K^+ uptake and root-to-shoot allocation in *Arabidopsis* require coordination of nitrate transporter1/peptide transporter family member NPF6.3/NRT1.1

Xian Zhi Fang[1,2], Xing Xing Liu[1,2], Ya Xing Zhu[1], Jia Yuan Ye[1], Chong Wei Jin[1, *]

[1]Ministry of Education Key Laboratory of Environmental Remediation and Ecosystem Health, College of Natural Resources and Environmental Science, Zhejiang University, Hangzhou, 310058, China

[2]These authors contributed equally to this work.

*To whom correspondence should be addressed.

Email: jincw@zju.edu.cn

Tel: +(86) 571 88982478

Abstract

K^+ and NO_3^- are the major forms of potassium and nitrogen that are absorbed by the roots of most terrestrial plants. In this study, we observed that the close relationship between NO_3^- and K^+ homeostasis was mediated by nitrate transporter1 (NRT1.1) in *Arabidopsis*. The *nrt1.1* mutants lacking NRT1.1 function showed disturbed K^+ uptake and root-to-shoot allocation, especially under K^+-limited conditions, and had a yellow-shoot sensitive phenotype on K^+-limited medium. The K^+ uptake and root-to-shoot allocation of these mutants were partially rescued by expressing NRT1.1 in the root epidermis-cortex and central vasculature by using *Sultr1;2* and *PHO1* promoters, respectively. Furthermore, two-way analysis of variance based on the K^+ content in *nrt1.1-1*/*akt1*, *nrt1.1-1*/*hak5-3*, *nrt1.1-1*/*kup7*, and *nrt1.1-1*/*skor-2* double mutants and their corresponding single mutants and wild-type plants revealed physiological interactions between NRT1.1 and K^+ channels located in the root epidermis-cortex and central vasculature. Taken together, these data suggest that the expression of NRT1.1 in the root epidermis-cortex coordinates with K^+ uptake channels to improve K^+ uptake, whereas its expression in the root central vasculature coordinates with the channels loading K^+ into the xylem to facilitate K^+ allocation from the roots to the shoot.

Key words: Potassium; Nitrate; NRT1.1; K^+ channels

33 **Introduction**

34 Potassium (K^+) is essential for plant growth and development, and it determines the yield
35 and quality of crops in agriculture production (Wang & Wu, 2013). However, the
36 concentration of soluble K^+ in most soils is relatively low, which often limits plant growth
37 (Maathuis, 2009). Crop production is increased by applying a large amount of potassic
38 fertilizers to agricultural fields, but about one-half of the applied fertilizers is unavailable
39 to plants and left behind as residues in soils, consequently leading to environmental
40 contamination (Meena *et al*., 2016). Thus, improving the K^+ utilization efficiency of
41 plants by thoroughly understanding the molecular mechanisms of K^+ transport and
42 regulation is urgently required. Therefore, in the past few decades, researchers have been
43 focusing on identifying K^+ channels and transporters as well as their regulation
44 mechanisms in plants.

45 In *Arabidopsis thaliana*, 71 K^+ channels and transporters have been identified and
46 categorized into three channel families (shaker, tandem-pore K^+, and Kir-like) and three
47 transporter families (KUP/HAK/KT, HKT, and CPA families) (Wang & Wu, 2010).
48 Among them, the shaker inward K^+ channel AKT1 and the KUP/HAK/KT K^+ transporter
49 HAK5 have been characterized as the two major components that mediate K^+ uptake from
50 the external environment into root cells, but they operate at different K^+ levels (Pyo *et al*.,
51 2010; Wang & Wu, 2013). AKT1 functions in plant K^+ uptake over a wide range of K^+
52 concentrations. Comparatively, HAK5 shows a high-affinity K^+ transport activity (Gierth
53 *et al*., 2005). After its uptake into root epidermal cells, K^+ is then distributed to different
54 organs or tissues of a plant. An *Arabidopsis* shaker-like outward-rectifying K^+ channel,
55 SKOR, was first identified to facilitate K^+ secretion from the stelar cells to the xylem sap
56 in the roots, which is a critical step for long-distance K^+ transport from the roots to the
57 shoots (Gaymard *et al*., 1998). Recently, *At*KUP7, one of the KT/HAK/KUP family
58 members, was functionally characterized as a novel K^+ transporter participating in both
59 K^+ uptake by roots from the growth medium and K^+ release into the xylem for its
60 allocation from roots to shoots, especially under K^+-limited conditions (Han *et al*., 2016).
61 However, the uptake affinity for K^+ of KUP7 is considerably lower than that of *At*HAK5
62 (Wang & Wu, 2017).

63 In addition to the several aforementioned K^+ channels and transporters, many other
64 nutrients such as Na^+, Ca^{2+}, and N also remarkably affect K^+ homeostasis in plants,

65 particularly the association of N with K^+, which has been explored for a long time because
66 N is the mineral element that is required in the greatest quantity by most plants and is the
67 most widely used fertilizer nutrient in crop production (Fageria & Baligar, 2005; Wang &
68 Wu, 2013; Shin, 2017). Since the 1960s, physiological studies have revealed a close
69 relationship between NO_3^- and K^+ with regard to uptake and translocation (Zioni *et al*.,
70 1971; Blevins *et al*., 1978; Barneix & Breteler, 1985; Drechsler *et al*., 2015). However,
71 how these two nutrients coordinate with each other in the transport pathways has not yet
72 been clearly explored at the molecular level. Interestingly, lines of evidence have shown
73 that NRT1.5 from the nitrate transporter1 (NRT1)/peptide transporter (PTR) transporter
74 family (NPF) functions as a component in controlling K^+ allocation in plants (Lin *et al*.,
75 2008; Drechsler *et al*., 2015; Li *et al*., 2017; Du *et al*., 2019), which was initially
76 identified as a pH-dependent bidirectional NO_3^- transporter and is mainly expressed in the
77 root pericycle cells mediating NO_3^- release into the xylem vessels (Lin *et al*., 2008). In
78 this study, we showed that the loss of another nitrate transporter1 member NRT1.1 in
79 *nrt1.1* mutants also led to the development of a more serious K-deficiency phenotype
80 under low-K stress. Further physiological and genetic evidences revealed that both the
81 uptake and root-to-shoot allocation of K^+ in plants require NRT1.1 in plants. However,
82 NRT1.1 acts as a coordinator rather than a K^+ channel/transporter in the above two K^+
83 nutrition issues, which could depend on its transport activity for NO_3^-. Our findings
84 suggest that the modification of NRT1.1 activity in crops by using biological engineering
85 approach might be a promising way for simultaneously improving the utilization
86 efficiencies of K and N fertilizers in agriculture production.

87 **Material and Methods**

88 **Plant material**

89 The mutants *chl1-5* (Huang et al., 1996), *nrt1.1-1* (salk_097431), *nrt1.2* (cs859605),
90 *nrt2.1* (cs859604), *nrt2.2* (salk_043543), *nrt2.5* (GK-213H10.06), *akt1* (salk_071803),
91 *hak5* (salk_130604), *kup7* (cs805085), and *skor* (cs2103489) and the transgenic plants
92 *pNRT1.1::NRT1.1-GFP* and *pNRT1.1::NRT1.1-GUS* (cs6513) are in a Columbia (Col-0)
93 background, whereas the *chl1-6* (cs6154) and *nrt2.4* (cs27332) lines are in a Landsberg
94 *erecta* (*Ler*) background. The seeds of the lines *chl1-5* and *pNRT1.1::NRT1.1-GFP* were
95 kindly gifted by Dr. Philippe Nacry (Biochimie et Physiologie Moléculaire des Plantes,
96 Montpellier, France). The double mutants *nrt1.1-1/akt1*, *nrt1.1-1/hak5-3*, *nrt1.1-1/kup7*,

97 and *nrt1.1-1/skor-2* were generated by crossing *nrt1.1-1* with *akt1*, *hak5-3*, *kup7*, and
98 *skor-2*, respectively. The mutants were verified using the primers listed in Table S1.

99 **Plant culture**

100 The seeds were surface-sterilized and sown on basal agar medium containing 1% sucrose
101 (w/v) and 0.9% (w/v) agar. The complete nutrients in the basal agar medium had the
102 following composition: 1.125 mM $Ca(NO_3)_2$, 500 μM $CaCl_2$, 500 μM $MgSO_4$, 750 μM
103 NaH_2PO_4, 375 μM $(NH_4)_2SO_4$, 25 μM Fe-EDTA, 10 μM H_3BO_3, 0.5 μM $MnSO_4$, 0.5 μM
104 $ZnSO_4$, 0.1 μM $CuSO_4$, and 0.1 μM $(NH_4)_6Mo_7O_{24}$, pH 6.5. After 4 d of germination, the
105 seedlings were used in studies, and the final concentrations of K^+ and NO_3^- were adjusted
106 using KCl and $Ca(NO_3)_2$, as described in the figure legends.

107 **Phenotypic analyses**

108 After 8 d of growth in agar medium containing 2 mM K^+ or 0.05 mM K^+, the phenotype
109 and fresh weight of plants were recorded. The chlorophyll of fresh leaves was extracted
110 with 80% (v/v) acetone and was colorimetrically measured according to Choi *et al*. (2014).
111 In addition, the chlorophyll fluorescence was analyzed using a pulse amplitude-
112 modulating imaging fluorometer (IMAGING-PAM; Germany). Briefly, after a 20 min
113 dark-adaption, the leaves were arranged on the fluorometer, and the maximum quantum
114 efficiency (Fv/Fm) and electron transport rate through PSII (ETR) were monitored.

115 **Measurement of K concentrations**

116 The harvested plants were separated into shoots and roots. Samples were dried at 75°C for
117 48 h, and then wet-digested as described by Luo *et al*. (2012). The digests were diluted
118 with ultrapure water, and the K concentrations were analyzed using MP-AES (Agilent
119 Technologies; USA).

120 **Green fluorescent protein and β-glucuronidase expression analysis**

121 The NRT1.1-green fluorescent protein (GFP) expression in the roots of
122 *pNRT1.1::NRT1.1-GFP* plants was observed using an epifluorescence microscope
123 (excitation, 488 nm; emission, 525–550 nm; Ni-U; Nikon). Histochemical assay of β-
124 glucuronidase (GUS) expression in the roots of *pNRT1.1::NRT1.1-GUS* plants was
125 performed as described by Fang *et al*. (2016), and the distribution and intensity of the blue
126 product were observed under a microscope.

127 **Complementation of *nrt1.1-1* with *pSultr1;2::NRT1.1* and *pPHO1::NRT1.1***

128 The 2 kb genomic fragments upstream of the *PHO1* and *Sultr1;2* initiation codons were,
129 respectively, PCR-amplified on genomic DNA from Arabidopsis by using the primers
130 listed in Table S1. Subsequently, the above two amplified products were cloned upstream
131 of the NRT1.1 coding region by using the pCAMBIA1300 vector. The constructs were
132 transformed in *nrt1.1-1* plants by using the Agrobacterium-mediated floral dip method.
133 Homozygous T4 transgenic plants were used.

134 **Measurement of NO_3^- and K^+ and Rb^+ uptake**

135 Net fluxes of NO_3^- and K^+ were measured in the roots by using a non-invasive
136 microelectrode ion flux measurement system (ipa-2; AE, USA). After 3 d of preculture in
137 the nutrient medium containing 2 mM or 0.05 mM K^+, the flux of NO_3^- and K^+ in each
138 roots section was monitored using NO_3^--selective and K^+-selective microelectrodes, as
139 described by Hawkins *et al.* (2008) and Li *et al.* (2012), respectively. For Rb^+ uptake, the
140 4-d-old seedlings were grown on complete nutrient medium containing 2 mM or 0.05 mM
141 K^+ replaced with Rb^+ for 12 h, and then these plants were harvested and used for Rb
142 concentration analysis.

143 **Transcription analysis**

144 Total RNA in roots was extracted using RNAisoPlus (TaKaRa, Otsu, Japan) and was
145 treated with DNase I to remove genomic DNA contamination. The first-strand cDNA was
146 synthesized from genomic DNA-removed RNA by using PrimeScript™ RT reagent kit
147 (TaKaRa). Next, the transcript levels of the corresponding genes were measured using
148 quantitative real-time PCR by using TB Green™ Premix Ex Taq™ II (TaKaRa). The
149 primers used are listed in Table S1.

