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# DELLAs Contribute to Set the Growth and Mineral Composition of *Arabidopsis thaliana* Plants Grown Under Conditions of Potassium Deprivation

**Sonia Oliferuk[1] · Reyes Ródenas[2] · Adriana Pérez[1] · Vicente Martínez[2] · Francisco Rubio[2] · Guillermo E. Santa-María[1]**

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**Abstract** DELLAs proteins play a major role in the modulation of plant responses to fluctuations in environmental conditions. In this work, we examined to what extent *Arabidopsis thaliana* plants lacking DELLAs activity (*5xdella* mutant) or carrying an altered function allele of one of the DELLAs coding genes (*gai-1* mutant) display differential responses, in terms of growth and shoot elemental composition, relative to *WT* plants when deprived of potassium (K). Studies with plants grown in hydroponic media unveiled that the shoot mineral composition of *gai-1* constitutively differs from that of *WT* and *5xdella* plants. Tolerance to K-deprivation, as estimated by the relative decline of biomass accumulation, followed the order *gai-1* > *WT* > *5xdella*. In turn, the degree of responsiveness of the shoot composition to the stress condition followed the order *5xdella* > *WT* > *gai-1*, suggesting a correspondence between the degree of injury and changes in the elemental composition. Internal efficiency of K-utilization was maximized in *WT* relative to *5xdella* plants. Interestingly, the acquisition of K was severely impaired in *gai-1* plants well supplied, or deprived of, K. Complementary studies indicated that influx and root-to-shoot transport of Rubidium, a K-analogue, were reduced in those plants. Furthermore, evidence obtained supports the view that the effect of altered DELLAs derives, at least partially, from controlling the accumulation of transcripts coding for the AtHAK5 transporter. These results, together with the observation that K-deprivation promotes the accumulation of a DELLA protein (RGA) fused to GFP in root cells, suggest a pivotal role of DELLAs in key plant responses to K-deprivation.

**Keywords** DELLA · Ionome · Potassium · Uptake · Utilization · *Arabidopsis thaliana*

## Introduction

Potassium, a major nutrient for plants, plays important roles both at cellular and whole plant levels. Deprivation of this nutrient severely impairs plant growth by interfering with critical physiological processes (White and Karley 2010; Anschütz and others 2014). Low K availability is a common limiting factor for crop productivity in soils with high-rate of K-leaching, which need the extensive use of potassium-rich fertilizers (Römheld and Kirkby 2010). To improve plant productivity in these soils, genetic improvement of K-acquisition, internal K-utilization, as well as tolerance to low K-supply are considered central components of the research agenda (Rengel and Damon 2008). Work performed over the last 25 years led to increased knowledge on the structural and regulatory components that contribute to K-acquisition. In this regard, the early isolation and further characterization of the AKT1 channel and the HAK1 transporter (named as HAK5 in *Arabidopsis thaliana*) allowed the identification of the entities responsible

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✉ Guillermo E. Santa-María
gsantama@intech.gov.ar

[1] Instituto Tecnológico Chascomús (INTECH), Consejo Nacional de Investigaciones Científicas y Técnicas, Universidad Nacional de San Martín (CONICET-UNSAM), Avenida Intendente Marino, km 8.2, Chascomús 7130, Buenos Aires, Argentina

[2] Centro de Edafología y Biología Aplicada del Segura (CEBAS), Consejo Superior de Investigaciones Científicas (CSIC), Campus Universitario de Espinardo, Espinardo, Murcia 30100, Spain

