion (K^+), as a major inorganic constituent of the plant cells and the most abundant
82 cation in the cytosol, is an essential macronutrient for growth and development. It is involved in
83 various functions including electrical neutralization of negative charges, control of cell
84 membrane polarization and osmoregulation (Clarkson and Hanson, 1980; Leigh and Wyn Jones,
85 1984). K^+ is thus the main cation absorbed by the roots and circulating within the plant at the
86 cellular or long distance levels. In guard cells, it is well known as a major contributor, with Cl^- ,
87 NO_3^- and malate, to the osmolarity (Raschke and Schnabl, 1978; Willner and Fricker, 1996).
88 Stomatal opening is initiated by activation of plasma membrane proton pumps in guard cells,
89 which promotes K^+ influx through voltage-gated inward K^+ channels, as well as anion uptake
90 through H^+ -anion symporters (Blatt, 1987a; Schroeder et al., 1987; Roelfsema and Prins, 1997;
91 Talbott and Zeiger, 1998; Guo et al., 2003; Jezek and Blatt, 2017). Conversely, stomatal closure
92 requires inhibition of proton pumping at the guard cell membrane and activation of both anion
93 channels and voltage-gated outward K^+ channels.

94 The molecular mechanisms responsible for inward and outward K^+ fluxes across the plasma
95 membrane have been extensively investigated in Arabidopsis. Shaker channel subunits, present as
96 a 9-member family in Arabidopsis, have been shown to form the major pathways for these fluxes
97 throughout the plant (Véry and Sentenac, 2003). In the Arabidopsis model species, four genes
98 encoding Shaker channel subunits have been identified as playing a major role in root to shoot K^+
99 translocation and in stomatal movements. The SKOR subunit, which is expressed in root
100 pericycle and xylem parenchyma, forms outwardly-rectifying channels involved in K^+ secretion
101 into the xylem sap (Gaymard et al., 1998). In stomata, the inward Shaker channel subunits KAT1
102 and KAT2 are involved in guard cell K^+ uptake, and the outward Shaker channel GORK
103 mediates guard cell K^+ release (Ache et al., 2000; Pilot et al., 2001; Szyroki et al., 2001; Hosy et
104 al., 2003; Lebaudy et al., 2008). Whereas these Shaker subunits have been deeply characterized
105 in Arabidopsis and the Shaker family, as a whole, can be considered as the best characterized
106 family of plant membrane transport systems, little information at the molecular genetic level is
107 yet available on this family outside Arabidopsis.

108 The stomatal complex in rice (*Oryza sativa* L.), the present model cereal, is very different
109 from that of Arabidopsis (Itoh et al., 2005; Franks and Farquhar, 2007; Roelfsema and Hedrich,

110 2009). In rice like in other cereals, it comprises two subsidiary cells in addition to the two guard
111 cells. Substantial differences may thus exist between rice and Arabidopsis in stomatal functioning
112 (Mumm et al., 2011). Rice possesses two putative outward Shaker channel subunits (Pilot et al.,
113 2003; Véry et al., 2014), named *OsK5.1* (or *OsSKOR*) and *OsK5.2* (or *OsGORK*) (Pilot et al.,
114 2003; Kim et al., 2015). *OsK5.1* was reported to be mainly expressed in root vasculature, just as
115 *SKOR* in Arabidopsis (Kim et al., 2015). In contrast, *OsK5.2* was found to be expressed both in
116 roots and shoots (Kim et al., 2015). The expression pattern of this gene at the tissue level has,
117 however, not been described. Here, we investigate the expression pattern and role of this rice
118 outward Shaker gene. *OsK5.2* is shown to play two important roles in rice plants: it mediates K^+
119 translocation into the xylem sap towards the shoots, and it is involved in K^+ release from guard
120 cells and stomatal movements.

121

122

123 RESULTS

124 The outward Shaker channel subfamily in Arabidopsis and rice

125 The Shaker group 5, which consists of the outward Shaker subunits, usually comprises 2 or 3
126 members in the plant genomes analyzed so far (Pilot et al., 2003; Véry et al., 2014). Figure 1
127 illustrates the phylogenetic relationships between the members of the Shaker group 5 present in
128 Arabidopsis and in 3 monocots, rice, maize (*Zea mays*) and *Brachypodium distachyon*. Based on
129 this phylogenetic tree, the rice OsK5.1 and OsK5.2 subunits appeared to belong to two different
130 subgroups within the Shaker group 5. The distance between these subgroups was larger than that
131 between GORK and SKOR from Arabidopsis.

132

133 Expression pattern of *OsK5.2*

134 Transgenic rice plants expressing *GUS* under the control of the *OsK5.2* promoter region
135 (Fig. 2A) were generated to investigate the expression of this Shaker gene (Fig. 2, B-J). *GUS*
136 staining revealed that *OsK5.2* was expressed in vascular tissue of root (Fig. 2, E-G), like *OsK5.1*
137 (Kim et al., 2015), as well as in the shoot vascular tissue (Fig. 2, B, D). In addition, the *OsK5.2*
138 promoter was active in root cortical cells (except at the root tip) (Fig. 2, E-G). Weak *GUS*
139 staining was also observed in flowers, in the pollen sacs and grains (Fig. 2, H-J). Analysis of
140 *GUS* staining in leaf epidermis revealed expression of *OsK5.2* in stomata, the staining being
141 stronger in guard cells than in subsidiary cells (Fig. 2, B-D).

142 The following experiments were aimed at investigating the role of *OsK5.2* in K⁺ translocation
143 towards the shoots and in stomatal movements.

144

145 Production of *osk5.2* loss-of-function mutant plants

146 Genotyping experiments performed on the progeny of the mutant lines ASJA08 and ASHF06
147 (Supplemental Figure 1A) allowed us to identify *osk5.2* homozygous mutant plants (*Tos17*
148 insertion alleles), as well as plants without insertion in the *OsK5.2* gene, hereafter named wild-
149 type controls. The offspring of the selected plants (*osk5.2* mutants and wild-type controls) was
150 used for in planta experiments. RT-PCR experiments on total RNA prepared from the *osk5.2*

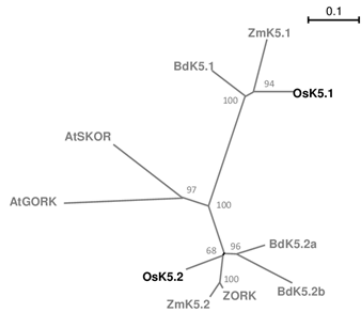


Figure 1. Phylogenetic analysis of outward Shaker channels in monocot species and *Arabidopsis thaliana*. The unrooted phylogenetic trees were generated with PhyML software (<http://www.atgc-montpellier.fr/phyml/binaries.php>) using the maximum-likelihood method and 1000 bootstrap replicates in Seaview application (<http://doua.prabi.fr/software/seaview>). The scale bar corresponds to a distance of 10 changes per 100 amino acid positions. The protein (GenBank) accession numbers are given in Supplemental Table S2. At, *Arabidopsis thaliana*; Bd, *Brachypodium distachyon*; Os, *Oryza sativa* (rice); Zm, *Zea mays* (maize).

151 mutants and the wild type control plants confirmed the loss of function of *OsK5.2* in the selected
 152 *osk5.2* homozygous mutant lines (Supplemental Figure 1B).

153

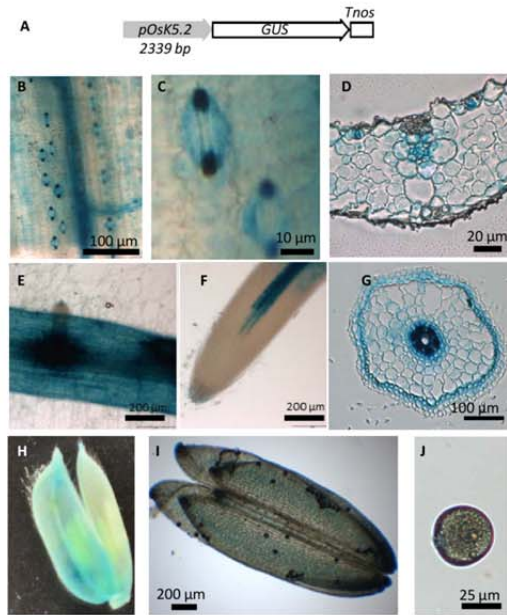


Figure 2. Expression pattern of *OsK5.2* outward Shaker channel gene in rice. A, *Promoter::GUS* construct (promoter region size: 2339 bp). B to J, GUS activity in transgenic rice plants, revealed by an overnight incubation in the presence of X-Gluc: leaf tissues (B), stomata (C), leaf cross-section (D), root (E), root tip (F), root cross-section (G), flower (H), anther (I), and pollen (J).