150 **RNA sequencing and functional enrichment analysis**

151 Total RNA was extracted from the roots of Col-0 and *nrt1.1-1* plants by using the MagZol
152 Reagent (Magen), according to manufacturer's protocol. Sequencing libraries were
153 prepared using NEBNext Ultra RNA Library Prep Kit for Illumina (NEB, USA), and the
154 quality was assessed using the Agilent 2100 Bioanalyzer. Sequencing was performed on a
155 Hiseq Xten (Illumina) at RIBOBIO (Guangzhou, China). Raw sequencing reads were
156 quality controlled and trimmed using Trimmomatic tools and FastQC, and then the clean

157 reads were aligned to the TAIR10 reference genome by using HISAT2. Significantly
158 differentially expressed genes were assessed using an adjusted *P*-value threshold of <0.05
159 and $|\log_2(\text{fold change})|$ of >1 by using DEGseq. All the genes with significant differential
160 expression were grouped based on k-means clustering and selected for subsequent GO
161 analyses by using R package clusterProfiler.

162 **Grafting of Arabidopsis plants**

163 Grafts between *nrt1.1-1* mutants and Col-0 plants were performed following the protocol
164 described by Andersen *et al*. (2014). Briefly, seedlings to be grafted were grown on
165 complete nutrient agar medium for 5 days. After cotyledons were removed, the seedlings
166 were separated into scion and rootstock by using a sterilized blade. The scion was then
167 grafted to the corresponding rootstock on new agar plates by using forceps under a
168 stereomicroscope. The grafted seedlings were left undisturbed for another 5–7 days to
169 form graft union. The adventitious roots emerging at or above the graft union were
170 eliminated, and then the successful unified seedlings were used in the study as described
171 in the legend of figure 4.

172 **Yeast complementation assay**

173 The protein coding sequence of NRT1.1 was constructed into the pDR196 vector by using
174 primers listed in Table S1. Next, the pDR196-NRT1.1 vector was transformed into the
175 yeast strain R5421 (*trk1Δ*, *trk2Δ*) by using the lithium acetate method (Ito *et al*., 1983).
176 Yeast strain R757 was used as a positive control. The picked single yeast cells were
177 precultured overnight at 30°C in 2 mL liquid YPDA medium containing 100 mM KCl.
178 The cells were collected the following day and washed with ultrapure water three times.
179 Subsequently, the yeast cells were resuspended in ultrapure water to an OD_{600} of 1, and
180 10-fold serial dilutions from OD_{600} of 1 to 10^{-3} were incubated on AP agar plates
181 supplemented with different K^+ concentrations for the experiments.

182 **Results**

183 **NO_3^- facilitates K^+ uptake by roots**

184 We first determined how the NO_3^- supply affects the K levels in Arabidopsis wild-type
185 (Col-0) plants. The presence of NO_3^- in the growth medium increased the K levels in both
186 roots and shoots compared with those in the NO_3^--free treatment (Fig. 1a,b). However, a

187 further increase of NO_3^- supply over 6 mM decreased the K levels in plants. These results
188 indicate that an appropriate NO_3^- supply might favor the uptake of K^+ by roots. Therefore,
189 we calculated the amount of K^+ absorbed per weight of roots and found that these values
190 also increased along with the supply of NO_3^- when the level was less than 6 mM (Fig. S1).
191 To further evaluate how NO_3^- influences K^+ uptake by Col-0 roots, we used the non-
192 invasive micro-test technology (NMT system) to measure the transmembrane K^+ influx
193 and efflux by root cells in a non-invasive manner (Li *et al*., 2012). Although the net K^+
194 influx by root cells was observed in the NO_3^--free medium (Fig. 1c), its average value was
195 only around -30 pmol cm^{-2} s^{-1} (Fig.1d, a negative value indicates influx). Interestingly,
196 addition of 6 mM NO_3^- to the rooting media resulted in a sudden increase in the net K^+
197 influx rate, which reached around -900 pmol cm^{-2} s^{-1} (Fig. 1c). Subsequently, the rate of
198 transmembrane K^+ influx gradually decreased to a relatively stable level, but the average
199 value was still about 7-fold higher than that of the NO_3^--free treatment (Fig. 1c,d). Taken
200 together, these results suggest that the uptake of K^+ by roots might require a coordination
201 of NO_3^- uptake.

202 **Resistance to K deficiency requires NRT1.1**

203 As the uptake of K^+ requires a coordination of NO_3^- uptake in roots, we intended to
204 determine whether the sensitivity of a plant to low K stress is also associated with the root
205 NO_3^- uptake. In *Arabidopsis*, six nitrate transporters, including NRT1.1, NRT1.2, NRT2.1,
206 NRT2.2, NRT2.4, and NRT2.5, have been characterized to be involved in the root NO_3^-
207 uptake (Wang *et al*., 2012; Léran *et al*., 2014; Lezhneva *et al*., 2014). Here we found that,
208 in the growth medium containing 6 mM NO_3^-, the two NRT1.1 knockout mutants *nrt1.1-1*
209 and *chl1-5* exhibited severer leaf senescence than in the wild-type plants (Col-0) after 8 d
210 of low K treatment (0.05 mM K), but these three plant lines did not show distinguishable
211 phenotypes under sufficient K treatment (2 mM K; Fig. 2a) Considering that the decrease
212 of chlorophyll content would affect the photosynthesis of plants, the maximum quantum
213 efficiency of photosystem II (Fv/Fm) and photosynthetic electron transport rate (ETR)
214 were also evaluated as indicators of the photosynthetic reactions in leaves. In the leaves of
215 *nrt1.1-1* and *chl1-5* plants, the Fv/Fm was almost completely abolished (Fig. 2c), and the
216 ETR also declined apparently compared with that in Col-0 plants under low-K conditions
217 (Fig. 2d). Therefore, the *nrt1.1* knockout mutants had both shorter roots and less biomass
218 than the Col-0 plants after low-K treatment (Figs 2e,f, S2). In addition, we examined a
219 third NRT1.1-null mutant, *chl1-6*, the fresh weight and root elongation of which were also

220 significantly reduced compared with those of its corresponding wild-type *Ler* under low-
221 K conditions (Fig. S3a,b). Since NRT1.1 can act as a high-affinity as well as low-affinity
222 system (Wang *et al*., 2012; Léran *et al*., 2014), the growth of these NRT1.1-null mutants
223 was also evaluated in low-K growth medium containing 0.2 mM NO_3^-. However, the
224 phenotypes of these mutants were indistinguishable from those of wild-type plants (Fig.
225 S4a,b). These results suggest that NRT1.1 plays an important role in plants to confer
226 resistance to K deficiency, but it requires a sufficient supply of NO_3^- in the growth
227 medium.

228 We next determined the roles of the other five NRTs in plant growth responses to low-K
229 stress. Since NRT2.1, NRT2.2, NRT2.4, and NRT2.5 act as high-affinity NO_3^-
230 transporters, the growth of these NRTs-null mutants was evaluated in a growth medium
231 containing 0.2 mM NO_3^-. However, the fresh weight and root elongation of *nrt2.1*, *nrt2.2*,
232 *nrt2.4*, and *nrt2.5* mutants were similar to those of the corresponding wild-type plants in
233 both low-K and sufficient-K treatments (Fig. S5). Although NRT1.2 is a low-affinity
234 NO_3^- transporter (Huang *et al*., 1999), the *nrt1.2* mutant showed similar fresh weight and
235 root elongation as those of wild-type plants in both low-K and sufficient-K growth
236 medium containing 6 mM NO_3^- (Fig. S5e,j). These results indicate that NRT1.2, NRT2.1,
237 NRT2.2, NRT2.4, and NRT2.5 do not act like NRT1.1 when conferring resistance to K
238 deficiency.

239 **NRT1.1 is required for homeostasis of K in plants**

240 We next emphasized on how NRT1.1 affects K nutrition in plants. In both sufficient-K
241 and low-K treatments, the K levels were significantly lower in *nrt1.1-1*, *chl1-5*, and *chl1-*
242 *6* mutants than in their crossponding wild-type plants (Figs 3a,b, S3c,d), indicating that
243 the lack of NRT1.1 function disturbed the K homeostasis in plants. To provide further
244 support for the role of NRT1.1 in K homeostasis, we measured the K levels in the
245 transgenic plants *pNRT1.1::NRT1.1-GFP*, which are in the *chl1-5* mutant background
246 (Krouk *et al*., 2010). In both sufficient-K and low-K treatments, the *pNRT1.1::NRT1.1-*
247 *GFP* transgenic plants contained higher K levels than those in the *chl1-5* mutants and
248 similar K levels as those in Col-0 plants (Fig. 3c,d). Therefore, phenotype analysis
249 showed that complementation of NRT1.1 conferred the transgenic plants tolerance to K^+
250 deficiency, as evidenced by the similar chlorophyll content, root elongation, and fresh
251 weight as those in Col-0 plants (Figs 3e, S6).

252 As described above, the uptake of K^+ by roots requires a coordination of NO_3^- uptake.
253 However, when the plants were grown in the medium containing low NO_3^- (0.2 mM), no
254 significant difference in both growth and K levels was noted between *NRT1.1-null*
255 mutants and wild-type plants in both sufficient-K and low-K treatments (Fig. S4). The
256 result suggested that the role of NRT1.1 in maintaining K homeostasis in plants depends
257 on sufficient NO_3^- supply. We also investigated the role of the other NRTs in maintaining
258 K homeostasis. Nevertheless, the K levels in the roots and shoots of *nrt1.2, nrt2.1*, *nrt2.2,*
259 *nrt2.4*, and *nrt2.5* mutants were not obviously different from those of their corresponding
260 wild-type plants in sufficient-K or low-K treatments (Fig. S8). Thus, NRT1.1 might
261 improve the K nutrition in a relatively specific manner.

262 **Root is the action point for NRT1.1 to improve K nutrition in plants, and low-K**
263 **stress stimulates the activity of NRT1.1 in roots**

264 NRT1.1 is expressed in both roots and shoots (Guo *et al*., 2001). Therefore, homografted
265 (Col-0 scion/Col-0 stock and *nrt1.1-1* scion/*nrt1.1-1* stock) and heterografted (Col-0
266 scion/*nrt1.1-1* stock and *nrt1.1-1* scion/Col-0 stock) plants were generated to clarify
267 whether either or both the roots and shoots are the action point for NRT1.1 to improve K
268 nutrition in plants. The leaves of Col-0/*nrt1.1-1* and *nrt1.1-1*/*nrt1.1-1* plants developed
269 severely chlorotic phenotype after 8 d of being grown on low-K medium (Fig. 4a).
270 However, *nrt1.1-1*/Col-0 and Col-0/Col-0 did not exhibit any chlorotic symptom. This
271 result suggested that the absence of the root-part function of NRT1.1 was primarily
272 responsible for the sensitive symptom. This conclusion was further supported by the
273 observation that K levels were clearly reduced in the shoots of the plants grafted with
274 *nrt1.1-1* root stock in low-K treatment (Fig. 4c). Furthermore, we found that the K levels
275 in both the shoots and roots of Col-0/*nrt1.1-1* and *nrt1.1-1*/*nrt1.1-1* plants were
276 significantly lower than those in the plants grafted with Col-0 rootstocks in the sufficient
277 K treatment (Fig. 4b). These results suggested that only the root part is the action point for
278 NRT1.1 to improve K nutrition in plants.

279 The role of root NRT1.1 in improving plant K nutrition prompted us to investigate how
280 NRT1.1 in roots responds to low-K stress. Real-time quantitative PCR analysis showed
281 that low-K treatment clearly increased the expression of *NRT1.1* in roots (Fig. 4d),
282 whereas the expression of the other five NO_3^- uptake transporter genes was either
283 decreased (*NRT2.1*) (Fig. S9a) or not significantly affected (*NRT1.2*, *NRT2.2*, *NRT2.4*,

284 and *NRT2.5*; Fig. S9b-e), suggesting that low-K stress might have a specific effect on the
285 action of NRT1.1. Consistent with gene expression, the NRT1.1-GFP protein expression
286 indicated by GFP-associated fluorescence in the roots of *pNRT1.1::NRT1.1-GFP*
287 transgenic plants was elevated in the low-K treatment than in the sufficient-K treatment
288 (Fig. 4e). The result was confirmed by GUS staining of the roots of *pNRT1.1::NRT1.1-*
289 *GUS* transgenic plants (Fig. 4e). These findings suggested that the activity of NRT1.1
290 could be up-regulated in response to low K^+ stress. Thus, we analyzed the rate of net NO_3^-
291 fluxes at the surface of the maturation, elongation, and meristematic zones of Col-0 roots
292 by using the NMT system. In all the three measured zones, the plants precultured with
293 low-K medium had a higher rate of net NO_3^- influx than in those precultured with
294 sufficient-K medium in the same testing medium (Fig. 4f), providing a direct evidence
295 that low-K stress stimulates NO_3^- uptake activity, which should be associated with an up-
296 regulation of NRT1.1 due to low-K stress.