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for the inward flux of K to roots from diluted K-solutions (Sentenac and others 1992; Santa-María and others 1997; Hirsch and others 1998; Gierth and others 2005). Following research leads to the identification of some mechanisms and processes that contribute to modulate their activities (Xu and others 2006; Li and others 2006; Fulgenzi and others 2008; Nieves-Cordones and others 2008; Hong and others 2013; Ragel and others 2016). Important advances have been also attained regarding the mechanisms that participate in controlling root architecture under conditions of variable K-supply (Shin and Schachtman 2004; Kellermeier and others 2013; Chen and others 2015, Song and others 2015). Although relevant efforts have been made in phenotyping for increased K-utilization efficiency (Damon and others 2007; White and others 2010; Yang and others 2003), the precise mechanisms that contribute to set it are only partially understood (White 2013), being almost unknown their underlying molecular components. On the other hand, work done by researchers allowed the identification of several hormones like ethylene (Jung and others 2009), cytokinins (Nam and others 2012), methyl jasmonate (Armengaud and others 2004, 2010), and auxins (Vicente-Agullo and others 2004; Song and others 2015) as key signaling components in plant responses to low K-supply. Although most of the works above mentioned used *Arabidopsis* as model plant, labor recently done with dwarf wheat mutants showed that *Rht-1* genes contribute to modulate senescence under conditions of limiting K-supply (Moriconi and others 2012). Interestingly, *Rht-1* genes encode DELLAs proteins, which participate in the Gibberellins (GAs)-Gibberellin Receptor-DELLAS (GAs-GID-DELLAs) module, a major point of integration of signals involved in setting plant responses to several stresses (Harberd and others 2009).

DELLAs proteins are members of a subgroup of the GRAS family of transcriptional regulators that pose a DELLA/TVHYNP motif in the N terminus. Research done over the last 15 years disclosed that DELLAs act as repressors of GAs signaling, contributing to modulate growth and development (Harberd and others 2009; Hauvermale and others 2012). GAs have the capacity to bind to GA-receptors, named GID, which once bounded to GAs are able to recruit DELLAs. This favors targeting of DELLAs for degradation at the proteasome, thus alleviating growth restriction (Harberd and others 2009). Plants lacking DELLAs display a slender phenotype, while plants carrying altered function alleles that lead to defective recruitment by GID display a dwarf phenotype. The activity of DELLAs on plant growth can also be modified in other alternative ways, indicating that they participate in both GAs-dependent and GAs-independent pathways (Harberd and others 2009; Hauvermale and others 2012). In addition to their important role in growth and development, as well as in plant responses to different stresses (that is, Achard and others 2006, 2008; Jiang and others 2007), it has been shown that in *Arabidopsis*, these proteins have the capacity to modulate the antioxidant response during plant acclimation to salinity and necrotrophic pathogen attack (Achard and others 2008). In wheat, *Rht-1* altered function mutants showed that DELLAs contribute to differentially modulate the antioxidant response of plants grown under conditions of low K-supply (Moriconi and others 2012). Interestingly, during that study, evidence was obtained showing that the concentration of calcium and sodium could be differentially modulated in those plants, leading to ask to what extent DELLAs could globally influence the mineral composition of plants. Despite the critical role played by the GA-GID-DELLAs module in setting plant growth, the effects of loss of DELLAs function as well as altered function alleles on the global plant elemental composition have not been previously examined. In the same way, to which extent DELLA participates in the modulation of K-utilization efficiency and K-capture remains unexplored.

*Arabidopsis* possesses five DELLAs, namely RGA, GAI, RGL1, RGL2, and RGL3. It is thought that the first two, and to a lesser extent RGL1, contribute to a different extent to vegetative growth and development (Gallego-Bartolomé and others 2010). In this study, we examined the possibility that DELLAs could contribute to set the shoot elemental composition, as well as the tolerance to, and the utilization and acquisition of K, during K-deprivation. For these studies, a quintuple DELLA mutant and an altered function mutant of the *GAI* gene have been used. Our results indicate a prominent role of DELLAs in plant responses to low K-supply.

## Materials and Methods

### Plant Material and Growth Conditions

Seeds of *A. thaliana* plants *WT* ecotype Landsberg erecta (Ler), the GA-insensitive gain of function (*gai-1*), and the *gai-t6 rga-t2 rgl1-1 rgl3-1 rgl2-1* quintuple della (*5xdella*) mutants made in the same background were surface sterilized in a 70% ethanol–water solution (v/v), followed by a 15-min wash out with 5% (v/v) bleach. Seeds were then washed with sterilized water four times and suspended in sterile water at 4 °C for 72 h. Seeds were next placed in plastic tubes filled with rock wool, which were positioned in plastic boxes (Supplemental Fig. 1) containing the culture solution below described following the procedure depicted by Rubio and others (2008), which does not include the use of additional substrates. The boxes were placed in a controlled growth chamber (8 h/16 h day/night cycle with a photon flux density of 150 µmol $m^{-2}$ $s^{-1}$ of photosynthetically active radiation,