154 **Impaired K^+ transport into the xylem sap and K^+ accumulation in shoots in *osk5.2* mutant**
 155 **plants**

156 K^+ was assayed in roots and shoots of 5 week-old plants hydroponically grown on standard
157 Yoshida medium. Comparison of *osk5.2* mutant plants with the corresponding wild type plants
158 revealed that the absence of *OsK5.2* functional expression did not affect K^+ accumulation in
159 roots, but resulted in significantly reduced K^+ contents (by ~25%) in shoots (Fig. 3, A and B, left
160 panels). The K^+ concentration measurements, in xylem sap samples which were collected after
161 leaf excision, revealed lower values, by 30 to 40%, in the mutant plants (Fig. 3, A and B, right
162 panels). Thus, the whole set of data indicated that the absence of *OsK5.2* functional expression
163 resulted in a decrease in K^+ translocation from roots to shoots via the xylem sap.

164

165 **Increased transpirational water loss due to impaired stomatal movements in *osk5.2* mutant**
166 **plants**

167 Water consumption (essentially due to transpirational water loss) was monitored in *osk5.2*
168 mutant plants and the corresponding wild-type control plants hydroponically grown for 5 weeks
169 on Yoshida medium using a multi-potometer (Supplemental Figure S2). Both mutant lines
170 displayed larger steady state rates of transpirational water loss than the corresponding wild-type
171 plants in the light, by about 25% (Fig. 4).

172 The transpiration rate is dependent on stomatal aperture (Pallas Jr, 1965; Hosy *et al.*, 2003).
173 Light to dark transitions were used to provoke stomatal closure. The decrease in transpiration rate
174 when light was switched off was slower in the mutant plants than in the corresponding wild-type
175 control plants (Fig. 5, A and B, top panels), revealing a slower rate of dark-induced stomatal
176 closure in absence of *OsK5.2* functional expression. Fitting the decrease in transpiration rates
177 with exponential functions yielded a time constant of approximately 10 min for the wild-type
178 control plants, and of 20-30 min for the mutants from the two *osk5.2* lines (Fig. 5, A and B,
179 bottom panels).

180 The kinetics of the light-induced increase in transpiration rate was also studied (Fig. 6). In
181 wild type plants, when light was switched on, the transpiration rate displayed a rapid increase,
182 which was followed by a transient decrease. The *osk5.2* mutant plants also displayed a rapid
183 increase in transpiration but the kinetics was slightly slower than in the wild type plants (Fig. 6, A
184 and B, bottom panels), and no following transient decrease in transpiration rate was observed
185 (Fig. 6).

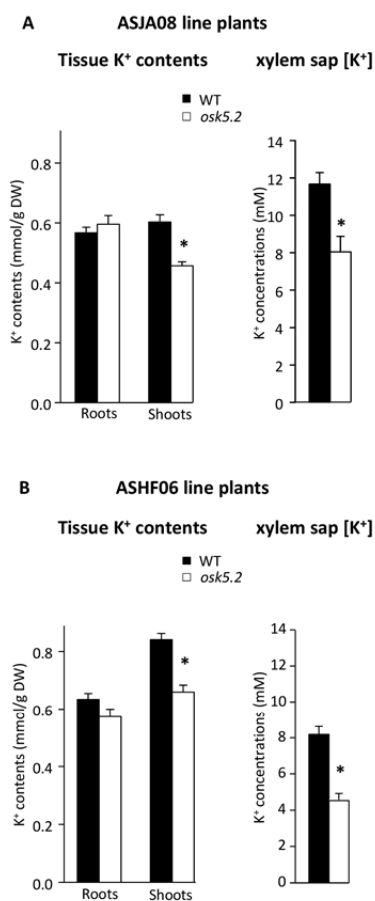


Figure 3. Tissue K⁺ contents and xylem sap K⁺ concentration in wild-type and *osk5.2* mutant plants. Five week-old plants were grown in standard Yoshida hydroponic solution. A and B, Root and shoot K⁺ contents (left) and xylem sap K⁺ concentration (right) in wild-type control (black bars) and *osk5.2* mutant (white bars) plants issued from the initial AJSA08 (A) or ASHF06 (B) lines. Assayed xylem sap exudates were collected in wild-type control and *osk5.2* mutant plants after 1-h exudation in the case of the plants issued from the AJSA08 line, and 2-h exudation in the case of the plants issued from the ASHF06 line. Data are means \pm SE ($n = 3$ or 4 for wild-type control plants, and 4 or 5 for *osk5.2* mutant plants in A, and $n = 4$ or 6 for wild-type control plants, and 5 or 6 for *osk5.2* mutant plants in B). An asterisk indicates that the corresponding difference in K⁺ content or concentration between the wild-type control and *osk5.2* mutant plants were statistically significant (Student's t test, $P < 0.05$).

186
 187 **Absence of Shaker-type outwardly rectifying K⁺ channel activity in guard cells of *osk5.2***
 188 **mutant plants**

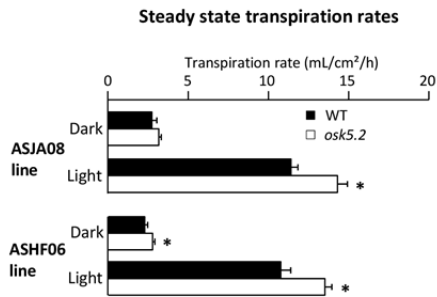


Figure 4. Steady-state transpiration rates in wild-type control and *osk5.2* mutant plants. Five week-old plants issued from the initial ASJA08 line (above) or ASHF06 line (below) were used for steady-state transpiration rate analyses in dark (ca. 5 h after light was switched off) and in light (ca. 3 h after light was switched on) conditions. The steady-state transpiration rate (in mL/cm²/h) was determined by dividing the average speed of water loss at steady-state (mean of 3 values) by the total area of the plant aerial parts. Black and white bars represent wild-type control and *osk5.2* mutant plants, respectively. Data are means \pm SE; $n = 6$ for wild-type control and 13 for *osk5.2* mutant plants in the case of the plants issued from the ASJA08 line, and $n = 7$ for wild-type control and 9 for *osk5.2* plant plants in the case of the plants issued from the ASHF06 line. An asterisk indicates that the corresponding difference between the wild-type control and mutant plants was statistically significant (Student's t test, $P < 0.05$).

189 The consequences of *OsK5.2* loss of function mutation on the K⁺ conductance of the guard
 190 cell membrane were analyzed *in planta*. Guard cells from two-week-old plants were impaled with
 191 double-barreled electrodes (Blatt, 1990; Thiel et al., 1992; Roelfsema et al., 2001) and inward

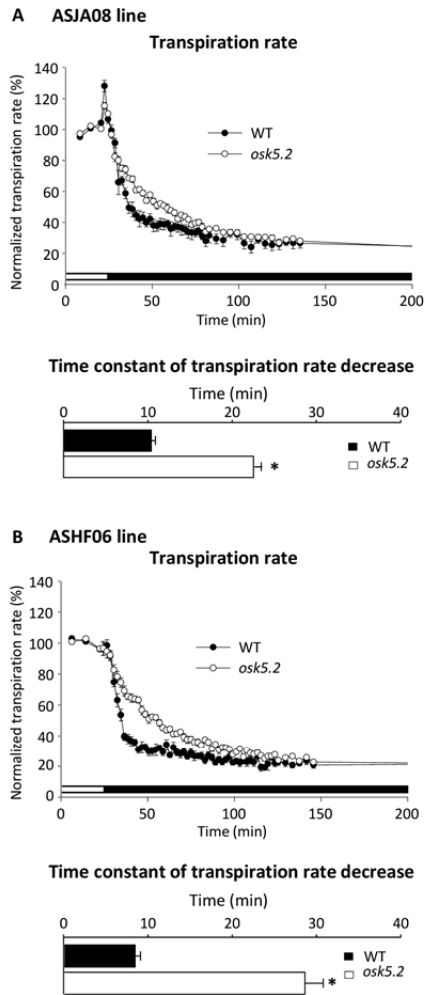


Figure 5. Kinetics of the decrease in transpiration rate upon dark-induced stomatal closure in wild-type and *osk5.2* mutant plants. A and B, Kinetics (top) and time constant (bottom) of the dark-induced decrease in transpiration rate in wild-type control (●, ■) and *osk5.2* mutant (○, □) plants issued from the initial ASJA08 (A) or ASHF06 (B) lines in standard Yoshida hydroponic solution. The light was switched off after 30 min of recording under light condition as indicated by the change from white to black in the bar below the graph. Transpiration rates were normalized by dividing absolute transpiration rates by the steady-state transpiration rate in light condition, and are presented as percentages (%). Time constants were derived by fitting the experimental dark-induced transpiration rate decrease kinetics with an exponential function (least-squares fitting). Black and white bars correspond to wild-type control and *osk5.2* mutant plants, respectively. Data are means \pm SE; $n = 6$ for wild-type control and 13 for *osk5.2* mutant plants in A, and $n = 7$ for wild-type control and 9 for *osk5.2* mutant plants in B. The asterisks indicate that the corresponding differences between the wild-type control and mutant plants were statistically significant (Student's *t* test, $P < 0.05$).