297 **Both uptake and root-to-shoot allocation of K require a coordination of NRT1.1**

298 We next determined how NRT1.1 improves K nutrition in plants by investigating its role
299 in the root K uptake. The alkaline ion Rb^+, the closest analog of K^+, was first used to
300 evaluate the K^+ uptake by roots. The rates of root Rb^+ uptake in *chl1-5* and *nrt1.1-1*
301 mutants were considerably less than those in Col-0 plants in the medium containing either
302 2 mM Rb^+ or 0.05 mM Rb^+ (Fig. 5a), indicating that the loss of NRT1.1 function might
303 disturb the uptake of K^+ by roots. To further evaluate the contribution of NRT1.1 in root
304 K^+ uptake, we measured the root K^+ fluxes of the above three plant lines. We found that,
305 at the elongation and maturation zones, the rates of net K^+ influx of *nrt1.1-1* and *chl1-5*
306 mutants were less than 50% of those obtained in the Col-0 plants in both 2 mM K^+ and
307 0.05 mM K^+ testing medium. However, the net K^+ influx rate in the meristematic zone did
308 not differ significantly among these plants (Fig. 5b,c). These results suggest that the
309 elongation and maturation zones, but not the meristematic zone, are the target regions for
310 NRT1.1 to contribute to K^+ uptake by root cells.

311 Consistent with its role in NO_3^- uptake, NRT1.1 was expressed in the epidermis and
312 cortex of roots (Huang *et al*., 1996). However, NRT1.1 was also expressed in the root
313 central vasculature (Remans *et al*., 2006), revealing its another function: allocating root
314 NO_3^- to shoot parts through xylem NO_3^- loading in the root stele (Léran *et al*., 2013). This
315 additional function suggests that NRT1.1 might also play a role in root-to-shoot allocation

316 of K. Therefore, the rates of K distributed in the root and shoot were calculated. However,
317 the proportion of K distributed in either shoots or roots did not differ between the Col-0
318 plants and *nrt1.1* knockout mutants when they were grown in sufficient-K medium (Fig.
319 5d). Interestingly, both *chl1-5* and *nrt1.1-1* mutants had less proportion of K distributed in
320 the shoots, but greater proportion of K distributed in the roots compared with those in
321 Col-0 plants grown in the low-K medium (Fig. 5d). These results indicated that a K-
322 insufficient status of plants allows NRT1.1 to play the distinct role in root-to-shoot
323 allocation of K.

324 **Action of NRT1.1 in favoring uptake and root-to-shoot allocation of K, respectively,**
325 **depends on its specific expression in the epidermis-cortex and central vasculature of**
326 **roots**

327 As NRT1.1 is expressed in both the epidermis-cortex and central vasculature of roots
328 (Huang *et al*., 1996; Remans *et al*., 2006), we intended to determine whether such
329 expression pattern correlates with its dual roles in coordinating the uptake and root-to-
330 shoot allocation of K^+. To address this question, we generated transgenic plants
331 expressing NRT1.1 under the control of the promoters of *Sultr1;2* and *PHO1* genes,
332 respectively, in the *nrt1.1-1* mutant background. *Sultr1;2* is expressed in the root
333 epidermis and cortex (Yoshimoto, 2002), whereas *PHO1* is expressed in the root central
334 vasculature (Hamburger *et al.,* 2002; Wege & Poirier, 2014). Therefore, the uses of these
335 two promoters to drive *NRT1.1* gene in transgenic plants could, respectively, ensure
336 NRT1.1 expression in the epidermis-cortex and central vasculature of roots, although their
337 expression levels might not well match with those in the wild-type plants. The
338 determination of K levels in two independent *pSultr1;2::NRT1.1* transgenic lines in
339 comparison to those in Col-0 and *nrt1.1-1* plants revealed partial recovery of K to wild-
340 type levels in the plants in both sufficient-K and low-K treatments (Fig. 6a,b). However,
341 the calculation of the ratio of K distributed in the root and shoot showed that
342 *pSultr1;2::NRT1.1* complementation in *nrt1.1-1* plants did not improve the root-to-shoot
343 allocation of K (Fig. 6c). Therefore, the expression of NRT1.1 in the epidermis and cortex
344 might only be responsible for improving the K uptake.

345 The measurement of K levels in other two independent *pPHO1::NRT1.1* transgenic lines
346 also showed partial recovery of K to Col-0 wild-type levels in the plants in both K
347 treatments, with the recovery being more distinct in the shoots (Fig. 6d,e). Furthermore,

348 the ratio of K distributed in the root and shoot revealed that the complementation of
349 *pPHO1::NRT1.1* in *nrt1.1-1* plants increased the K transport from root to shoot (Fig. 6f),
350 suggesting that the expression of NRT1.1 in the central vasculature of roots is responsible
351 for supporting the root-to-shoot allocation of K.

352 **Action of NRT1.1 in favoring the uptake and root-to-shoot allocation of K,**
353 **respectively, depends on the K^+ channels in the epidermis-cortex and central**
354 **vasculature of roots**

355 Next, we investigated how the expression of NRT1.1 in the epidermis-cortex and central
356 vasculature, respectively, coordinated the uptake and root-to-shoot allocation of K. In
357 addition to a role in mediating nitrate transport across the plasmalemma, NRT1.1 was
358 found to act as a singling component in regulating the expression of numerous genes in
359 plants (Ho *et al*., 2009; Bouguyon *et al*., 2015; Wang *et al*., 2018). Therefore, the
360 difference in whole-genome gene expression profiles between Col-0 plants and *nrt1.1*
361 mutants in response to different K treatments were compared using RNA-seq. Gene
362 ontology (GO) analysis revealed that the differentially expressed genes (DEGs) were
363 involved in many physiological processes such as response to nutrient levels, response to
364 reactive oxygen species, and response to salicylic acid (Fig. S10). However, among the
365 genes associated with K uptake and trafficking, only the expression of *SKOR*, which
366 mediates K^+ loading into the xylem (Gaymard *et al*., 1998; Drechsler *et al*., 2015), was
367 inhibited by NRT1.1 mutation in both sufficient-K and low-K treatments, whereas that of
368 the other genes was either increased or not affected (Fig. 7a). This result cannot explain
369 the decreased root K uptake due to the loss of function of NRT1.1. Therefore, we also
370 investigated whether NRT1.1 acts a K^+ channel. To functionally test the role of NRT1.1
371 in K^+ transport activity in a heterologous expression system, we used the yeast
372 (*Saccharomyces cerevisiae*) mutant R5421, lacking the main K^+ uptake systems (*trk1Δ,*
373 *trk2Δ*; Gaber *et al*., 1988; Han *et al*., 2016). However, NRT1.1 could not complement the
374 growth retardation of R5421 under K deficiency (Fig. 7b), refuting the K^+ transport
375 activity of NRT1.1.

376 Theoretically, the flux of anions across the membrane should be accompanied by an
377 orthokinetic flux of cations so as to maintain the ionic balance, and this process might
378 depend on the cooperation between anion transporters/channels and cation
379 transporters/channels. In this context, the action of NRT1.1-mediated NO_3^- flux across the

380 plasmalemma might collaborate wih K^+ channels. To test this hypothesis, we generated
381 *nrt1.1-1/akt1*, *nrt1.1-1/hak5-3*, *nrt1.1-1/skor-2*, and *nrt1.1-1/kup7* double mutants by
382 crossing the NRT1.1-null mutant *nrt1.1-1* with *akt1*, *hak5-3*, *skor-2*, and *kup7* mutants
383 and measured K levels in these plants. AKT1, HAK5, and KUP7 are the three
384 characterized components that contribute to K^+ uptake in *Arabidopsis* roots, and the latter
385 two channels only have a significant contribution in K uptake under K-limited condition
386 (Lagarde *et al*., 1996; Gierth *et al*., 2005; Han *et al.,* 2016). In addition, KUP7 was found
387 to be involved in K^+ loading into the xylem in addition to the SKOR channel (Han *et al*.,
388 2016). Two-way analysis of variance (ANOVA) showed that the difference of K levels in
389 the shoots and roots between *akt1* and *akt1/nrt1.1-1* mutants was significantly less than
390 that between Col-0 and *nrt1.1-1* in both K treatments (Fig. 8a,b), suggesting that the
391 reduced K levels due to the loss of NRT1.1 was associated with the action of AKT1.
392 Similarly, two-way ANOVA based on the data obtained in Col-0, *nrt1.1-1, hak5-3*,
393 *nrt1.1-1/hak5-3*, *kup7*, and *nrt1.1-1/kup7* plants showed that the reduced K levels due to
394 the loss of NRT1.1 were also associated with HAK5 and KUP7 in low-K medium (Fig.
395 8c-e). These results indicated that NRT1.1 should interplay with the K uptake channels in
396 the root epidermis-cortex to improve the plant K nutrition. Next, we determined whether
397 the KUP7 and SKOR channels are required for the action of NRT1.1 in favoring root-to-
398 shoot allocation of K under low K condition. Two-way ANOVA showed that the
399 difference in the proportion of K distributed from the roots to shoots either between *skor-*
400 *2* and *nrt1.1-1/skor-2* or between *kup7* and *nrt1.1-1/kup7* was significantly less than that
401 between Col-0 and *nrt1.1-1* (Fig. 8f,h). These results indicate that the action of NRT1.1 in
402 favoring root-to-shoot allocation of K depends on the K^+ channels that mediate K^+
403 loading into the xylem.

404

405 **Discussion**

406 K^+ and NO_3^- are the major forms of potassium and nitrogen that are absorbed by roots of
407 most terrestrial plants. In addition, they, respectively, represent the most abundant
408 inorganic cation and anion sources in plant cells, and are thus also the major forms of
409 potassium and nitrogen that are transported within plants (Du *et al*., 2019). In this study,
410 we revealed a close relationship between NO_3^- and K^+ homeostasis, which was mediated
411 by the NO_3^- transporter NRT1.1, in the root tissues of *Arabidopsis*.

412 NRT1.1 was initially characterized as an NO_3^- transporter involved in root NO_3^- uptake
413 from the growth medium (Tsay *et al*., 1993). Further, it was found to be a bidirectional
414 transporter (Remans *et al*., 2006), thereby acting as a component involved in the xylem
415 NO_3^- loading in the root stele, which confers the root-to-shoot allocation of NO_3^- (Léran *et*
416 *al*., 2013). Several studies have revealed many additional functions such as regulation of
417 another NO_3^- transporter NRT2.1 (Ho *et al*., 2009), auxin transport (Krouk *et al*., 2010),
418 and regulation of tolerance to NH_4^+ toxicity (Jian *et al*., 2018). These functions of NRT1.1
419 are independent of its NO_3^- transport activity, because the absence of NO_3^- supply in the
420 growth medium did not affect the above functions. However, our finding that wild-type
421 plants did not show higher K levels in both shoots and roots than in *nrt1.1* knockout
422 mutants when NH_4^+ was the sole nitrogen source shows that the activity of NO_3^- transport
423 across the plasmalemma is probably necessary for NRT1.1 to coordinate K^+ homeostasis
424 in plants (Fig. S7c,d). Theoretically, NO_3^- transport-coordinated K^+ homeostasis in plants
425 follows a general mechanism. However, the other nitrate uptake NRTs did not act like
426 NRT1.1 when coordinating K^+ homeostasis in plants. Previous studies showed that
427 NRT1.1 contributes to > 70% of the total root NO_3^- uptake in NO_3^--sufficient medium
428 (Huang *et al*., 1996; Wang *et al*., 1998), suggesting that the other NRTs (i.e., NRT1.2,
429 NRT2.1, NRT2.2, NRT2.4, and NRT2.5) might just be responsible for < 30% of NO_3^-
430 uptake. Therefore, an insufficient NO_3^- transport of any of these five NRTs might explain
431 why none of them plays an evident role in improving K^+ homeostasis in plants. This also
432 suggests that the action of NRT1.1 to improve K^+ homeostasis might require a higher
433 NO_3^- supply to ensure sufficient NO_3^- transport. This notion was supported by the
434 observation that the increase of NO_3^- supply elevated the K levels in wild-type plants that
435 have a normal NRT1.1 function (Fig. 1a,b). In addition, the finding that NRT1.1-null
436 mutants did not have distinguishable growth phenotype and K levels compared with those
437 of wild-type plants in low NO_3^- growth medium (0.2 mM NO_3^-; Fig S4) further supports
438 the above notion.