being the temperature 22 °C day/night). The 8 h photoperiod was chosen to maintain plants in vegetative stage as long as possible, which helped to maximize vegetative biomass accumulation. The composition of the culture solution corresponded to a modified one-fifth-strength complete Hoagland solution containing the following macronutrients (mM): 1.4 KCl, 1.4 $Ca(NO_3)_2$, 0.35 $MgSO_4$, and 0.1 $Ca(H_2PO_4)_2$, and the following micronutrients (μM): 50 $CaCl_2$, 12.5 $H_3BO_3$, 1 $MnSO_4$, 1 $ZnSO_4$, 0.5 $CuSO_4$, 0.1 $H_2MoO_4$, 0.1 $NiSO_4$, and 10 Fe-EDDHA. The pH was brought to $5.6 \pm 0.1$ by the addition of $Ca(OH)_2$. Solutions were renewed once a week. In the experiments specifically designed to explore the elemental composition, during the fifth week, K-starvation was imposed to half of the plants by transferring them to a solution with no KCl added for a week. Each experiment typically included 4–6 replicates per harvest. Each replicate corresponds to 3–4 individual plants grown in an individual container. To study the long-term effect of K-deprivation on plant growth, plants of the three genotypes were grown in the complete solution described above for 21 days. Half of the plants were then exposed to K-deprivation for 9 or 13 days being the other half maintained in a complete solution.

Studies on solid medium were also performed with *Arabidopsis* Ler plants *RGAp::GFP-RGA* to analyze the effect of K-deprivation on the accumulation of the GFP-RGA protein. In these experiments, made under otherwise identical conditions to those formerly described, sterilized seeds were sown onto vertical plates containing a solid medium with the same composition that above described for hydroponic studies, but with the addition of noble agar (Difco) 1% (W/V). During the third day, after sowing, seedlings were transferred either to plates with complete medium (+K), plates with no KCl added (−K), and plates with complete medium with 1 μM paclobutrazol (PAC) for a week. Two independent experiments on agar plates were made. Roots of six plants were photographed for each growing condition.

### Biomass, Relative Growth Rate (RGR), and Chlorophyll Measurements

Two harvests were typically made, one just before the treatment application and other at the end of the experimental period. Plants were harvested and the fresh weight was determined. Then, the plant material was dried at 65 °C for 4 days and dry weights recorded. The relative growth rate (RGR) for each genotype and treatment was calculated as:

$$\mathrm{RGR} = \left(\ln\left(W_f\right) - \ln\left(W_i\right)\right)/\left(t_f - t_i\right),$$

where $W$ corresponds to whole plant dry biomass; $t$, time. The subscripts $i$ and $f$ denote the initial and final harvests, respectively.

For determination of chlorophyll in the aerial tissue, plant material was immediately frozen and later powdered in liquid nitrogen. Following extraction with acetone 80%, the amount of chlorophyll was determined as described by Inskeep and Bloom (1985).

### Mineral Composition Determination

For elemental composition analysis, dried material was digested with $HNO_3$:$HClO_4$ (2:1 v:v) and the concentrations of multiple elements determined in the digests by ICP spectrometry using an Iris Intrepid II ICP spectrometer (Thermo Electron Corporation, Franklin, USA). Among all elements measured, only K, Ca, Mg, P, S, Fe, Zn, Cu, Mn, Mo, Sr, and Al were quantitatively determined. Three independent experiments were performed with 4–6 replicates, at the final and initial harvests, respectively, for each genotype. C and N concentrations were determined in two additional independent experiments. For these analyses, dry material was explored with an elemental analyzer ELEMENTAL LECO TruSpec CN. The error obtained in root samples for several elements was considerably higher than that obtained for shoots, thus precluding the possibility to solve unequivocally the elemental composition of the below ground part of plants as well as to perform comparative analyses between both organs.

When the effect of 13 days, K-deprivation period was studied, dried material was digested with HCl 1N to allow the release of free cations, and, after a week, the concentration of K in the extracts was estimated by Atomic Absorption Spectrometry, emission mode, through the use of a Perkin Elmer AAnalyst 100 spectrometer.