192 and outward currents through the membrane were elicited by voltage clamp pulses (Fig. 7A). In
 193 wild-type guard cells, depolarizing voltage pulses elicited slowly activating outward currents
 194 (Fig. 7B, left panel, and Fig. 7C), clearly reminiscent of those mediated by the outwardly-

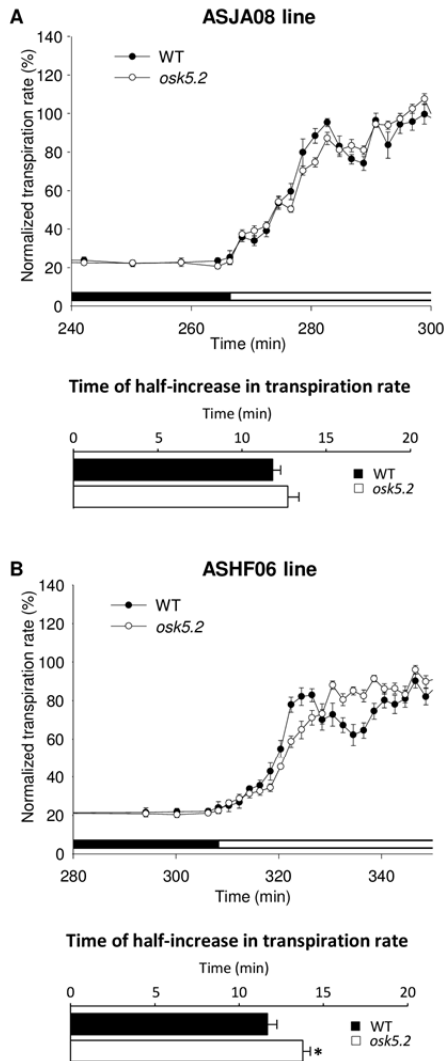


Figure 6. Kinetics of the increase in transpiration rate upon light-induced stomatal opening after a long period of darkness in wild-type and *osk5.2* mutant plants. A and B, Kinetics of light-induced increase in transpiration rate (top) and time of the corresponding half-increase in transpiration rate (bottom) in wild-type control (●, ■) and *osk5.2* mutant (○, □) plants from the initial ASJA08 (A) and ASHF06 (B) lines in standard Yoshida hydroponic solution. The change from black to white in the bar below the curves indicates the time at which light was switched on after the dark period. The normalized transpiration rate (%) was determined as described in Figure 5. Times of half light-induced increase in transpiration rate were derived from the fit of the experimental data up to the end of the initial rapid phase of light-induced transpiration rate increase to a sigmoidal equation (least-squares fitting). Data are means \pm SE; $n = 6$ for wild-type control and 13 for *osk5.2* mutant plants in A, and $n = 7$ for wild-type control and 9 for *osk5.2* mutant plants in B). An asterisk indicates that the corresponding difference between the wild-type control and mutant plants in time of half-increase in transpiration rate was statistically significant (Student's *t* test, $P < 0.05$).

195 rectifying K^+ channels from the Shaker family (Gaymard et al., 1998; Hosy et al., 2003; Hedrich,
 196 2012; Véry et al., 2014). Slowly activating outward currents were lacking in *osk5.2* mutant plants
 197 (Fig. 7, D and E). In contrast, the inward conductance appeared to be very similar in *osk5.2*

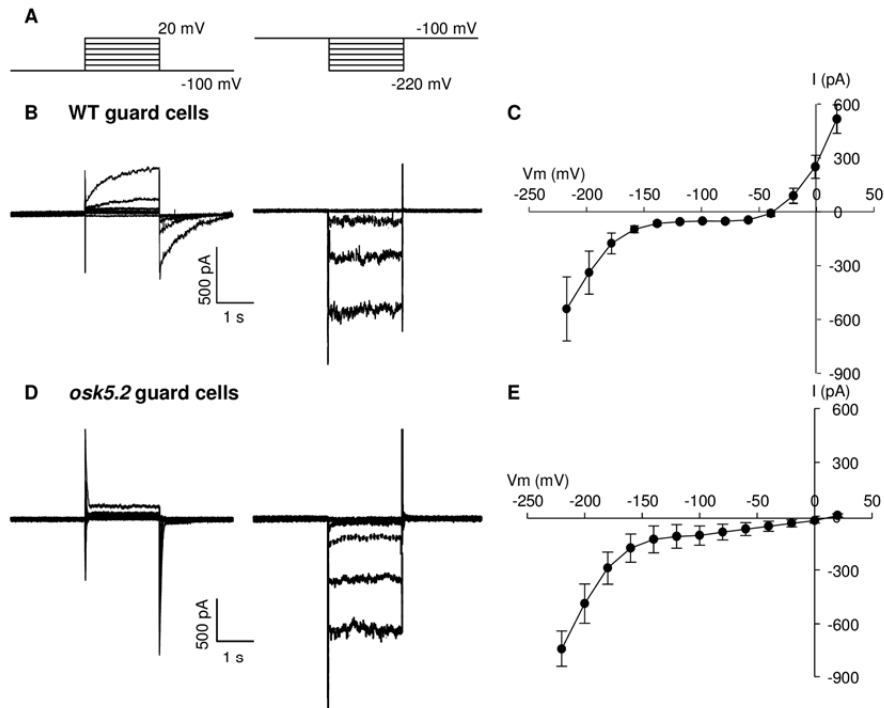


Figure 7. Voltage-clamp experiments with guard cells in intact rice plants. A, Voltage-clamp protocols used to record either outward (left) or inward (right) currents in rice guard cells. Starting at a holding value of -100 mV, the voltage was shifted in 20 mV steps to either +20 mV, or -220 mV. B and D, Representative outward (left) and inward (right) current traces recorded in guard cells from ASJA08 line wild-type control (B) or *osk5.2* mutant (D) plants. C and E, Average current-voltage relationships obtained in wild-type control (C) or mutant (E) guard cells. Data are means \pm SE ($n = 6$ in C, and $n = 4$ in E).

198 mutant and wild type guard cells (Fig. 7, B and D, right panels). This suggested that, although *in*
 199 *planta* voltage-clamp analyses can give rise to experimental drawbacks (Blatt, 1987b; Roelfsema
 200 et al., 2001), comparison between wild type and mutant guard cell currents was valid in our

201 conditions, and thus that *OsK5.2* encodes slowly-activating outwardly rectifying K^+ channels in
202 rice guard cells. It was also observed that the absence of *OsK5.2* channel activity caused a
203 positive shift of the free running membrane potential (cross point with X-axis in the current-
204 voltage relationship), by about 50 mV (Fig. 7, C and E).

205

206

207 **DISCUSSION**

208 **Comparison of the expression patterns of the outward Shaker genes in rice and Arabidopsis**

209 In rice, as in Arabidopsis, the outward Shaker channel group comprises two members.
210 However, based on the phylogenetic tree displayed in Figure 1, OsK5.1 and OsK5.2 cannot be
211 simply considered as the orthologs of the Arabidopsis outward Shaker channels SKOR and
212 GORK. OsK5.2 and OsK5.1 belong to two separate subgroups within the group of outward
213 Shaker channels from cereal plant species (Fig. 1).

214 In Arabidopsis, *SKOR* has been reported to be essentially expressed in the root vasculature
215 (Gaymard et al. 1998), and GORK in root periphery cells (except in the root apex) and in stomata
216 (Ache et al., 2000; Ivashikina et al., 2001). The expression pattern of *OsK5.1* in rice is
217 reminiscent of that of *SKOR* in Arabidopsis, with strong expression in root vasculature (Kim et
218 al., 2015). In contrast, *OsK5.2* displays high expression levels in both the root vasculature (Fig. 2
219 E-G) and the stomata (Fig. 2 B-D), and thus its expression pattern clearly encompasses the
220 patterns reported for both *SKOR* and *GORK* in Arabidopsis (Gaymard et al., 1998; Ache et al.,
221 2000; Ivashikina et al., 2001). It is not yet known whether the expression patterns of *OsK5.1* and
222 *OsK5.2* are shared by genes belonging to the same outward Shaker subgroups from other
223 monocots. The expression pattern of only one maize gene from *OsK5.2* subgroup has been
224 partially characterized so far, by qRT-PCR analyses (Büchschütz et al., 2005). Like *OsK5.2*,
225 this maize outward Shaker gene is expressed in both stomata and roots, and thus its expression
226 pattern may be similar to that of *OsK5.2*.