439 Interestingly, the coordination of K^+ homeostasis by NRT1.1 in *Arabidopsis*, including
440 root uptake and root-to-shoot allocation processes, depend on the nature of NRT1.1
441 expression pattern in the root epidermis-cortex and central vasculature, respectively.
442 Hence, determining how such expression pattern of NRT1.1 in root tissues improves the
443 uptake and root-to-shoot allocation of K^+ becomes necessary. Previously, NRT1.1 and
444 other NRT transporters were shown to be a kind of proton-coupled transporters that

445 transport NO_3^- across the plasmalemma together with an equal amount of proton (H^+)
446 influx via a symport mechanism (Tsay *et al*., 1993; Fang *et al*., 2016). However, the H^+
447 levels in the regular growth media were considerably less than those of NO_3^-. Thus, an H^+
448 efflux mechanism of root cells should be required to compensate for the consumed H^+ due
449 to NO_3^- uptake, which consequently ensures the subsequent NO_3^- uptake. The uptake of
450 K^+ across the plasmalemma by K^+ channels of the root cortex cells is known to be
451 electrically coupled to H^+-ATPase-mediated H^+ efflux (Behl & Raschke, 1987; Wang &
452 Wu, 2013). Since K^+ is the most abundant inorganic cation absorbed by roots, the amount
453 of H^+ efflux owing to K^+ uptake could be a key compensation to the consumed H^+ by
454 NO_3^- uptake, which might also be a mechanism to retain the charge balance during NO_3^-
455 –K^+ fluxes across the plasmalemma and thus to ensure a normal electrophysiological
456 environment in the cells. In this context, NRT1.1 is probably coordinated by K^+ channels
457 in the root epidermis-cortex. Our two-way ANOVA based on K levels in *nrt1.1*/*akt1*,
458 *nrt1.1*/*hak5*, and *nrt1.1*/*kup7* double mutants and their corresponding monomutants and
459 wild-type plants supported the above notion, that is, the NRT1.1 interplay with K^+
460 channels in the root epidermis-cortex could improve the plant K uptake (Fig. 8a,b,c,e). As
461 all the investigated K^+ channels could physiologically interplay with NRT1.1 to improve
462 K uptake, we suggest that these interplays should function in a nonspecific manner.

463 The two-way ANOVA based on K distribution in the shoot and root tissues of *nrt1.1/skor*
464 and *nrt1.1*/*kup7* double mutants and their corresponding monomutants and wild-type
465 plants also revealed a physiological interaction between NRT1.1 and K^+ channels in the
466 root central vasculature, which improves the root-to-shoot allocation of K (Fig. 8e-h). In
467 addition to this mechanism, transcript regulation by NRT1.1 might also play a role in
468 improving the root-to-shoot allocation of K, because the expression of *SKOR* was
469 positively regulated by NRT1.1 (Fig. 7a). We also analyzed the K distribution in the shoot
470 and root tissues of *nrt1.1/akt1* and *nrt1.1/hak5* double mutants and their corresponding
471 monomutants and wild-type plants, but no physiological interaction was noted between
472 NRT1.1 and AKT1 or HAK5 in improving the root-to-shoot allocation of K, as revealed
473 by the two-away ANOVA (Fig. S11c,d), indicating that the K^+ uptake channels might not
474 be involved in the NRT1.1-coordinated root-to-shoot allocation of K. Notably, the
475 contribution of NRT1.1 to the root-to-shoot allocation of K^+ is only remarkable under the
476 K-limited condition, but not under the K-sufficient condition (Fig. 5d). Our finding that
477 insufficient NO_3^- supply did not efficiently support the role of NRT1.1 in the uptake and

478 root-to-shoot allocation of K^+ (Fig. 4e) indicated that a higher ratio of NO_3^- to K^+
479 transport across the cell plasmalemma might be a prerequisite for NRT1.1 to remarkably
480 contribute the root-to-shoot allocation of K^+ in plants. As the K^+ in plants could be easily
481 allocated to shoots after its root uptake from the growth medium (De Boer, 1999), the
482 amount of K^+ loading into the root xylem needs to be very higher under the K-sufficient
483 condition, which results in a lower ratio of NO_3^- to K^+ transport across the cell
484 plasmalemma in the root central vasculature. This might explain why the contribution of
485 NRT1.1 to the root-to-shoot allocation of K^+ is not remarkable under the K-sufficient
486 condition.

487 As both the uptake and root-to-shoot allocation of K^+ were significantly improved by
488 NRT1.1 under the K-limited condition (Figs 3b, 5d), the *nrt1.1* knockout mutants had
489 shorter roots, less biomass, and severer leaf senescence, compared with the wild-type
490 plants in the low-K growth medium (Fig. 2e,f,a), indicating that NRT1.1 is required for
491 plants to resist K deficiency. Owing to the limited availability of K^+ in most natural soils,
492 plants often suffer from K^+-deficiency stress (Wang & Wu, 2013). Therefore, plants have
493 to evolve a complex signaling and physiological regulatory network to adapt to K^+-
494 deficient environments, which helps them survive under K^+-deficiency stress (Ashley *et*
495 *al.,* 2006; Tsay *et al*., 2011). We found that the activity of NRT1.1-mediated NO_3^- uptake
496 was significantly up-regulated in response to low-K^+ stress (Fig. 4f). Considering a role of
497 NRT1.1 in improving both root uptake and root-to-shoot allocation of K^+ in plants, the
498 up-regulation of NRT1.1 activity by low-K^+ stress could be recognized as an adaption
499 mechanism for plants to help them survive in K^+-deficient environments. Therefore, for
500 crop cultivation, designing other more practical methods for improving NRT1.1-mediated
501 NO_3^- uptake and increasing the utilization efficiency of K fertilizers is necessary.
502 However, how low-K^+ stress up-regulates NRT1.1 activity is not yet known. In our study,
503 we observed that the rate of root NO_3^- uptake in low-K medium was clearly less than that
504 in sufficient-K medium (Fig. 4f). Therefore, an insufficient K supply might result in mild
505 nitrogen starvation in plants, which could favor an up-regulation of NRT1.1 to
506 compensate for the decreased NO_3^- uptake. Nevertheless, further studies are required to
507 experimentally verify theis assumption.

508 In conclusion, we showed that the NO_3^- transport activity of NRT1.1 plays an important
509 role in the K nutrition in plants: its expression in root epidermis-cortex coordinates with
510 the K^+ uptake channels such as AKT1, HAK5, and KUP7 to improve the root K^+ uptake

511 from the growth medium, whereas its expression in the root central vasculature
512 coordinates with the channels that load K^+ into the xylem, such as SORK and KUP7, to
513 facilitate the root-to-shoot allocation of K^+ (Fig. 9). In the current agricultural practices in
514 China, excessive N and K fertilizers are usually applied by farmers; this reduces the
515 utilization efficiencies of these fertilizers and results in environmental pollution (Guo *et*
516 *al*., 2010; Zhang, 2017). Our findings indicate that designing practical methods for
517 enhancing NRT1.1 activity in crops might be possible to improve simultaneously the
518 utilization efficiencies of N and K fertilizers in agricultural production.

519 **Acknowledgments**

520 The authors thank Dr. Philippe Nacry for sharing seeds and Dr. Yi Wang (China
521 Agricultural University) for providing the yeast strains. This work was financially
522 supported by the Natural Key R&D Program of China [2016YFD0200103], the Natural
523 Science Foundation of China [31622051 and 31670258], and the Fundamental Research
524 Funds for the Central Universities [2017XZZX002-06].

525 **Author Contributions**

526 X.Z.F., X.X.L. and C.W. J. conceived the project and designed the experiments; C.C. and
527 X.X.L performed most of the experiments and analyzed data; Y.X.Z. and J.Y.Y. helped
528 with generation of double mutants; X.Z.F. and C.W. J. wrote the article with contributions
529 from all authors.

530 **Supporting Information**

531 **Fig. S1** Effect of NO_3^- on the amount of K^+ absorbed per weight of roots in Col-0 plants.
532 **Fig. S2** Root growth of *Arabidopsis thaliana* Col-0, *nrt1.1-1*, and *chl-5* plants to low-K^+
533 stress.
534 **Fig. S3** Comparisons of growth and K concentration between *Ler* and *chl1-6* plants.
535 **Fig. S4** Comparisons of growth and K concentrations between Col-0 and *nrt1.1* plants in
536 low nitrate medium.
537 **Fig. S5** Growth responses of Col-0, *nrt1.2*, *nrt2.1*, *nrt2.2*, *nrt2.5*, *Ler*, and *nrt2.4* plants to
538 low-K^+ stress.
539 **Fig. S6** Comparison of low-K sensitivity between *chl1-5* mutants and complementation
540 line.

541 **Fig. S7** Comparisons of growth and K concentration between Col-0 and *nrt1.1* plants in
542 nitrate-free medium.
543 **Fig. S8** The K concentrations in Col-0, *nrt1.2*, *nrt2.1*, *nrt2.2*, *nrt2.5*, *Ler*, and *nrt2.4* plants.
544 **Fig. S9** Effect of low-K^+ stress on the expression of *NRTs* in the root of Col-0 plants.
545 **Fig. S10** Heatmap analysis of changes in gene expression regulated by K in the roots of
546 Col-0 and *nrt1.1-1* plants.
547 **Fig. S11** Roles of K^+ channels in NRT1.1-imrpoved K nutrition is associated with the
548 biological function of K^+ channels.
549 **Table S1** Primers used in this study.
550

551 **References**

552 **Andersen TG, Liang D, Halkier BA, White R. 2014.** Grafting *Arabidopsis*. Bio-
553 protocol 4(13): e1164. DOI: 10.21769/BioProtoc.1164.

554 **Ashley MK, Grant M, Grabov A. 2006.** Plant responses to potassium deficiencies: a
555 role for potassium transport proteins. *Journal of Experimental Botany* **57:** 425–36.

556 **Barneix AJ, Breteler H. 1985.** Effect of cations on uptake, translocation and reduction of
557 nitrate in wheat seedlings. *New Phytologist* **99:** 367-379.

558 **Behl R, Raschke K. 1987.** Close coupling between extrusion of H^+ and uptake of K^+ by
559 barley roots. *Planta* **172(4):** 531-538.

560 **Blevins DG, Barnett NM, Frost WB. 1978.** Role of potassium and malate in nitrate
561 uptake and translocation by wheat seedlings. *Plant Physiology* **62:** 784-788.

562 **Bouguyon E, Brun F, Meynard D, Kubeš M, Pervent M *et al.* 2015.** Multiple
563 mechanisms of nitrate sensing by *Arabidopsis* nitrate transceptor NRT1. 1. *Nature Plants*
564 **1(3):** 15015.

565 **Choi WG, Toyota M, Kim SH, Hilleary R, Gilroy S. 2014.** Salt stress-induced Ca^{2+}
566 waves are associated with rapid, long distance root-to-shoot signaling in plants. *Proc. Natl.*
567 *Acad. Sci. USA* **111:** 6497–6502.

568 **De Boer AH. 1999.** Potassium translocation into the root xylem. *Plant Biology* **1(01):** 36-
569 45.

570 **Drechsler N, Zheng Y, Bohner A, Nobmann B, von Wirén N, Kunze R, Rausch C**
571 **2015.** Nitrate-dependent control of shoot K homeostasis by the nitrate
572 transporter1/peptide transporter family member NPF7. 3/NRT1. 5 and the stelar K^+
573 outward rectifier SKOR in Arabidopsis. *Plant Physiology* **169(4):** 2832-2847.

574 **Du XQ, Wang FL, Li H, Jing S, Yu M *et al.* 2019.** The transcription factor MYB59
575 regulates K^+/NO_3^- translocation in the *Arabidopsis* response to low K^+ stress. *The Plant*
576 *Cell* tpc-00674.

577 **Fageria NK, Baligar VC. 2005.** Enhancing nitrogen use efficiency in crop
578 plants. *Advances in Agronomy* **88:** 97-185.

579 **Fang XZ, Tian WH, Liu XX, Lin XY, Jin CW, Zheng SJ. 2016.** Alleviation of proton
580 toxicity by nitrate uptake specifically depends on nitrate transporter 1.1 in *Arabidopsis*.
581 *New Phytologist* **211(1):** 149-158.

582 **Gaber RF, Styles CA, Fink GR**. **1988.** TRK1 encodes a plasma membrane protein
583 required for high-affinity potassium transport in *Saccharomyces cerevisiae*. *Molecular*
584 *and Cellular Biology* **8:** 2848–2859.

585 **Gaymard F, Pilot G, Lacombe B, Bouchez D, Bruneau D *et al.* 1998.** Identification
586 and disruption of a plant shaker-like outward channel involved in K^+ release into the
587 xylem sap. *Cell* **4:** 647–655.

588 **Gierth M, Mäser P, Schroeder JI. 2005.** The potassium transporter AtHAK5 functions
589 in K^+ deprivation-induced high-affinity K^+ uptake and AKT1 K^+ channel contribution to
590 K^+ uptake kinetics in *Arabidopsis* roots. *Plant Physiology* **137(3):** 1105-1114.