### Potassium Utilization Efficiency (EKU) Estimation

Potassium Utilization Efficiency (EKU), which attempts to inform on the amount of biomass produced by each unit of K present in plant tissues, was estimated using the following indicators (Santa María and others 2015):

$$\mathrm{EKUo} = WQK^{-1},$$

$$\mathrm{EKUu} = W^2QK^{-1},$$

$$\mathrm{EKUi} = \left(W_f - W_i\right)/\left[\left(t_f - t_i\right)QK\right],$$

$$\mathrm{EKUe} = \left[\ln\left(W_f\right) - \ln\left(W_i\right)\right]/\left[\left(t_f - t_i\right)QKW^{-1}\right],$$

where $QK$ is the amount of K in the whole plant, whereas $W$ and $t$ as well as the subscripts $f$ and $i$ are as above described.

### Specific Absorption Rate (SAR) and Specific Translocation Rate (STR) Estimates

The K-specific absorption rate (SARK) was calculated according to the formula:

$$\mathrm{SARK} = \left(QK_f - QK_i\right) / \left[\left(t_f - t_i\right) \times \left(Wroot_f + Wroot_i\right)/2\right]$$

In this case, *Wroot* corresponds to the root dry weight. To calculate the K-specific translocation rate (STRK), the formula above was modified by including the amount in shoots instead of that whole plant amount of K.

### Rubidium-Depletion Assays

The potential capacity of roots of K-deprived plants to acquire K was estimated through the depletion technique. For this purpose, 36-day-old plants grown over the last 7 days in a solution without K-addition were placed in small volume recipients containing the solution above described, without K, containing 50 μM of Rb, a known analogue for K (Santa-María and others 1997; Rubio and others 2000), provided as RbCl. From the start, at fixed intervals, the culture solution was sampled and Rb determined in the samples with an AAnalyst 100 spectrometer. Curves of Rb-content-decay in the solution with time were next used to estimate the initial rate of Rb-uptake.

### Rubidium-Uptake Studies

Experimental procedures were also performed to estimate the influx and root-to-shoot transport of Rb in plants grown under the conditions above mentioned. The experimental procedure started 4 h after the beginning of the light period. Roots of intact plants of the three genotypes were carefully rinsed for 30 s with a complete nutrient solution to which no K was added. After that procedure, plants were transferred to the same solution, which contained either 0.1 or 1.4-mM RbCl, depending on the specific purpose of the experiment. The loading period extended for 15 and 30 min and was followed for a 3-min washout period with a solution without Rb. Shoot and root fractions were subjected to acid extraction, as above described, and Rb determined.

### Gene Expression Analysis

Samples of roots were collected, ground in liquid nitrogen, and the RNA extracted using the RNeasy Plant mini kit (Qiagen Science, Maryland, USA). The RNA was next treated with TURBO DNA-Free Kit (Ambion, Life technologies) and its integrity and purity evaluated. Total RNA was subsequently used as a template for the synthesis of cDNA, which was carried out using Superscript II Reverse Transcriptase (Invitrogen) according to the manufacturer's instructions. Amplification of *AtHAK5, AKT1, GORK, SKOR*, and either *alpha-elongation factor* or *actin* fragments was performed using the primers detailed in the Supplementary Table 1. Amplification was performed using a StepOnePlus Real-Time PCR system (Life Technologies). Ct values for each gene in each biological sample were estimated with three technical replications for each one of them. Fold change values reported correspond to $2^{-\Delta\Delta Ct}$.

### Statistical Methods

Data obtained for the plants contained in a single growth recipient, for a given harvest, were considered as a replicate. A factorial analysis of variance was applied to comparing variables among DELLAs mutants grown with and without K-addition, considering each experiment as a block. Data were tested for homogeneity of variance using the Levene test and for normality of distribution using the Shapiro–Wilk test. When necessary data were *ln* transformed. Post hoc comparisons were made through the Tukey test. In elemental composition studies, the alpha level was set at 0.01 to consider the error derived from multiple comparisons. A principal component analysis of 12 elements, plus water, was performed for shoot. Data were previously standardized to unit variance. All the analyses were performed with InfoStat version 2014 (FCA, Universidad Nacional de Córdoba, Argentina; http://www.infostat.com.ar).