227

228 **Functional properties of OsK5.2 channels**

229 Members of Shaker group 5 have been cloned in several plant species and successfully
230 expressed in heterologous systems (*Xenopus* oocyte essentially) for electrophysiological analysis
231 of their functional properties (Véry et al., 2014). They all behave as slowly-activating outwardly-
232 rectifying K⁺-selective channels. However, so far, all the channels for which such functional
233 analyses have been reported belong to dicotyledonous species. We attempted to express OsK5.2
234 in *Xenopus* oocytes, but no exogenous current in the oocytes was detected using the classical
235 two-electrode voltage-clamp technique (Véry et al., 1995; Tounsi et al., 2016). This might

236 indicate that these outward K^+ channels have to be co-expressed with regulatory protein partners
237 to be active at the cell membrane, as reported for number of members of the inwardly rectifying
238 Shaker channel group 1 from dicots (Xu et al., 2006; Honsbein et al., 2009; Cuéllar et al., 2010;
239 Cuéllar et al., 2013) and monocots (Boscari et al., 2009; Geiger et al., 2009).

240 Our voltage-clamp measurements with intact guard cells from wild type plants and *osk5.2*
241 mutants (Fig. 7) indicate that *OsK5.2* displays functional features reminiscent of group 5 Shaker
242 channels as determined upon expression in heterologous systems (Ache et al., 2000; Langer et al.,
243 2002; Johansson et al., 2006; Sano et al., 2007), especially a strong outward rectification, a slow
244 activation upon membrane depolarization and slow deactivation of the so-called tail currents
245 when the membrane is repolarized. Finally, the fact that these Shaker-type outward currents were
246 no longer observed in guard cells from the *osk5.2* mutants indicates that *OsK5.2* is the major
247 contributor to the expression of such a channel activity at the guard cell membrane.

248

249 **Comparison of the roles of *OsK5.2* and *SKOR* in K^+ translocation towards the shoots**

250 Both ASJA08 and ASHF06 *osk5.2* loss-of-function mutations caused reduced leaf K^+
251 contents, by 21 to 24% (Fig. 3). Taking into account that *OsK5.2* displays expression in root
252 vascular tissues, the simplest explanation is that *OsK5.2*-encoded channel activity contributes to
253 K^+ secretion into the xylem sap in roots, like *SKOR* in Arabidopsis, and thereby in K^+
254 translocation towards the shoots. This hypothesis received support from the assay of K^+ in xylem
255 sap exudates from excised roots (Fig. 3), which revealed a marked concentration decrease (by
256 >30%) in the *osk5.2* loss-of-function mutants.

257 In Arabidopsis, the absence of *SKOR* channel activity due to a loss-of-function mutation has
258 been shown to lead to a decrease in the concentration of K^+ in the xylem sap by about 40%, and
259 to a decrease in the accumulation of K^+ in shoots by about 50% (Gaymard et al., 1998). Thus, the
260 contribution of *SKOR* to the control of K^+ translocation and accumulation in leaves seems a bit
261 higher than that of *OsK5.2* in rice. Since *SKOR* is the only outward Shaker channel gene
262 expressed in the root stele, K^+ -permeable channels belonging to other families and/or other types
263 of transport systems (e.g., from the KUP/HAK family; Han et al., 2016), have to significantly
264 contribute to K^+ secretion into the xylem sap in Arabidopsis. The situation may be different in
265 rice, where both outward Shaker channel genes *OsK5.1* and *OsK5.2* are expressed in the plant

266 vasculature (Fig. 2; Kim et al., 2015). In other words, besides *OsK5.2*, another outward Shaker
267 channel, *OsK5.1*, could contribute to K^+ translocation towards the shoots.

268
269 **Comparison of the roles of OsK5.2 and GORK in control of stomatal aperture and**
270 **transpirational water loss**

271 Altogether, the present data indicate that *OsK5.2* enhances the rate of stomatal closure by
272 mediating K^+ efflux through the guard cell membrane, just as its counterpart GORK in
273 Arabidopsis (Hosy et al., 2003). The kinetics of dark-induced decrease in transpiration rate
274 indicated that stomata can close in absence of this outwardly rectifying Shaker-type channel
275 activity in rice, like in Arabidopsis, but the closure kinetics is slowed down. It is likely that
276 poorly selective cation channels (Hedrich, 2012) substitute for Shaker channels as K^+ efflux
277 systems, but stomatal closure is then less efficient. The defect in stomatal closure in *osk5.2*
278 mutant plants is also likely to result from the large difference in free running membrane potential,
279 by approximately 50 mV, between *osk5.2* and wild type guard cells (Fig. 7). Indeed, in *osk5.2*
280 guard cells, the free running voltage across the plasma membrane was shifted towards the
281 reversal potential of anion channels, which was approximately +25 mV (as in Kollist et al.,
282 2014). Thus, the driving force for anion efflux (difference between the free running membrane
283 potential and reversal potential of anion channels) was reduced in *osk5.2* guard cells. This is
284 expected to cause a decrease in anion efflux, and thus in the rate of stomatal closure.

285 Besides its impact on the rate of dark-induced stomatal closure and steady-state stomatal
286 aperture in darkness, the absence of Shaker-type outward K^+ channel activity in rice is also likely
287 to result in larger steady-state stomatal aperture in light, based on the observation that the steady
288 state transpiration rate was increased (Fig. 4). This would mean that *OsK5.2* outwardly-rectifying
289 channel activity displays an inhibitory effect on steady-state stomatal aperture in light. The fact
290 that the leaf K^+ content was lower in the *osk5.2* mutant plants (Fig. 3) is likely to lead to a
291 decrease in turgor of the leaf cells. A decrease in the turgor of epidermal and subsidiary cells
292 more important than that of guard cells could result in reduced back-pressure on stomatal guard
293 cells and, thus, in increased steady state stomatal aperture in the light. The impact of *OsK5.2*
294 activity on steady-state transpiration rate in light could be understood within the framework of
295 this latter hypothesis.

296

297 **CONCLUSION**

298 By controlling the transpirational water loss at leaf surface, stomata also regulate the flux of
299 water from roots to shoots within the xylem vasculature, and thereby contribute to the regulation
300 of nutrient ion translocation towards the shoots. In Arabidopsis, various ion transport systems
301 play a role in both ion translocation into the xylem sap towards the shoots and ion transport
302 across the guard cell membrane and control of stomatal aperture. This is the case, for example, of
303 the anion channel SLAH3 (Geiger et al., 2011; Cubero-Font et al., 2016), the nitrate transporter
304 NRT1.1 (Guo et al., 2003; Wang et al., 2012) and the phosphate transporter PHO1 (Hamburger *et*
305 *al.*, 2002; Zimmerli *et al.*, 2012). In contrast, regarding K^+ , some kind of specialization has
306 occurred within the Shaker group 5 in Arabidopsis since K^+ release from guard cells for stomatal
307 closure has been strictly assigned to GORK, and K^+ secretion into the xylem sap to SKOR. In
308 rice, OsK5.2 contributes to both K^+ release by guard cells for stomatal closure and K^+
309 translocation towards the shoots. The physiological significance of such evolutionary differences
310 would be interesting to clarify. The organization and the roles of the Shaker family are strongly
311 conserved in plants, allowing one to assume functional equivalence between orthologs, but the
312 present data clearly indicate that the biological variability deserves to be investigated since
313 differences between plant species in the use of this gene family are likely to play a role in major
314 physiological functions and in adaptation to environmental constraints.