591 **Guo FQ, Wang R, Chen M, Crawford NM. 2001.** The *Arabidopsis* dualaffinity nitrate
592 transporter gene AtNRT1.1 (CHL1) is activated and functions in nascent organ
593 development during vegetative and reproductive growth. *The Plant Cell* **13:** 1761–1777.

594 **Guo JH, Liu XJ, Zhang Y, Shen JL., Han WX *et al.* 2010.** Significant acidification in
595 major Chinese croplands. *Science* **327:** 1008-1010.

596 **Hamburger D, Rezzonico E, Petétot JMC, Somerville C, Poirier Y. 2002.**
597 Identification and characterization of the *Arabidopsis* PHO1 gene involved in phosphate
598 loading to the xylem. *The Plant Cell* **14(4):** 889-902.

599 **Han M, Wu W, Wu WH, Wang Y. 2016.** Potassium transporter KUP7 is involved in K^+
600 acquisition and translocation in *Arabidopsis* root under K^+-limited conditions. *Molecular*
601 *Plant* **9(3):** 437-446.

602 **Hawkins BJ, Boukcim H, Plassard C. 2008.** A comparison of ammonium, nitrate and
603 proton net fluxes along seedling roots of *Douglas-fir* and *lodgepole pine* grown and
604 measured with different inorganic nitrogen sources. *Plant, Cell and Environment* **31:**
605 278–287.

606 **Ho CH, Lin SH, Hu HC, Tsay YF. 2009.** CHL1 functions as a nitrate sensor in plants.
607 *Cell* **138(6):** 1184-1194.

608 **Huang NC, Chiang CS, Crawford NM, Tsay YF. 1996.** CHL1 encodes a component of
609 the low-affinity nitrate uptake system in *Arabidopsis* and shows cell type-specific
610 expression in roots. *The Plant Cell* **8:** 2183-2191.

611 **Huang NC, Liu KH, Lo HJ, Tsay YF. 1999.** Cloning and functional characterization of
612 an *Arabidopsis* nitrate transporter gene that encodes a constitutive component of low-
613 affinity uptake. *The Plant Cell* **11(8):** 1381-1392.

614 **Ito H, Fukuda Y, Murata K, Kimura A. 1983.** Transformation of intact yeast cells
615 treated with alkali cations. *Journal of Bacteriology* **153:** 163-168.

616 **Jian S, Liao Q, Song H, Liu Q, Lepo JE, Guan C, Zhang J, Ismail AM, Zhang Z.**
617 **2018.** NRT1. 1-Related NH_4^+ toxicity is associated with a disturbed balance between
618 NH_4^+ uptake and assimilation. *Plant physiology* **178(4):** 1473-1488.

619 **Krouk G, Lacombe B, Bielach A, Perrine-Walker F, Malinska K *et al.* 2010.** Nitrate-
620 regulated auxin transport by NRT1. 1 defines a mechanism for nutrient sensing in plants.
621 *Developmental Cell* **18(6):** 927-937.

622 **Lagarde D, Basset M, Lepetit M, Conejero G, Gaymard F, Astruc S, Grignon C.**
623 **1996.** Tissue-specific expression of *Arabidopsis* AKT1 gene is consistent with a role in
624 K^+ nutrition. *The Plant Journal* **9(2):** 195-203.

625 **Léran S, Muños S, Brachet C, Tillard P, Gojon A, Lacombe B. 2013.** *Arabidopsis*
626 NRT1. 1 is a bidirectional transporter involved in root-to-shoot nitrate translocation.
627 *Molecular Plant* **6(6):** 1984-1987.

628 **Léran S, Varala K, Boyer JC, Chiurazzi M, Crawford N, Daniel-Vedele F *et al.* 2014.**
629 A unified nomenclature of nitrate transporter1/peptide transporter family members in
630 plants. *Trends in Plant Science* **19(1):** 5-9.

631 **Lezhneva L, Kiba T, Feria-Bourrellier A B, Lafouge F, Boutet-Mercey S *et al.* 2014.**
632 The *Arabidopsis* nitrate transporter NRT 2.5 plays a role in nitrate acquisition and
633 remobilization in nitrogen-starved plants. *The Plant Journal* **80(2):** 230-241.

634 **Li H, Yu M, Du XQ, Wang ZF, Wu WH, Quintero FJ, Jin XH, Dong JH, Wang Y.**
635 **2017.** NRT1. 5/NPF7. 3 functions as a proton-coupled H^+/K^+ antiporter for K^+ loading
636 into the xylem in *Arabidopsis*. *The Plant Cell* **29(8):** 2016-2026.

637 **Lin SH, Kuo HF, Canivenc G, Lin CS, Lepetit M *et al.* 2008.** Mutation of the
638 *Arabidopsis* NRT1. 5 nitrate transporter causes defective root-to-shoot nitrate transport.
639 *The Plant Cell* **20(9):** 2514-2528.

640 **Li S, Yu J, Zhu M, Zhao F, Luan S. 2012.** Cadmium impairs ion homeostasis by
641 altering K^+ and Ca^{2+} channel activities in rice root hair cells. *Plant, Cell and Environment*
642 **35(11):** 1998-2013.

643 **Luo BF, Du ST, Lu KX, Liu WJ, Lin XY, Jin CW. 2012.** Iron uptake system mediates
644 nitrate-facilitated cadmium accumulation in tomato (*Solanum lycopersicum*) plants.
645 *Journal of Experimental Botany* **63:** 3127–3136.

646 **Maathuis FJM. 2009.** Physiological functions of mineral macronutrients. *Current*
647 *Opinion in Plant Biology* **12:** 250–258.

648 **Meena VS, Maurya BR, Verma JP, Meena RS. 2016.** Potassium solubilizing
649 microorganisms for sustainable agriculture. New Dehli: Springer.

650 **Pyo YJ, Gierth M, Schroeder JI, Cho MH. 2010.** High-affinity K^+ transport in
651 *Arabidopsis*: HAK5 and AKT1 are vital for seedling establishment and postgermination
652 growth under low-potassium conditions. *Plant Physiology* **153:** 863–875.

653 **Remans T, Nacry P, Pervent M, Filleur S, Diatloff E, Mounier E, Tillard P, Forde**
654 **BG, Gojon A. 2006.** The *Arabidopsis* NRT1. 1 transporter participates in the signaling
655 pathway triggering root colonization of nitrate-rich patches. *Proc. Natl. Acad. Sci. USA*
656 **103(50):** 19206-19211.

657 **Shin R. 2017.** Potassium sensing, signaling, and transport: toward improved potassium
658 use efficiency in plants. *In Plant Macronutrient Use Efficiency*. Academic Press, 149-163.

659 **Tsay YF, Ho CH, Chen HY, Lin SH. 2011.** Integration of nitrogen and potassium
660 signaling. *Annual Review of Plant Biology* **62:** 207–226.

661 **Tsay YF, Schroeder JI, Feldmann KA, Crawford NM. 1993.** The herbicide sensitivity
662 gene CHL1 of *Arabidopsis* encodes a nitrate-inducible nitrate transporter. *Cell* **72:** 705–
663 713.

664 **Wang R, Liu D, Crawford NM. 1998.** The Arabidopsis CHL1 protein plays a major role
665 in high-affinity nitrate uptake. *Proc. Natl. Acad. Sci. USA* **95:** 15134–15139.

666 **Wang Y, Wu WH. 2010.** Plant sensing and signaling in response to K^+-deficiency.
667 *Molecular Plant* **3:** 280–287.

668 **Wang Y, Wu WH. 2013.** Potassium transport and signaling in higher plants. *Annual*
669 *Review of Plant Biology* **64:** 451-476.

670 **Wang Y, Wu WH. 2017.** Regulation of potassium transport and signaling in
671 plants. *Current Opinion in Plant Biology* **39:** 123-128.

672 **Wang YY, Cheng YH, Chen KE, Tsay YF. 2018.** Nitrate transport, signaling, and use
673 efficiency. *Annual Review of Plant Biology* **69:** 85-122.

674 **Wang, Y.Y., Hsu, P.K., and Tsay, Y.F. 2012.** Uptake, allocation and signaling of nitrate.
675 *Trends in Plant Science* **17:** 458–467.

676 **Wege S, Poirier Y. 2014.** Expression of the mammalian Xenotropic Polytropic Virus
677 Receptor 1 (XPR1) in tobacco leaves leads to phosphate export. *FEBS Letters* **588:** 482-
678 489.

679 **Yoshimoto N, Takahashi H, Smith FW, Yamaya T, Saito K. 2002.** Two distinct high-
680 affinity sulfate transporters with different inducibilities mediate uptake of sulfate in
681 *Arabidopsis* roots. *The Plant Journal* **29(4):** 465-473.

682 **Zhang X. 2017.** A plan for efficient use of nitrogen fertilizers. *Nature* **543:** 322-323.

683 **Zioni AB, Vaadia Y, Lips SH. 1971.** Nitrate uptake by roots as regulated by nitrate
684 reduction products of the shoot. *Physiologia Plantarum* **24(2):** 288-290.

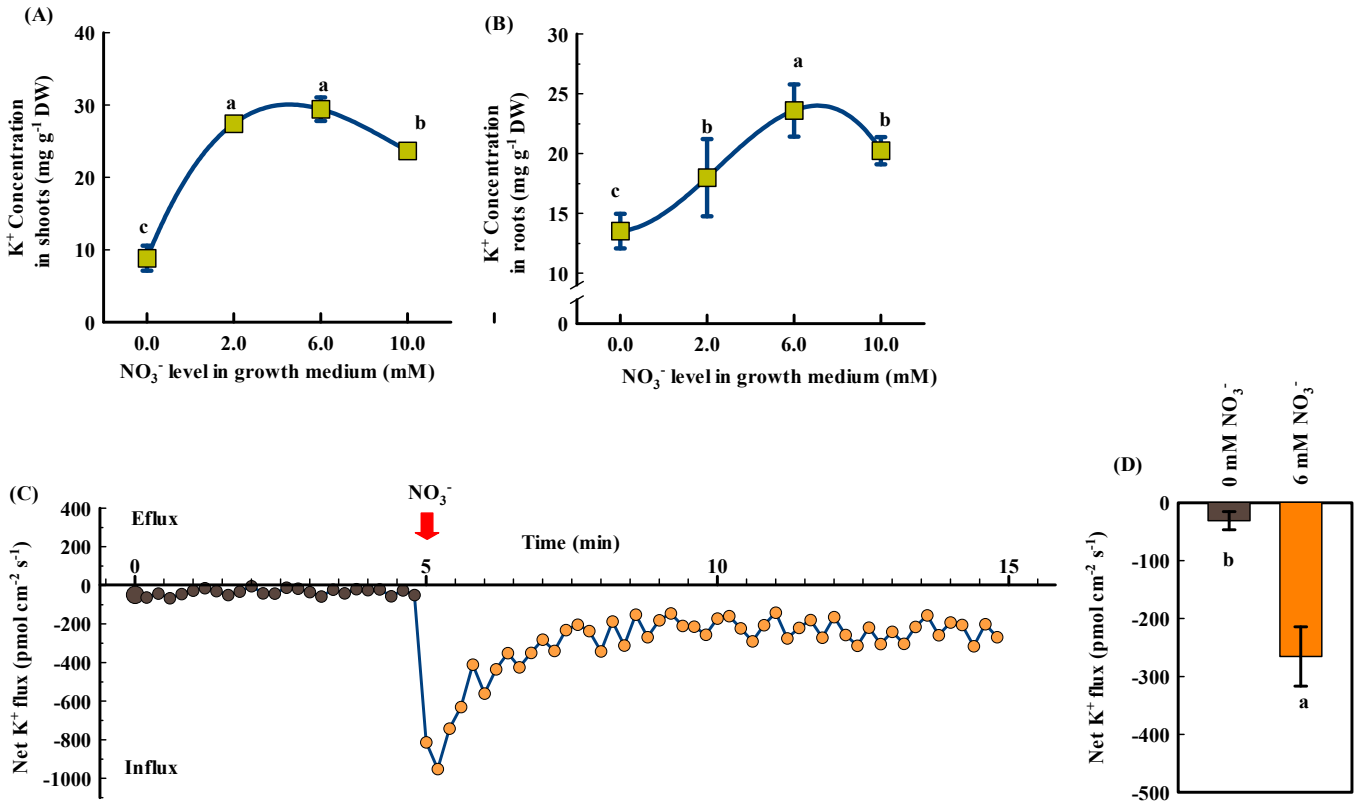

Figure 1. Increase of NO_3^- supply improves K nutrition in *Arabidopsis* Col-0 plants

(A) and (B) The K concentration in the shoots and roots. After 4 d of germination on the basal agar media, as described in the Materials and Methods, the seedlings were transplanted to the basal agar media containing different NO_3^- levels as indicated. The K concentrations were analyzed 8 d after seedling transfer. Bars represent the SD (n = 5 biological replicates). Different letters above bars indicate significant differences at $P < 0.05$ (LSD test). DW, dry weight. (C) Kinetics of net K^+ flux in Col-0 roots in response to NO_3^- addition. The net K^+ flux in Col-0 roots was first measured for 5 min in the absence of NO_3^-, and then it was measured in the presence of 6 mM NO_3^- (indicated by the red arrow). (D) The average values of root K^+ fluxes calculated from Figure c. Bars represent the mean ± SD. Different letters above bars indicate significant differences at $P < 0.05$ (LSD test). DW, dry weight.