## Results

### Potassium Deprivation Promotes the Accumulation of GFP-RGA Protein

The possible effect of K-deprivation on the accumulation of DELLAs proteins was evaluated using *A. thaliana RGAp::GFP-RGA* Landsberg erecta (Ler) plants grown on agar plates with (1.4 mM) or without the addition of KCl to the growth media. Observation of plants with a confocal microscope unveiled that while fluorescence associated with RGA in root cells was low when grown with adequate K-supply, it increased after a week of culture without K addition. The same effect was observed in the presence of PAC, which is an inhibitor of GAs accumulation and thus favors DELLAs stabilization (Fig. 1). These data indicate that an increase of GFP-RGA accumulation takes place in the root zone studied during K-deprivation.

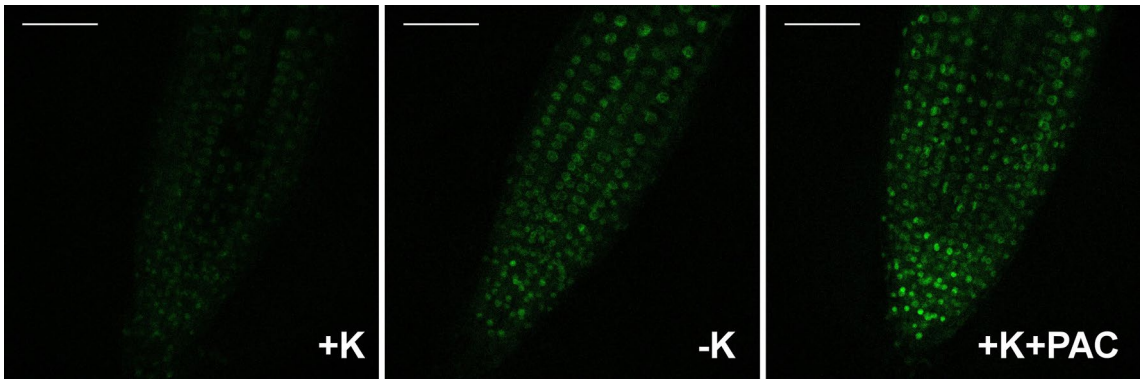


**Fig. 1** Representative confocal microscope images of primary roots of *WT* Landsberg erecta plants expressing *RGAp::GFP-RGA*. Plants were grown on agar plates either with a sufficient K-level (1.4-mM KCl, *left panel*), deprived of K for a week (*center panel*) or at 1.4-mM KCl in the presence of 1-µM paclobutrazol (PAC) for a week (*right panel*). Images were obtained with a Leica SP5 laser scanning confocal microscope; using an X63 objective at an excitation wavelenght of 488 nM. All images were captured and processed in the same manner. The *scale bars* correspond to 50 µm

### Loss of Function and Altered Function DELLAs Mutants Differentially Contribute to Set Biomass Accumulation in Plants Deprived of K

To assess the relevance of DELLAs on plant performance under conditions of K-scarcity, the biomass of wild type (*WT*), quintuple DELLA loss of function mutant (*5xdella*), and the gain of function *gai-1* mutant were analyzed for plants grown in a hydroponic medium (Supplementary Fig. 1) to which 1.4-mM K or no K was added, being it present just as a contaminant. It was observed that, at the end of the vegetative growing periods here analyzed, total biomass of *5xdella* plants was similar to (Fig. 2a), or slightly lower (Fig. 2b) than for, *WT* plants cultured in a medium containing 1.4-mM K, while it was always significantly lower for *gai-1* plants (Fig. 2a, b). Experiments, in which