315

316 **MATERIALS AND METHODS**

317 **Plant material and growth conditions**

318 Rice Nipponbare cultivar (*Oryza sativa* L. var. Nipponbare) was used in all the experiments.
319 For transpiration analysis, seeds were germinated in Petri dishes containing deionized water.
320 One-week-old plants were then transferred onto Yoshida hydroponic solution in 10 L containers
321 (0.7 mM KNO_3 , 0.8 mM KH_2PO_4 , 1.2 mM $Ca(NO_3)_2$, 0.5 mM $(NH_4)_2SO_4$, 1.6 mM $MgSO_4$, 60
322 $\mu M Na_2FeEDTA$, 45 $\mu M H_3BO_3$, 20 $\mu M MnSO_4$, 1.6 $\mu M CuSO_4$, 1.4 $\mu M ZnSO_4$, and 0.3 μM
323 $(NH_4)_6Mo_7O_{24}$, pH adjusted to 5.5). Plants were grown in growth chambers (day/night 12 h/12 h
324 photoperiod, 28 °C/25 °C, photon flux density 500 $\mu mol.m^{-2}.s^{-1}$, 80% relative humidity).

325

326 **Construction of reporter gene constructs and transgenic plants**

327 *OsK5.2* promoter region (2339 bp) was amplified by PCR from rice Nipponbare genomic
328 DNA using the primers listed in table S1. The PCR product was then cloned into pGemTeasy
329 vector for sequencing before sub-cloning into destination vector. The promoter was then
330 transferred upstream the *GUS* reporter gene in pCAMBIA1301 vector, where it replaced
331 CAMV35S promoter. The resulting plasmid was introduced into *Agrobacterium tumefaciens*
332 EHA105 strain for rice transformation, as described by Sallaud et al. (2002). Briefly, dehulked
333 rice seeds were rinsed in ethanol 70% for 90 seconds and treated with disinfection solution
334 (50 g/L of sodium hypochlorite) for 30 minutes. After sterilization, the seeds were transferred to a
335 callus induction medium leading to calluses development from the scutellum region. Rice grains
336 with developing calluses were incubated with bacteria suspension for 90 to 180 seconds and co-
337 cultured during 3 days. After the co-culture period, developing calluses were washed with
338 carbenicillin (500 mg/L), separated, divided into small pieces and transferred to shoot induction
339 medium containing hygromycin for transgenic tissue selection. Shoots regenerated from calluses
340 were separated and transferred to rooting medium. Four-week-old plantlets were transferred to
341 soil and grown in greenhouse for multiplication and selection of homozygous plants in the next
342 generation using antibiotics leaf painting method (Cotsaftis et al., 2002).

343

344 **Histochemical analysis of GUS activity**

345 Leaf samples of 4 week-old plants and flowers were collected from homozygous plants grown
346 in a greenhouse. Root samples were collected from 1 week-old plants grown in standard Yoshida
347 hydroponic medium. All samples were submerged in GUS solution (50 mM Phosphate Buffer
348 pH 7, 2.5 mM ferricyanide, 2.5 mM ferrocyanide, 0.05% v/v Triton X100 and 1 mM X-Gluc)
349 contained in a 24-well plate, the leaves having been previously quickly divided into 1x1 cm
350 pieces. The plate was placed under vacuum for 30 minutes to facilitate the penetration of GUS
351 solution into the tissues, then the samples were incubated overnight at 37°C. After the incubation
352 step, green samples were washed with 70% ethanol solution to remove chlorophyll. Leaf samples

353 were furthermore treated overnight with chloral hydrate (2.5 mg/L in 30% glycerol) before being
354 observed under microscope (BH2, Olympus) for GUS activity detection.

355 Thin (8- μ m thick) sections were prepared, using a RM 2165 microtome (Leica, Germany),
356 from GUS-stained fixed samples (pre-fixation in 50 mM phosphate buffer pH 7, 1.5%
357 formaldehyde, 0.05% Triton X100; fixation in 75 mM phosphate buffer, 2% paraformaldehyde,
358 0.5% glutaraldehyde) embedded in Technovit 7100 resin (Kulzer, Wehrheim, Germany).

359

360 **Selection of KO mutant and wild type control plants**

361 *Tos17* insertion mutant lines (cv Nipponbare background) displaying insertion in *OsK5.2* gene
362 were ordered from OTL collection (CIRAD-Genoscope, France; www.oryzatagline.cirad.fr;
363 Piffanelli et al., 2007). Homozygous mutant plants as well as “wild-type” plants for *OsK5.2* gene
364 were selected by PCR on genomic DNA extracted from plantlets issued from received seeds,
365 using Phire® Plant Direct PCR Kit (Thermo Fischer Scientific) and specific primers hybridizing
366 on the target gene and on the *Tos17*. Total RNA of 2 plants selected in the progeny of a
367 homozygous mutant, or of a plant displaying a wild-type genotype for *OsK5.2* (hereafter
368 generically named wild-type control plants), were isolated using RNeasy kit (QIAGEN) and used
369 as templates to synthesize total cDNA. PCR experiments, using primers hybridizing downstream
370 from the *Tos17* insertion sites (listed in Supplemental Table S1), allowed us to check the
371 presence (in the wild-type control plants) or absence (in the *osk5.2* mutants) of *OsK5.2*
372 transcripts.

373

374 **Voltage-clamp recordings on intact plant guard cells**

375 Two-electrode voltage-clamp experiments were performed on intact guard cells (Roelfsema et
376 al., 2001; Mumm et al., 2011) of intact plants grown on standard Yoshida hydroponic solution for
377 two weeks. A plant (wild type control or homozygous *osk5.2* mutant) was transferred into a 15
378 mL tube (Falcon type) containing about 5 mL of standard Yoshida solution, the tube being
379 inclined so that the root system was fully immersed in the solution. Outside of the tube, the
380 youngest fully developed leaf was attached to a Plexiglas block with double-sided adhesive tape,
381 the plant remaining intact. The whole plant, attached with its leaf to the plexiglass, was carefully

382 mounted onto the table of an upright microscope (Axioskop 2FS, Zeiss). About 0.5 to 1 mL of
383 bath solution (5 mM potassium citrate, 5 mM KCl, 0.1 mM CaCl₂, pH 5.0) was placed between
384 the leaf and the water immersion objective (Achromplan 40/0.80W, Zeiss), used to visualize the
385 stomata. Stomatal opening was induced by light provided by the microscope lamp. Guard cells
386 were impaled using double-barreled electrodes prepared from 1-mm-diameter glass capillaries
387 (1403547, Hilgenberg, Germany), filled with 300 mM KCl. A glass capillary filled with 300 mM
388 KCl and plugged with 300 mM KCl in 2% agarose was placed in the drop of solution between
389 the leaf and the objective and served as a reference electrode. The series resistance present
390 between leaf apoplast and reference electrode was estimated by measuring the resistance between
391 two drops of bath solution placed on the leaf surface, at a distance of approximately 1 cm. This
392 approach revealed a series resistance (which was not different between wild type and *osk5.2*
393 mutant plants) of 1.3 ± 0.1 (n=5) MOhm at the abaxial leaf face. With guard cell currents lower
394 than 1 nA (Fig. 7), such a series resistance leads to an overestimation of the applied voltage of
395 less than 2 mV. The potential difference between the bath solution and guard cell apoplast
396 ("surface" potential) was -10 ± 2 mV (n = 6). Because the series resistance and potential
397 difference between the external solution and the apoplast appeared as low, no attempt was made
398 to correct their impact on current-voltage plots. The Win WCP V5.1.6 software (J. Dempster,
399 Strathclyde University, Glasgow, UK) was used to setup the protocol and record applied voltage-
400 and current traces, using an ITC-16 interface (Instrutech Corp., Elmont, NY) and a GeneClamp
401 500 (Axon Instruments) amplifier.

402

403 **Analysis of stomatal movements induced by light or darkness**

404 Wild type control and homozygous *osk5.2* mutant plants were grown in parallel in standard
405 Yoshida hydroponic medium for five weeks. One day before the experiment, plants were
406 transferred into a multi-potometer device (Supplemental Figure S2), the root system bathed in
407 standard Yoshida hydroponic solution. On the day of the experiment, transpiration rates started to
408 be recorded after 2 h under light condition. A dark treatment was imposed after 30 minutes of
409 recording and was maintained for 4 h before switching back to light condition.

410

411 **Analysis of K⁺ content in tissues and xylem sap**

412 Root and shoot K⁺ contents were assayed in plants grown as described above for the analysis
413 of stomatal movements, i.e., hydroponic conditions for 5 weeks on standard Yoshida solution.
414 After excision, roots and shoots were weighed, dried (60°C during 2 days) and weighed again.
415 Ions were extracted from the tissues with 0.1 N HCl during 2 days. K⁺ content in the extracts was
416 determined by flame spectrophotometry (SpectrAA 220FS, Varian).