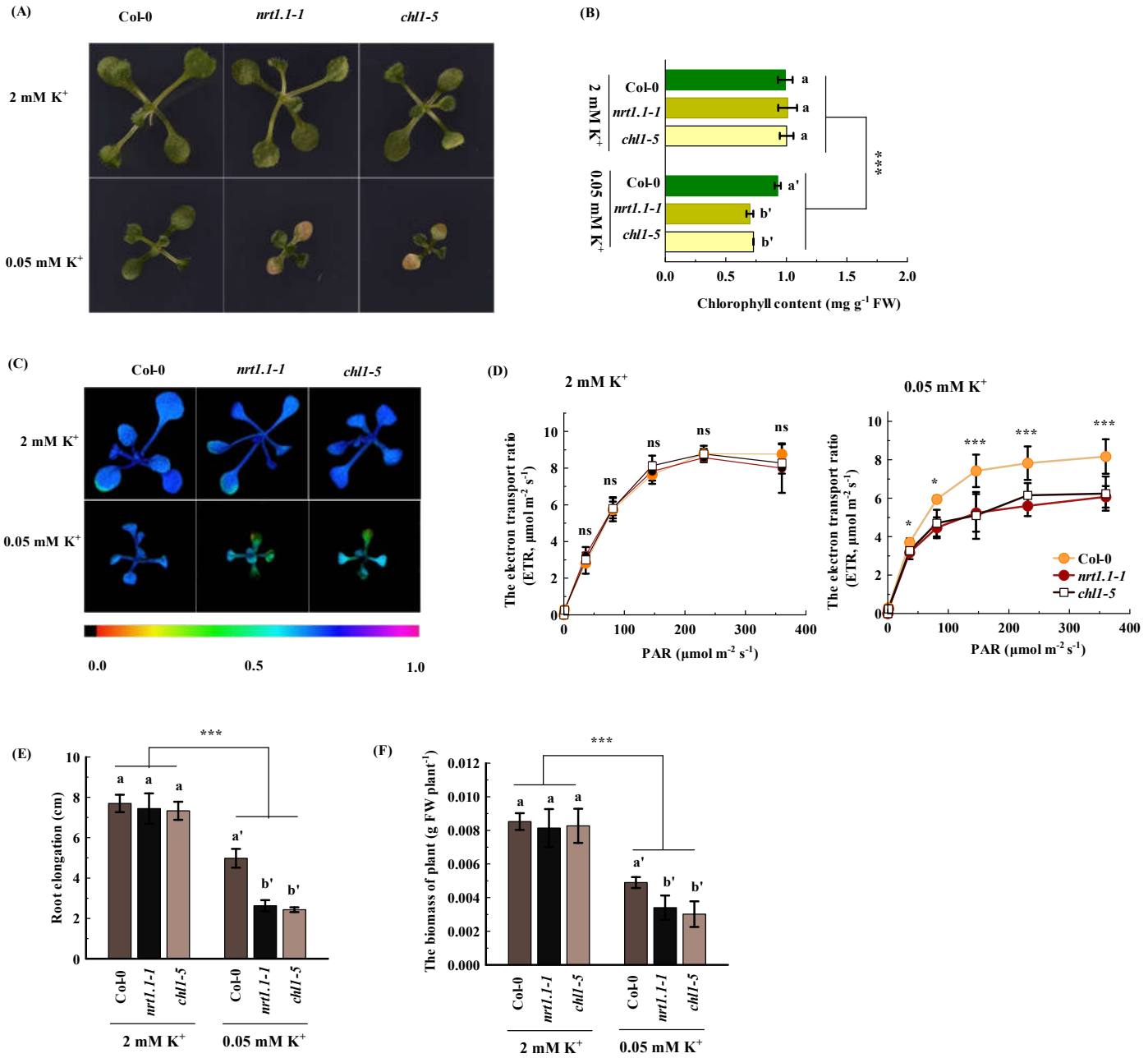

Figure 2. Loss of NRT1.1 function in *Arabidopsis nrt1.1* mutant leads to hypersensitivity to low-K stress

(A) Phenotypic photographs of plants; (B) chlorophyll content of leaves; (C) maximal quantum efficiency of PS; (D) electron transport rate (ETR); (E) root elongation; (F) biomass of plants. The 4-d-old seedlings of Col-0, *nrt1.1-1*, and *chl1-5* were transferred to an agar medium containing 2 mM or 0.05 mM K^+, as described in the Materials and Methods section. The analyses were performed 8 d after seedling transfer. Bars represent the mean ± SD (n = 4–5). (B), (E), and (F) Different letters above bars indicate significant differences at $P < 0.05$ (LSD test); asterisks indicate a significant genotype by treatment interaction (*** $P < 0.001$, two-way ANOVA). (D) Asterisks show significant differences compared with the Col-0; ns, non-significant (* $P < 0.05$, *** $P < 0.001$, two-tailed Student's *t*-test).

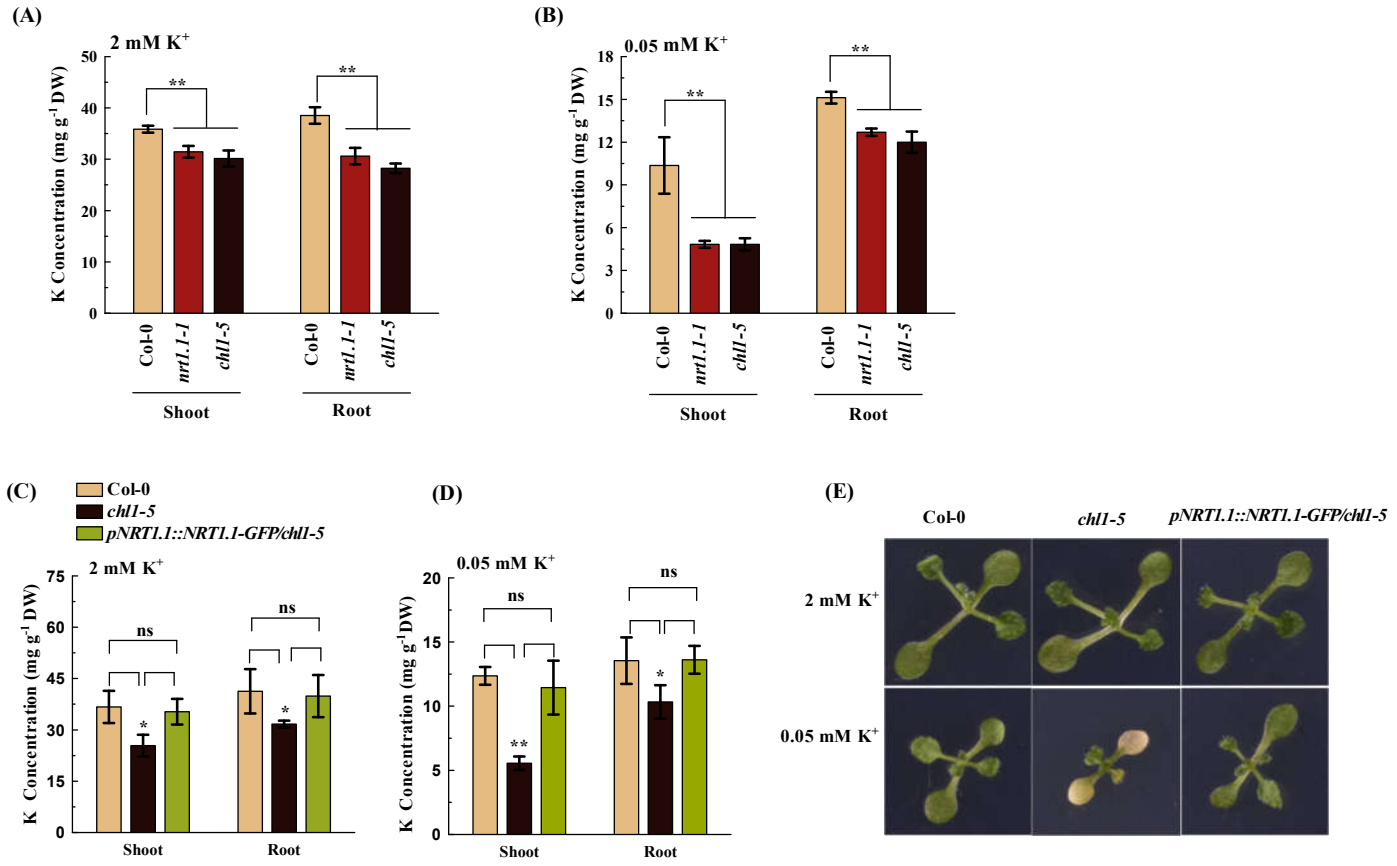

Figure 3. NRT1.1 is required for ensuring K nutrition in *Arabidopsis*

(A) and (B) Shoot and root K concentrations of Col-0, *nrt1.1-1*, and *chl-5* plants. (C) and (D) Shoot and root K concentrations of Col-0, *chl1-5*, and *pNRT1.1::NRT1.1-GFP* plants. (E) Phenotypic photographs of Col-0, *chl-5*, and *pNRT1.1::NRT1.1-GFP* plants. The 4-d-old seedlings were transferred to an agar medium containing 2 mM K^+ or 0.05 mM K^+, as described in the Materials and Methods section. The analyses were performed 8 d after seedling transfer. Bars represent the mean ± SD (n = 5). Asterisks indicate significant differences between genotypes; ns, non-significant (* $P < 0.05$, ** $P < 0.01$, two-tailed Student's *t*-test). DW, dry weight.

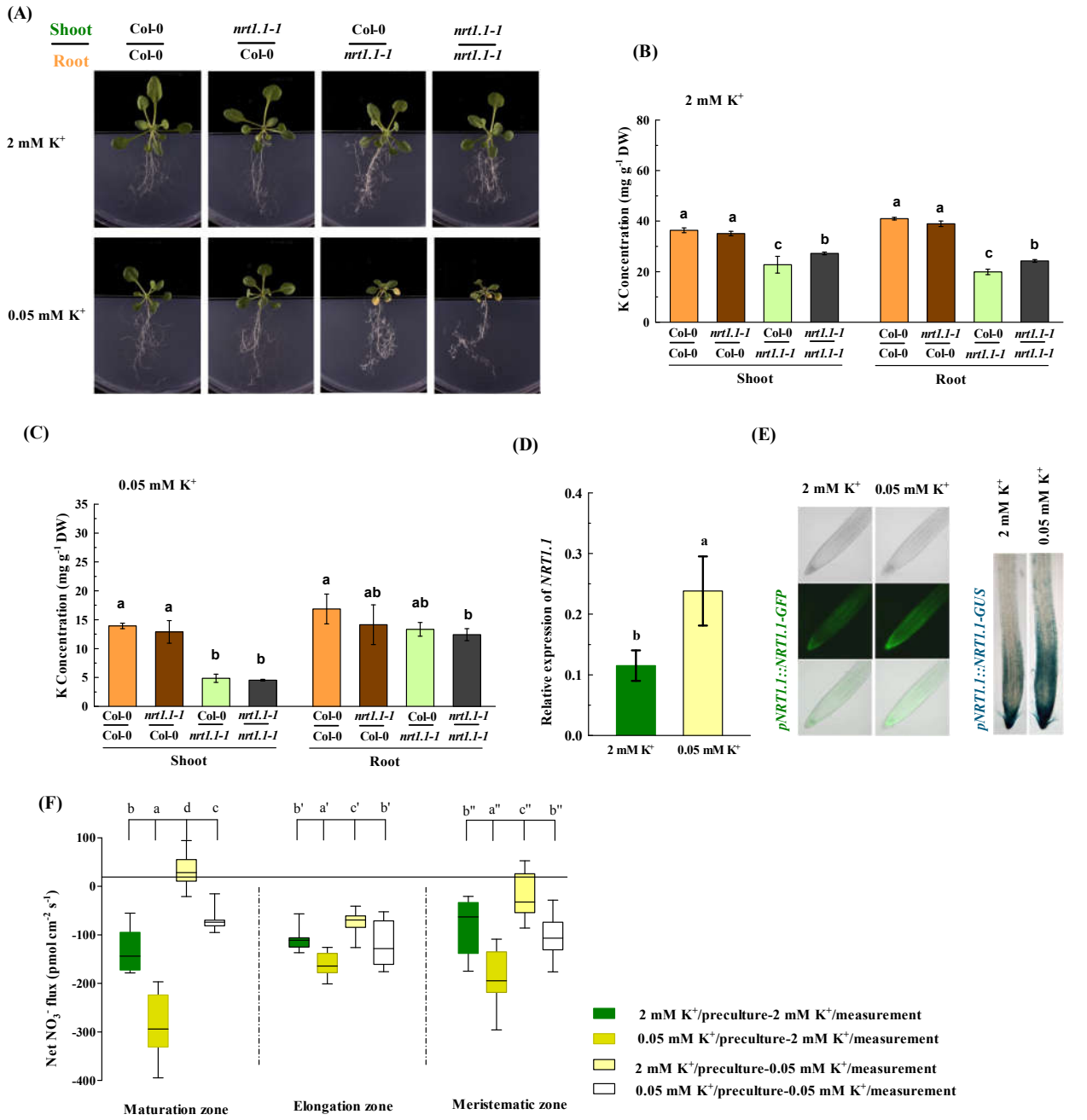

Figure 4. Improvement of K nutrition by NRT1.1 in *Arabidopsis* is a root behavior, and low-K stress stimulates NRT1.1 activity in the roots