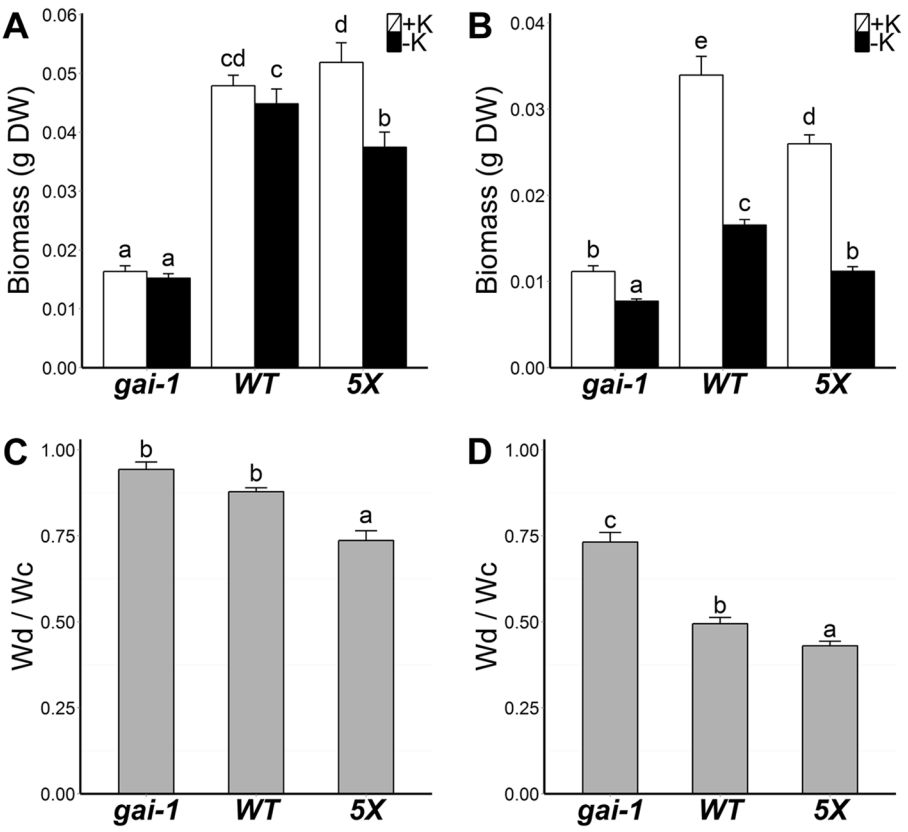


**Fig. 2** Biomass accumulation in *WT, gai-1* and *5xdella* plants exposed to adequate K-supply or exposed to K-deprivation for 7 days (**a**) or 13 days (**b**). The relative performances of K-deprived plants relative to plants grown at adequate K-supply either after 7 days (**c**) or 13 days of K-deprivation (**d**) are also shown. *White columns* correspond to plants grown at 1.4-mM KCl, whereas *black columns* correspond to K-deprived plants, respectively. Results shown correspond to the mean value (±SE) of 12 replicates (**a, c**) or 4 replicates (**b, d**). *Values* labeled with the *same letter* are not significantly different (alpha = 0.05) according to the Tukey test

shoot and root biomass were determined, showed that a similar statement holds true for both organs (Supplementary Fig. 2A, B). Interestingly, after a 7-day period (plants 6-week old at harvest) of K-deprivation, *5xdella* plants displayed a significant decrease of total biomass when compared to plants non-deprived of K (Fig. 2a). No significant reduction of total biomass was detected for *WT* neither for *gai-1* plants over that period, suggesting that lack of DELLAs activity affects the early acclimation of plants to conditions of K-scarcity. Lack of response of *WT* and *gai-1* plants to no K-addition may result from a short period of K-deprivation. Consistently, an additional experiment revealed that after a 13-day period of K-deprivation (plants 5-week old at harvest), the three genotypes experienced a significant reduction of total biomass relative to K-well supplied plants (Fig. 2b). In turn, no effect of K-deprivation on chlorophyll content was observed for any of the genotypes after 9 days of K-deprivation when biomass accumulation decayed for both *WT* and *5xdella* plants but not for *gai-1* plants (Supplementary Fig. 3). To examine to which extent the differences in biomass among genotypes were companied by growth differences over the precise period since the beginning of K-deprivation, relative growth rates (RGR) were calculated. Results obtained indicate that both over 7 and 13 days, there were no significant differences in the RGR between *5xdella* and *WT* plants grown at 1.4 mM K (Supplementary Fig. 4A, B), whereas it was significantly lower for *gai-1* plants than *for WT* plants. In turn, the effect exerted by K-deprivation on RGR for each genotype resembled that above reported for total biomass (Supplementary Fig. 4A, B).