417 K⁺ concentration in xylem sap was analyzed in 5 week-old plants grown in standard Yoshida
418 hydroponic solution. Excision at 1 cm above the crown allowed collection of the xylem sap.
419 Exudation duration before collection (identical in control and *osk5.2*mutant plants) lasted for 1 to
420 2 h. Collected samples were diluted in 0.1 N HCl and the concentration of K⁺ was determined
421 like that of tissue extracts.

422

423 **Accession numbers**

424 Sequence data for *OsK5.2* Shaker gene can be found in the GenBank library under accession
425 number Os06g0250600.

426

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431 transpiration rate data.

432

433 **FIGURE LEGENDS**

434 **Figure 1.** Phylogenetic analysis of outward Shaker channels in monocot species and *Arabidopsis*
435 *thaliana*. The unrooted phylogenetic trees were generated with PhyML software
436 (<http://www.atgc-montpellier.fr/phyml/binaries.php>) using the maximum-likelihood method and
437 1000 bootstrap replicates in Seaview application (<http://doua.prabi.fr/software/seaview>). The

438 scale bar corresponds to a distance of 10 changes per 100 amino acid positions. The protein
439 (GenBank) accession numbers are given in Supplemental Table S2. At, *Arabidopsis thaliana*; Bd,
440 *Brachypodium distachyon*; Os, *Oryza sativa* (rice); Zm, *Zea mays* (maize).

441 **Figure 2.** Expression pattern of *OsK5.2* outward Shaker channel gene in rice. A, *Promoter::GUS*
442 construct (promoter region size: 2339 bp). B to J, GUS activity in transgenic rice plants, revealed
443 by an overnight incubation in the presence of X-Gluc: leaf tissues (B), stomata (C), leaf cross-
444 section (D), root (E), root tip (F), root cross-section (G), flower (H), anther (I), and pollen (J).

445 **Figure 3.** Tissue K⁺ contents and xylem sap K⁺ concentration in wild-type and *osk5.2* mutant
446 plants. Five week-old plants were grown in standard Yoshida hydroponic solution. A and B, Root
447 and shoot K⁺ contents (left) and xylem sap K⁺ concentration (right) in wild-type control (black
448 bars) and *osk5.2* mutant (white bars) plants issued from the initial AJSA08 (A) or ASHF06 (B)
449 lines. Assayed xylem sap exudates were collected in wild-type control and *osk5.2* mutant plants
450 after 1-h exudation in the case of the plants issued from the AJSA08 line, and 2-h exudation in
451 the case of the plants issued from the ASHF06 line. Data are means ± SE ($n = 3$ or 4 for wild-
452 type control plants, and 4 or 5 for *osk5.2* mutant plants in A, and $n = 4$ or 6 for wild-type control
453 plants, and 5 or 6 for *osk5.2* mutant plants in B). An asterisk indicates that the corresponding
454 difference in K⁺ content or concentration between the wild-type control and *osk5.2* mutant plants
455 were statistically significant (Student's t test, $P < 0.05$).

456 **Figure 4.** Steady-state transpiration rates in wild-type control and *osk5.2* mutant plants. Five
457 week-old plants issued from the initial ASJA08 line (above) or ASHF06 line (below) were used
458 for steady-state transpiration rate analyses in dark (*ca.* 5 h after light was switched off) and in
459 light (*ca.* 3 h after light was switched on) conditions. The steady-state transpiration rate (in
460 mL/cm²/h) was determined by dividing the average speed of water loss at steady-state (mean of 3
461 values) by the total area of the plant aerial parts. Black and white bars represent wild-type control
462 and *osk5.2* mutant plants, respectively. Data are means ± SE; $n = 6$ for wild-type control and 13
463 for *osk5.2* mutant plants in the case of the plants issued from the ASJA08 line, and $n = 7$ for
464 wild-type control and 9 for *osk5.2* plant plants in the case of the plants issued from the ASHF06
465 line. An asterisk indicates that the corresponding difference between the wild-type control and
466 mutant plants was statistically significant (Student's t test, $P < 0.05$).

467 **Figure 5.** Kinetics of the decrease in transpiration rate upon dark-induced stomatal closure in
468 wild-type and *osk5.2* mutant plants. A and B, Kinetics (top) and time constant (bottom) of the
469 dark-induced decrease in transpiration rate in wild-type control (●, ■) and *osk5.2* mutant (○, □)
470 plants issued from the initial ASJA08 (A) or ASHF06 (B) lines in standard Yoshida hydroponic
471 solution. The light was switched off after 30 min of recording under light condition as indicated
472 by the change from white to black in the bar below the graph. Transpiration rates were
473 normalized by dividing absolute transpiration rates by the steady-state transpiration rate in light
474 condition, and are presented as percentages (%). Time constants were derived by fitting the
475 experimental dark-induced transpiration rate decrease kinetics with an exponential function
476 (least-squares fitting). Black and white bars correspond to wild-type control and *osk5.2* mutant
477 plants, respectively. Data are means ± SE; *n* = 6 for wild-type control and 13 for *osk5.2* mutant
478 plants in A, and *n* = 7 for wild-type control and 9 for *osk5.2* mutant plants in B. The asterisks
479 indicate that the corresponding differences between the wild-type control and mutant plants were
480 statistically significant (Student's *t* test, *P* < 0.05).

481 **Figure 6.** Kinetics of the increase in transpiration rate upon light-induced stomatal opening after
482 a long period of darkness in wild-type and *osk5.2* mutant plants. A and B, Kinetics of light-
483 induced increase in transpiration rate (top) and time of the corresponding half-increase in
484 transpiration rate (bottom) in wild-type control (●, ■) and *osk5.2* mutant (○, □) plants from the
485 initial ASJA08 (A) and ASHF06 (B) lines in standard Yoshida hydroponic solution. The change
486 from black to white in the bar below the curves indicates the time at which light was switched on
487 after the dark period. The normalized transpiration rate (%) was determined as described in
488 Figure 5. Times of half light-induced increase in transpiration rate were derived from the fit of
489 the experimental data up to the end of the initial rapid phase of light-induced transpiration rate
490 increase to a sigmoidal equation (least-squares fitting). Data are means ± SE; *n* = 6 for wild-type
491 control and 13 for *osk5.2* mutant plants in A, and *n* = 7 for wild-type control and 9 for *osk5.2*
492 mutant plants in B). An asterisk indicates that the corresponding difference between the wild-type
493 control and mutant plants in time of half-increase in transpiration rate was statistically significant
494 (Student's *t* test, *P* < 0.05).

495 **Figure 7.** Voltage-clamp experiments with guard cells in intact rice plants. A, Voltage-clamp
496 protocols used to record either outward (left) or inward (right) currents in rice guard cells.

497 Starting at a holding value of -100 mV, the voltage was shifted in 20 mV steps to either +20 mV,
498 or -220 mV. B and D, Representative outward (left) and inward (right) current traces recorded in
499 guard cells from ASJA08 line wild-type control (B) or *osk5.2* mutant (D) plants. C and E,
500 Average current-voltage relationships obtained in wild-type control (C) or mutant (E) guard cells.
501 Data are means \pm SE ($n = 6$ in C, and $n = 4$ in E).

502

503 **Supplemental Material**

504 **Supplemental Figure S1.** Selection of knock-out mutant plants for *OsK5.2* gene.

505 **Supplemental Figure S2.** Potometer device. The multi-potometer system used for plant
506 transpiration rate monitoring.

507 **Supplemental Table S1.** Primer sequences used for cloning, genotyping, and verification of KO
508 *osk5.2* mutant plants.

509 **Supplemental Table S2.** GenBank accession numbers of Shaker polypeptides present in
510 phylogenetic trees

511

512 **Supplemental Figure S1.** Selection of knock-out mutant plants for *OsK5.2* gene. A, *Tos17*
513 insertion sites in the *osk5.2* mutant ASJA08 (FST accession CU316012) and ASHF06 (FST
514 accession CU316066) lines. B, Lack of *OsK5.2* transcripts in homozygous *osk5.2* mutants issued
515 from ASJA08 and ASHF06 lines. RT-PCR experiments were performed for *OsK5.2* and *eEF1- β*
516 (internal control) using total mRNA isolated from roots of 5 week-old wild-type control (WT) or
517 homozygous mutant (ho) plants issued from each insertion line.