(A) The phenotypic photographs; (B) and (C) the K concentrations of grafted plants. The unified seedlings were transferred to an agar medium containing 2 mM K^+ or 0.05 mM K^+. The analyses were performed 8 d after seedling transfer.

(D) Relative expression of *NRT1.1*. (E) NRT1.1-GFP expression and GUS staining in *pNRT1.1::NRT1.1-GFP* and *pNRT1.1::NRT1.1-* GUS transgenic plants, respectively. (F) Average values of net NO_3^- fluxes in the meristematic, elongation, and maturation zones of Col-0 roots. The 4-d-old seedlings were transferred to an agar medium containing 2 mM K^+ or 0.05 mM K^+, as described in the Materials and Methods section. The analyses were performed 5 d after seedling transfer. The relative expression levels were normalized to the levels of *UBQ10*. The negative value in (F) indicates net influx. Bars represent the mean ± SD (n = 5); different letters above bars indicate significant differences at $P < 0.05$ (LSD test). DW, dry weight.

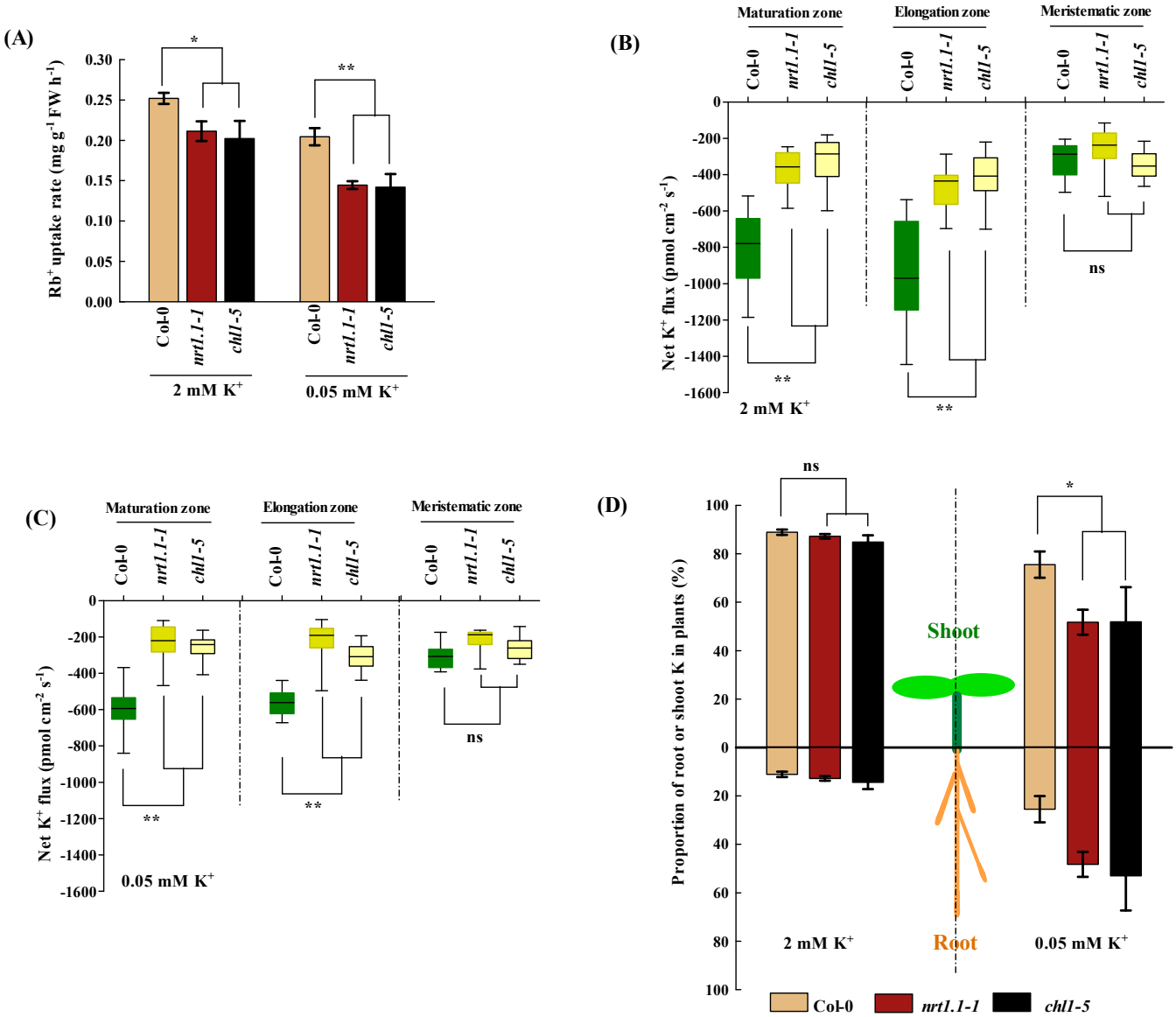

Figure 5. Both the uptake and root-to-shoot allocation of K^+ require an action of NRT1.1 in *Arabidopsis*

(A) Rb^+ uptake rate of roots. The 4-d-old seedlings were transferred to an agar medium containing 2 mM Rb^+ or 0.05 mM Rb^+ for 12 h, as described in the Materials and Methods section. Bars represent the mean ± SD (n = 5).

(B) and (C) The net K^+ fluxes in root. The 4-day-old seedlings were used to measure the net K^+ fluxes in the meristematic, elongation, and maturation zones under 2 mM K^+ or 0.05 mM K^+ treatment. Negative indicates net influx; bars represent the mean ± SD (n = 15).

(D) Proportions of K distributed in the shoots and roots. The 4-d-old seedlings were transferred to an agar medium containing 2 mM K^+ or 0.05 mM K^+, as described in the Materials and Methods section. The analyses were performed 8 d after seedling transfer. Bars represent the mean ± SD (n = 5); asterisks indicate significant differences compared with Col-0 plants; ns, non-significant (* $P < 0.05$, ** $P < 0.01$, two-tailed Student's *t*-test).

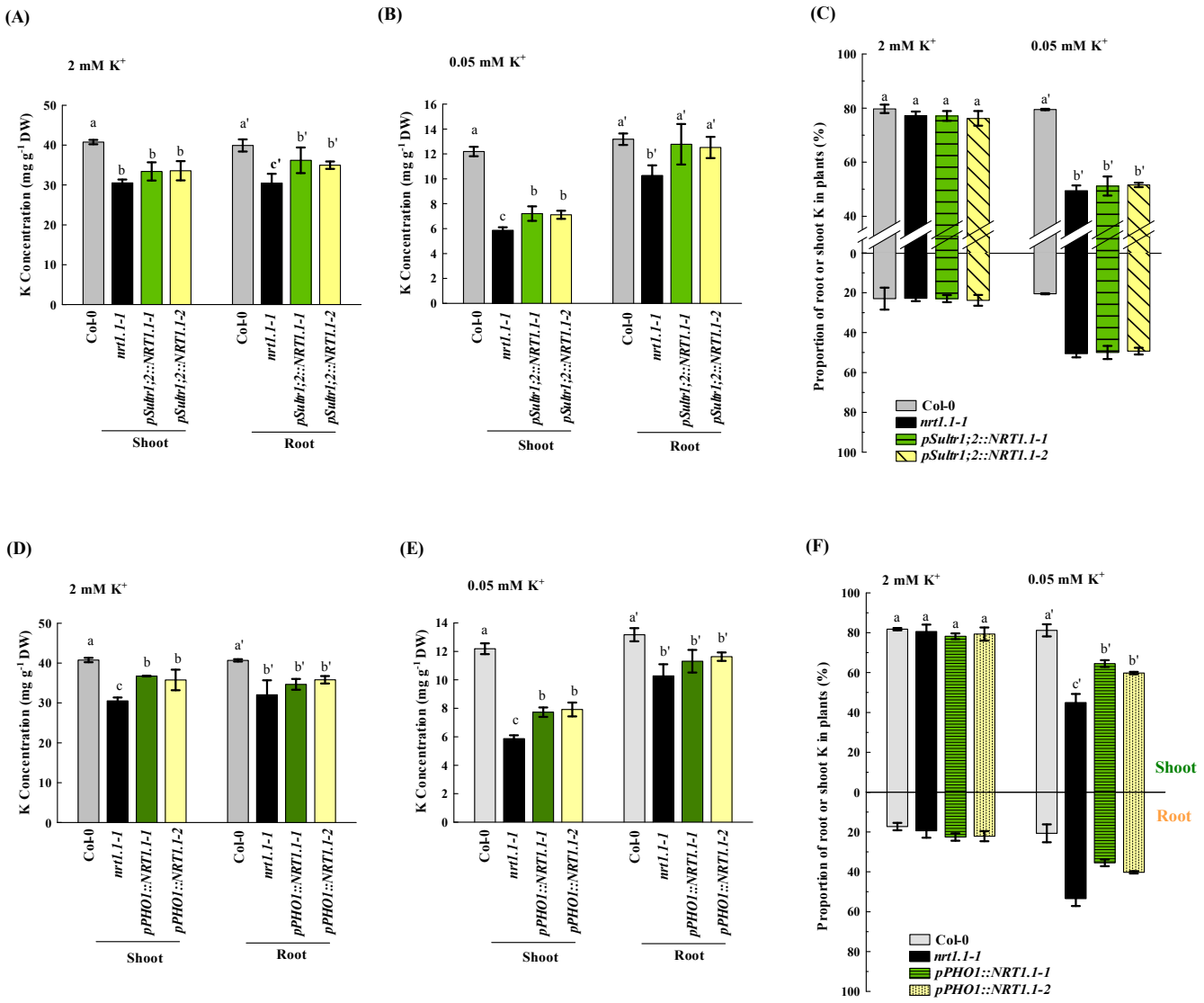

Figure 6. *NRT1.1* expression driven by *Sultr1;2* and *PHO1* promoters partially rescued the uptake and root-to-shoot allocation of K in *nrt1.1* mutants, receptively.

(A) and (B) K concentrations and (C) distributions in Col-0, *nrt1.1-1*, and two independent *pSultr1;2:NRT1.1*-transformed *nrt1.1-1* lines (*pSultr1;2:NRT1.1-1/-2*).

(D) and (E) K concentrations and (F) distributions in Col-0, *nrt1.1-1*, and two independent *pPHO1:NRT1.1*-transformed *nrt1.1-1* lines (*pPHO1:NRT1.1-1/-2*). The 4-d-old seedlings were transferred to an agar media containing 2 mM K^+ or 0.05 mM K^+ for 8 d, as described in the Materials and Methods section. Bars represent the mean ± SD (n = 5); different letters above bars indicate significant differences at $P < 0.05$ (LSD test).