518

519 **Supplemental Figure S2.** Potometer device. The multi-potometer system used for plant
520 transpiration rate monitoring consisted of twenty 50 mL syringes (Terumo corporation,
521 Philippines) vertically fixed into a polystyrene plaque laid over an empty container, and
522 connected to graduated 1 mL serological plastic pipettes (“1 mL Stripette”, Costar Corporation,
523 Cambridge, MA) via thin silicone tubes. A camera allowed to record the water level in the

524 pipettes over time. Four to five week-old plants were inserted through the black rubber plunger of
525 the syringes via a 4 mm hole at the plunger center, and sealed by dental polyvinylsiloxane-based
526 elastomer (Coltène® PRESIDENT light body). After polymerization of the elastomer, the
527 plungers were placed to the syringes filled with experimental solution (Yoshida medium). The
528 pipettes and connecting tubes were filled with the same solution, ensuring that no air bubble was
529 present in the system. The transpiration rate was calculated from the decrease in level of the
530 solution in the pipettes recorded over time.

531
532 **Supplemental Table S1.** Primer sequences used for cloning, genotyping, and verification of KO
533 *osk5.2* mutant plants.

534
535 **Supplemental Table S2.** GenBank accession numbers of Shaker polypeptides present in
536 phylogenetic trees

537

538

539

Parsed Citations

Ache P, Becker D, Ivashikina N, Dietrich P, Roelfsema MRG, Hedrich R (2000) GORK, a delayed outward rectifier expressed in guard cells of *Arabidopsis thaliana*, is a K⁺-selective, K⁺-sensing ion channel. FEBS Lett 486: 93-98

Pubmed: [Author and Title](#)

CrossRef: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Boscari A, Clement M, Volkov V, Goldack D, Hybiak J, Miller AJ, Amtmann A, Fricke W (2009) Potassium channels in barley: cloning, functional characterization and expression analyses in relation to leaf growth and development. Plant, cell & environ 32: 1761-1777

Pubmed: [Author and Title](#)

CrossRef: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Blatt MR (1987a) Electrical characteristics of stomatal guard cells: The contribution of ATP-dependent "electrogenic" transport revealed by current-voltage and difference-current-voltage analysis. J Membr Biol 98: 257-74

Pubmed: [Author and Title](#)

CrossRef: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Blatt MR (1987b) Electrical characteristics of stomatal guard cells: The ionic basis of the membrane potential and the consequence of potassium chloride leakage from microelectrodes. Planta 170: 272-287

Pubmed: [Author and Title](#)

CrossRef: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Blatt MR (1990) Potassium channel currents in intact stomatal guard cells: rapid enhancement by abscisic acid. Planta 180: 445-455

Pubmed: [Author and Title](#)

CrossRef: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Büchenschütz K, Marten I, Becker D, Philippar K, Ache P, Hedrich R (2005) Differential expression of K⁺ channels between guard cells and subsidiary cells within the maize stomatal complex. Planta 222: 968-976

Pubmed: [Author and Title](#)

CrossRef: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Chen Z-H, Hills A, Bätz U, Amtmann A, Lew VL, Blatt MR (2012) Systems dynamic modeling of the stomatal guard cell predicts emergent behaviors in transport, signaling, and volume control. Plant Physiol 159: 1235-1251

Pubmed: [Author and Title](#)

CrossRef: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Clarkson DT, Hanson JB (1980) The mineral nutrition of higher plants. Annu Rev Plant Physiol 31: 239-298

Pubmed: [Author and Title](#)

CrossRef: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Cotsaftis O, Sallaud C, Breitler JC, Meynard D, Greco R, Pereira A, Guiderdoni E (2002) Transposon-mediated generation of T-DNA and marker-free rice plants expressing a Bt endotoxin gene. Mol Breeding 10: 165-180

Pubmed: [Author and Title](#)

CrossRef: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Cubero-Font P, Maierhofer T, Jaslan J, Rosales MA, Espartero J, Díaz-Rueda P, Müller HM, Hürter A-L, AL-Rasheid KA, Marten I (2016) Silent S-Type anion channel subunit SLAH1 gates SLAH3 open for chloride root-to-shoot translocation. Curr Biol 26: 2213-2220

Pubmed: [Author and Title](#)

CrossRef: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Cuéllar T, Azeem F, Andrianteranagna M, Pascaud F, Verdeil JL, Sentenac H, Zimmermann S, Gaillard I (2013) Potassium transport in developing fleshy fruits: the grapevine inward K⁺ channel VvK1. 2 is activated by CIPK-CBL complexes and induced in ripening berry flesh cells. Plant J 73: 1006-1018

Pubmed: [Author and Title](#)

CrossRef: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Cuéllar T, Pascaud F, Verdeil JL, Torregrosa L, Adam-Blondon AF, Thibaud JB, Sentenac H, Gaillard I (2010) A grapevine Shaker inward K⁺ channel activated by the calcineurin B-like calcium sensor 1-protein kinase CIPK23 network is expressed in grape berries under drought stress conditions. Plant J 61: 58-69

Pubmed: [Author and Title](#)

CrossRef: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Franks PJ, Farquhar GD (2007) The mechanical diversity of stomata and its significance in gas-exchange control. Plant Physiol 143: 78-87

Pubmed: [Author and Title](#)

CrossRef: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Gaynard F, Pilot G, Lacombe B, Bouchez D, Bruneau D, Boucherez J, Michaux-Ferriere N, Thibaud J-B, Sentenac H (1998) Identification and disruption of a plant Shaker-like outward channel involved in K⁺ release into the xylem sap. *Cell* 94: 647-655

Pubmed: [Author and Title](#)

CrossRef: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Geiger D, Becker D, Vosloh D, Gambale F, Palme K, Rehers M, Anschuetz U, Dreyer I, Kudla J, Hedrich R (2009) Heteromeric AtKC1.AKT1 channels in Arabidopsis roots facilitate growth under K⁺-limiting conditions. *J Biol Chem* 284: 21288-21295

Pubmed: [Author and Title](#)

CrossRef: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Geiger D, Maierhofer T, AL-Rasheid KA, Scherzer S, Mumm P, Liese A, Ache P, Wellmann C, Marten I, Grill E (2011) Stomatal closure by fast abscisic acid signaling is mediated by the guard cell anion channel SLAH3 and the receptor RCAR1. *Sci Signal* 4: ra32-ra32

Pubmed: [Author and Title](#)

CrossRef: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Guo F-Q, Young J, Crawford NM (2003) The nitrate transporter AtNRT1.1 (CHL1) functions in stomatal opening and contributes to drought susceptibility in Arabidopsis. *Plant Cell* 15: 107-117

Pubmed: [Author and Title](#)

CrossRef: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Hamburger D, Rezzonico E, Petétot JM-C, Somerville C, Poirier Y (2002) Identification and characterization of the Arabidopsis PHO1 gene involved in phosphate loading to the xylem. *Plant Cell* 14: 889-902

Pubmed: [Author and Title](#)

CrossRef: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Han M, Wu W, Wu WH, Wang Y (2016) Potassium transporter KUP7 is involved in K⁺ acquisition and translocation in Arabidopsis root under K⁺-limited conditions. *Mol Plant* 9: 437-446

Pubmed: [Author and Title](#)

CrossRef: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Hedrich R (2012) Ion channels in plants. *Physiol Rev* 92: 1777-1811

Pubmed: [Author and Title](#)

CrossRef: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Hills A, Chen Z-H, Amtmann A, Blatt MR, Lew VL (2012) OnGuard, a computational platform for quantitative kinetic modeling of guard cell physiology. *Plant physiol* 159: 1026-1042

Pubmed: [Author and Title](#)

CrossRef: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Honsbein A, Sokolovski S, Grefen C, Campanoni P, Pratelli R, Paneque M, Chen Z, Johansson I, Blatt MR (2009) A tripartite SNARE-K⁺ channel complex mediates in channel-dependent K⁺ nutrition in Arabidopsis. *Plant Cell* 21: 2859-2877

Pubmed: [Author and Title](#)

CrossRef: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Hosy E, Vavasseur A, Mouline K, Dreyer I, Gaynard F, Porée F, Boucherez J, Lebaudy A, Bouchez D, Véry A-A (2003) The Arabidopsis outward K⁺ channel GORK is involved in regulation of stomatal movements and plant transpiration. *Proc Natl Acad Sci USA* 100: 5549-5554

Pubmed: [Author and Title](#)

CrossRef: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Itoh J-I, Nonomura K-I, Ikeda K, Yamaki S, Inukai Y, Yamagishi H, Kitano H, Nagato Y (2005) Rice plant development: from zygote to spikelet. *Plant Cell Physiol* 46: 23-47

Pubmed: [Author and Title](#)

CrossRef: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Ivashikina N, Becker D, Ache P, Meyerhoff O, Felle HH, Hedrich R (2001) K⁺ channel profile and electrical properties of Arabidopsis root hairs. *FEBS Lett* 508: 463-469

Pubmed: [Author and Title](#)

CrossRef: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Jezeq M, Blatt MR (2017) Guard cell photosynthesis is critical for stomatal turgor production, yet does not directly mediate CO₂- and ABA-induced stomatal closing. *Plant Physiol* 174: 487-519

Pubmed: [Author and Title](#)

CrossRef: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Johansson I, Wulfetange K, Porée F, Michard E, Gajdanowicz P, Lacombe B, Sentenac H, Thibaud JB, Mueller-Roeber B, Blatt MR (2006) External K⁺ modulates the activity of the Arabidopsis potassium channel SKOR via an unusual mechanism. *Plant J* 46: 269-281

Pubmed: [Author and Title](#)

CrossRef: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Kim HY, Choi E-H, Min MK, Hwang H, Moon S-J, Yoon I, Byun M-O, Kim B-G (2015) Differential gene expression of two outward-rectifying shaker-like potassium channels OsSKOR and OsGORK in rice. *J Plant Biol* 58: 230-235

Pubmed: [Author and Title](#)

CrossRef: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Kollist H, Nuhkat M, Roelfsema MRG (2014) Closing gaps: linking elements that control stomatal movement. *New Phytol* 203: 44-62

Pubmed: [Author and Title](#)

CrossRef: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Langer K, Ache P, Geiger D, Stinzinger A, Arend M, Wind C, Regan S, Fromm J, Hedrich R (2002) Poplar potassium transporters capable of controlling K⁺ homeostasis and K⁺-dependent xylogenesis. *Plant J* 32: 997-1009

Pubmed: [Author and Title](#)

CrossRef: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Lawson T, Blatt MR (2014) Stomatal size, speed, and responsiveness impact on photosynthesis and water use efficiency. *Plant physiol* 164: 1556-1570

Pubmed: [Author and Title](#)

CrossRef: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Lebaudy A, Vavasseur A, Hosy E, Dreyer I, Leonhardt N, Thibaud J-B, Véry A-A, Simonneau T, Sentenac H (2008) Plant adaptation to fluctuating environment and biomass production are strongly dependent on guard cell potassium channels. *Proc Natl Acad Sci USA* 105: 5271-5276

Pubmed: [Author and Title](#)

CrossRef: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Leigh R, Wyn Jones R (1984) A hypothesis relating critical potassium concentrations for growth to the distribution and functions of this ion in the plant cell. *New Phytol* 97: 1-13

Pubmed: [Author and Title](#)

CrossRef: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Marschner H, Kirkby E, Cakmak I (1996) Effect of mineral nutritional status on shoot-root partitioning of photoassimilates and cycling of mineral nutrients. *J Exp Bot* 47: 1255-1263

Pubmed: [Author and Title](#)

CrossRef: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Mumm P, Wolf T, Fromm J, Roelfsema MRG, Marten I (2011) Cell type-specific regulation of ion channels within the maize stomatal complex. *Plant Cell Physiol* 52: 1365-1375

Pubmed: [Author and Title](#)

CrossRef: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Piffanelli P, Droc G, Mieulet D, Lanau N, Bes M, Bourgeois E, Rouvière C, Gavory F, Cruaud C, Ghesquière A, Guiderdoni E (2007) Large-scale characterization of Tos17 insertion sites in a rice T-DNA mutant library. *Plant Mol Biol* 65: 587-601. Pallas Jr JE (1965) Transpiration and stomatal opening with changes in carbon dioxide content of the air. *Science* 147: 171-173

Pubmed: [Author and Title](#)

CrossRef: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Pilot G, Lacombe B, Gaymard F, Cherel I, Boucherez J, Thibaud JB, Sentenac H (2001) Guard cell inward K⁺ channel activity in Arabidopsis involves expression of the twin channel subunits KAT1 and KAT2. *J Biol Chem* 276: 3215-3221

Pubmed: [Author and Title](#)

CrossRef: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Pilot G, Pratelli R, Gaymard F, Meyer Y, Sentenac H (2003) Five-group distribution of the Shaker-like K⁺ channel family in higher plants. *J Mol Evol* 56: 418-434

Pubmed: [Author and Title](#)

CrossRef: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Raschke K, Schnabl H (1978) Availability of chloride affects the balance between potassium chloride and potassium malate in guard cells of *Vicia faba* L. *Plant Physiol* 62: 84-87

Pubmed: [Author and Title](#)

CrossRef: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Roelfsema MRG, Hedrich R (2009) Stomata. eLS

Pubmed: [Author and Title](#)

CrossRef: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Roelfsema MR, Prins HB (1997) Ion channels in guard cells of Arabidopsis thaliana (L.) Heynh.. Planta 202: 18-27

Pubmed: [Author and Title](#)

CrossRef: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Roelfsema MRG, Steinmeyer R, Staal M, Hedrich R (2001) Single guard cell recordings in intact plants: light-induced hyperpolarization of the plasma membrane. Plant J 26: 1-13

Pubmed: [Author and Title](#)

CrossRef: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Sallaud C, Meynard D, Van Boxtel J, Gay C, Bes M, Brizard J-P, Larmande P, Ortega D, Raynal M, Portefaix M (2003) Highly efficient production and characterization of T-DNA plants for rice (Oryza sativa L.) functional genomics. Theor Appl Genet 106: 1396-1408

Pubmed: [Author and Title](#)

CrossRef: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Sano T, Becker D, Ivashikina N, Wegner LH, Zimmermann U, Roelfsema MRG, Nagata T, Hedrich R (2007) Plant cells must pass a K⁺ threshold to re-enter the cell cycle. Plant J 50: 401-413

Pubmed: [Author and Title](#)

CrossRef: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Szyroki A, Ivashikina N, Dietrich P, Roelfsema MRG, Ache P, Reintanz B, Deeken R, Godde M, Felle H, Steinmeyer R (2001) KAT1 is not essential for stomatal opening. Proc Natl Acad Sci USA 98: 2917-2921

Pubmed: [Author and Title](#)

CrossRef: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Talbott L, Zeiger E (1998) The role of sucrose in guard cell osmoregulation. J Exp Bot 49: 329-337

Pubmed: [Author and Title](#)

CrossRef: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Thiel G, MacRobbie E, Blatt M (1992) Membrane transport in stomatal guard cells: the importance of voltage control. J Membr Biol 126: 1-18

Pubmed: [Author and Title](#)

CrossRef: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Tounsi S, Ben Amar S, Masmoudi K, Sentenac H, Brini F, Véry A-A (2016) Characterization of two HKT1; 4 transporters from Triticum monococcum to elucidate the determinants of the wheat salt tolerance Nax1 QTL. Plant Cell Physiol 57: 2047-2057

Pubmed: [Author and Title](#)

CrossRef: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Véry A-A, Nieves-Cordones M, Daly M, Khan I, Fizames C, Sentenac H (2014) Molecular biology of K⁺ transport across the plant cell membrane: what do we learn from comparison between plant species? J Plant Physiol 171: 748-769

Pubmed: [Author and Title](#)

CrossRef: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Véry A-A, Sentenac H (2003) Molecular mechanisms and regulation of K⁺ transport in higher plants. Annu Rev Plant Biol 54: 575-603

Pubmed: [Author and Title](#)

CrossRef: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Véry A-A, Sentenac H (2002) Cation channels in the Arabidopsis plasma membrane. Trends Plant Sci 7: 168-175

Pubmed: [Author and Title](#)

CrossRef: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Véry AA, Gaynard F, Bosseux C, Sentenac H, Thibaud JB (1995) Expression of a cloned plant K⁺ channel in Xenopus oocytes: analysis of macroscopic currents. Plant J 7: 321-332

Pubmed: [Author and Title](#)

CrossRef: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Wang Y-Y, Hsu P-K, Tsay Y-F (2012) Uptake, allocation and signaling of nitrate. Trends Plant Sci 17: 458-467

Pubmed: [Author and Title](#)

CrossRef: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Willmer C, Fricker M (1996) Topics in plant functional biology: 2- Stomata, M Black & B Charlwood eds (2nd ed), Chapman and Hall publishers, London, UK, 375p

Xu J, Li H-D, Chen L-Q, Wang Y, Liu L-L, He L, Wu W-H (2006) A protein kinase, interacting with two calcineurin B-like proteins, regulates K⁺ transporter AKT1 in Arabidopsis. Cell 125: 1347-1360

Pubmed: [Author and Title](#)

CrossRef: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Zimmerli C, Ribot C, Vavasseur A, Bauer H, Hedrich R, Poirier Y (2012) PHO1 expression in guard cells mediates the stomatal response to abscisic acid in Arabidopsis. Plant J 72: 199-211

Pubmed: [Author and Title](#)

CrossRef: [Author and Title](#)

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)