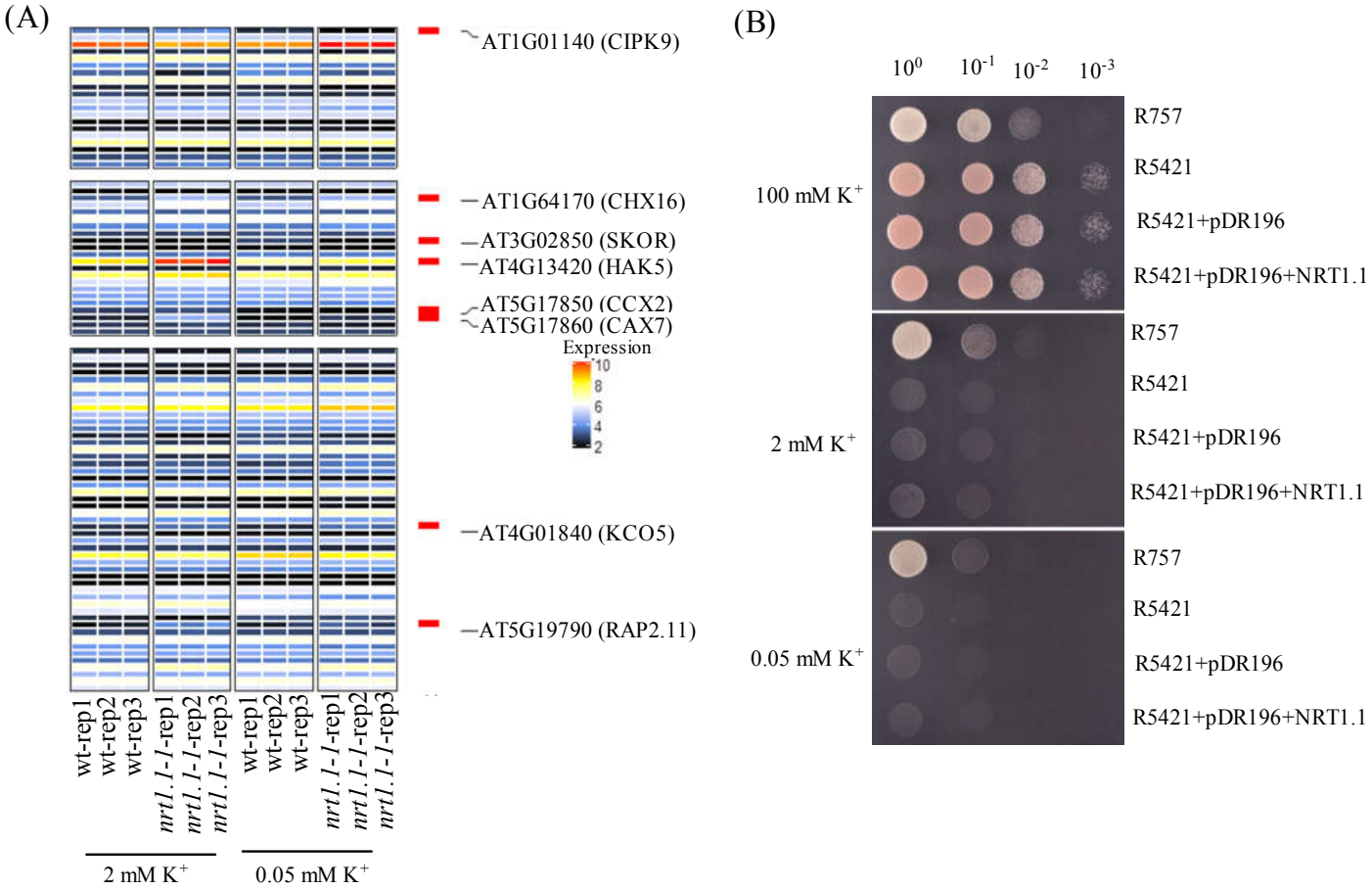

Figure 7. Both the regulation of gene expression and K^+ transport activity might not explain the effect of NRT1.1 in improving K nutrition in *Arabidopsis*

(A) Heatmap analysis of genes enriched in the GO terms: K^+ homeostasis, K^+ transport, and other processes of K^+ metabolism in the roots of *Arabidopsis* Col-0 and *nrt1.1-1* plants. The 4-d-old seedlings were transferred to a media containing 2 mM or 0.05 mM K^+ for 5 d, and roots were collected to perform transcriptome analysis, as described in the Materials and Methods section. Red color represents significantly up- or down- regulated genes compared with those in the control group. Changes in response to K treatment with a *p*-value of <0.05 were selected for GO analysis.

(B) Functional analysis of NRT1.1 in *Saccharomyces cerevisiae*. K^+ uptake activity was analyzed in yeast R757 strain and the K^+ import mutant strain R5421 (*trk1Δ*, *trk2Δ*), which were transformed with the expression constructs, as described in the Materials and Methods section. pDR196, empty vector.

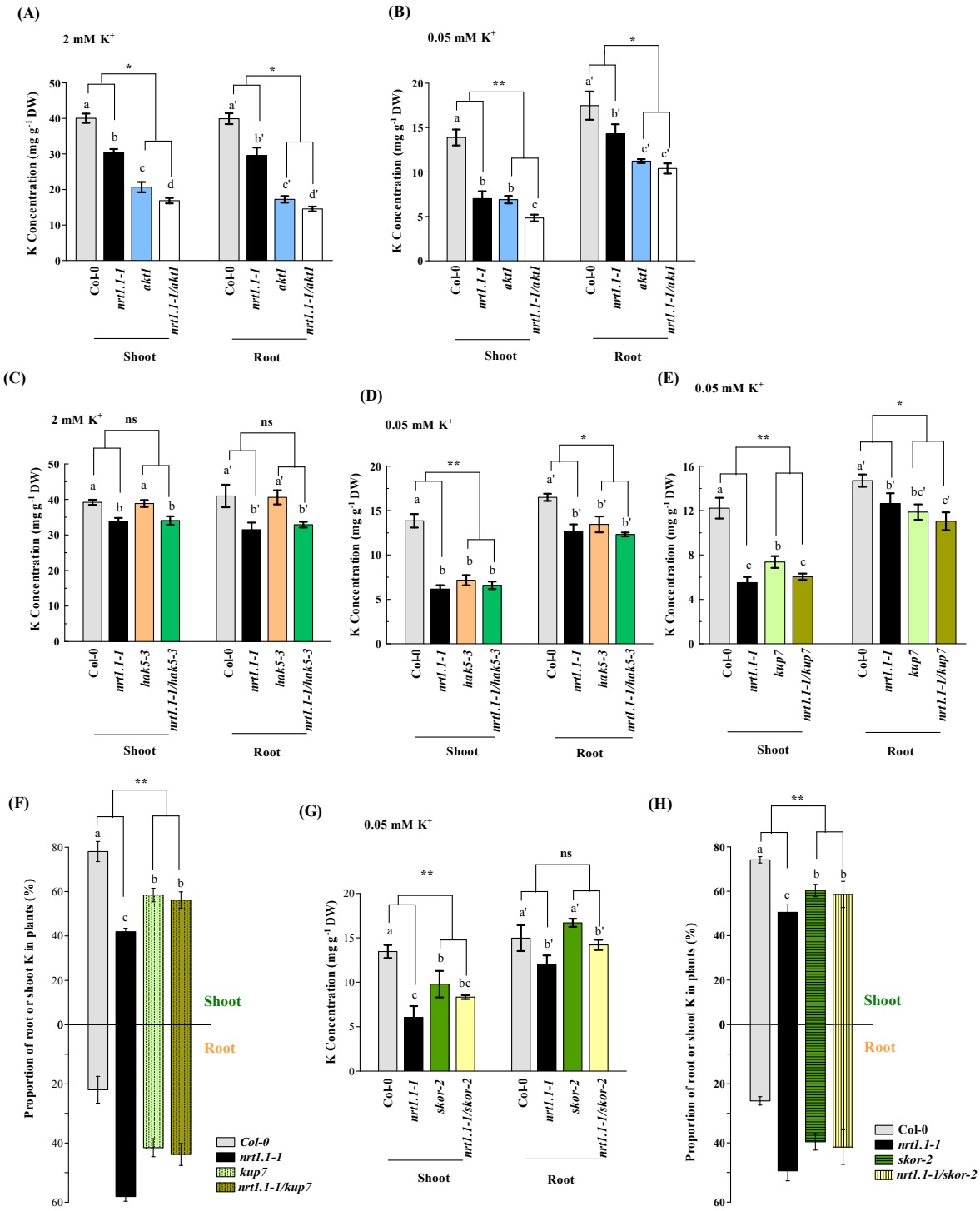

Figure 8. Physiological interactions between NRT1.1 and K^+ channels

(A) and (B) The K concentrations in Col-0, *nrt1.1-1*, *akt1,* and *nrt1.1-1*/*akt1* plants. (C) and (D) The K concentrations in Col-0, *nrt1.1-1*, *hak5-3*, and *nrt1.1-1*/*hak5-3* plants. (E) K concentrations and (F) distributions in Col-0, *nrt1.1-1*, *kup7*, and *nrt1.1-1*/*kup7* plants. (G) K concentrations and (H) distributions in Col-0, *nrt1.1-1*, *skor-2*, and *nrt1.1-1*/*skor-2* plants. The 4-d-old seedlings were transferred to an agar medium containing 2 mM K^+ or 0.05 mM K^+ for 8 d and subsequently analyzed for K levels, as described in the Materials and Methods section. Bars represent the mean ± SD (n = 5). Different letters above bars indicate significant differences at $P < 0.05$ (LSD test). Asterisks indicate a significant genotype by genotype interaction; ns, non-significant (* $P < 0.05$, ** $P < 0.01$, two-way ANOVA).

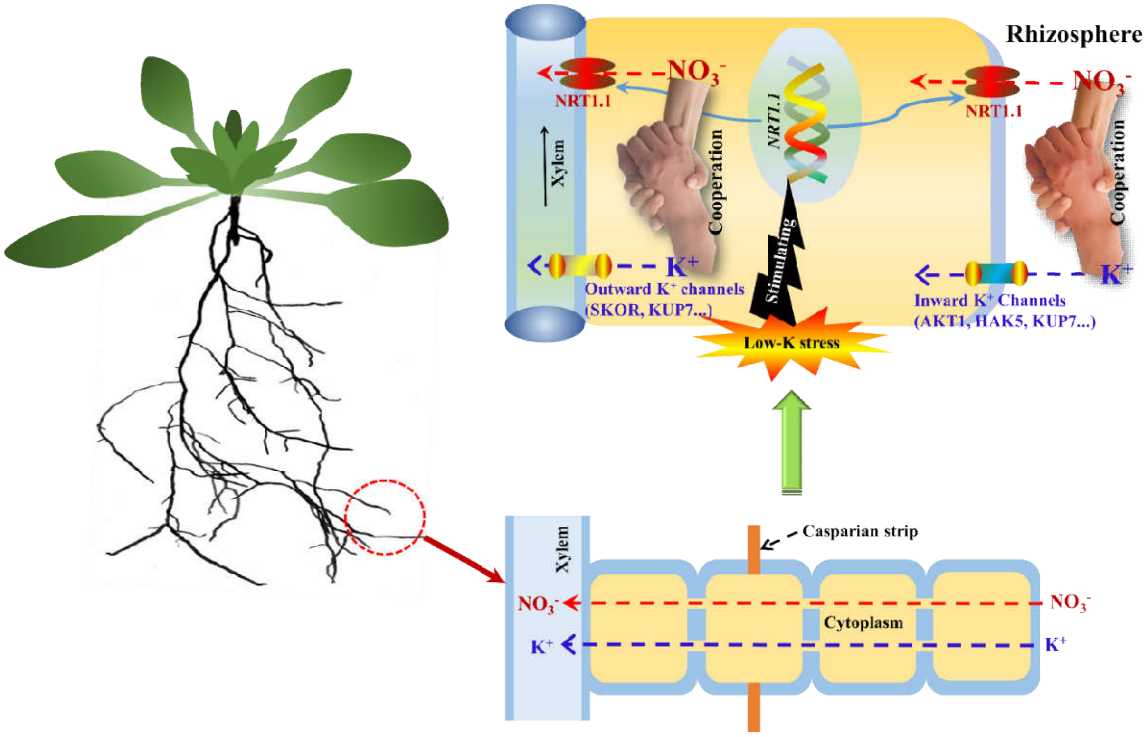

Figure 9. **Schematic model of how NRT1.1 responds to low-K stress in *Arabidopsis*.**

Under insufficient K growth conditions, the expression of the *NRT1.1* gene and its encoding protein in the root epidermis-cortex and central vasculature was induced, which coordinated with the K^+ uptake channels (such as AKT1, HAK5, and KUP7) and xylem K^+ loaders (such as SORK and KUP7) to improve root K^+ uptake from the external environment and facilitate K^+ root-to-shoot allocation, respectively.