Given that plants of the genotypes under study may differ in biomass and RGR when grown with an adequate provision of K, comparisons of absolute values could be misleading to estimate plant performance under conditions of K-scarcity. Therefore, to attain a more accurate comparison, the quotients between the total biomass determined for plants deprived of K (*Wd*) and that determined for plants grown in the presence of 1.4 mM KCl (*Wc*) were calculated. It was observed that after a 7-day period of K-deprivation, the *Wd*/*Wc* quotient was not significantly different between *gai-1* and *WT*, being significantly lower for *5xdella* plants (Fig. 2c). After a 13-day period of K-deprivation, when growth of all genotypes was significantly impaired, that quotient became higher for *gai-1* than for *WT*, and higher for *WT* than for *5xdella* plants (Fig. 2d). These results indicated that while loss of DELLAs activity led to lack of tolerance to K-scarcity, the presence of a DELLA stabilized version (*gai-1*) helped to diminish (in relative terms) the growth restriction that took place in plants suffering K-scarcity. Examination of the quotient *RGRd*/*RGRc* provided further supports to this conclusion (Supplementary Figs. 4C, D).

## Principal Components Analysis Unveils an Important Effect of Gain of DELLAs Function on the Shoot Elemental Composition

As above stated, an additional objective of this work was to explore the potential role of DELLAs in setting the mineral composition of plants grown either at sufficient levels or insufficient levels of K-supply. Therefore, the elemental composition of shoots of *WT, 5xdella* and *gai-1*, was examined over a K-deprivation period during which growth was not severely impaired (7 days). To obtain a comprehensive picture, we performed a principal component analysis (PCA) that included all elements measured, except C and N, which were determined in separate experiments. This analysis unveiled (Fig. 3) that when two components were considered, they represented 65.2% of the variation in the elemental composition among genotypes and levels of K-supply, component I being responsible for 45.0% and component II for 20.2%. The first component (Supplementary Table 2) negatively correlated with K and $H_2O$ concentration and positively with other elements, namely Ca, Mg, Mn, Mo, S, and Cu, being also present a contribution of Sr and Zn. The second component, in turn, was mainly related with Al,

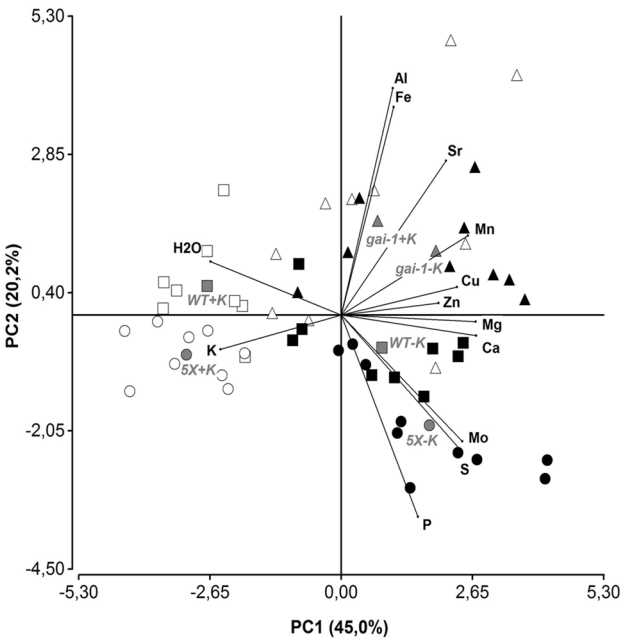


**Fig. 3** Principal components analysis of the shoot ionome of *WT, gai-1*, and *5xdella* plants grown either in the presence of 1.4 mM KCl or without K-addition for 7 days. Data correspond to three experiments. Symbols are as follows: *squares* correspond to *WT, triangles* to *gai-1*, and *circles* to *5xdella* plants, respectively. *White symbols* correspond to plants grown in the presence of 1.4 mM KCl, whereas *black symbols* correspond to plants grown without KCl addition. *Grey symbols* correspond to the centroid for each genotype at each growing condition as indicated in the figure

Fe, P, and marginally with Sr (Supplementary Table 2). Two interesting findings emerged from the analysis performed: first, that the elemental composition of *gai-1* plants was sharply separated from that of *WT* and *5xdella* plants grown in the presence of K; second, that while K-starvation exerted a major effect on the elemental composition of *5xdella* and to a lesser extent of *WT* shoots involving mainly the component I, the response of the *gai-1* elemental composition to this stress condition was considerably reduced in comparison to *WT* and *5xdella*. These data indicated that the constitutive elemental composition of *gai-1* plants was different from that of *WT* and *5xdella* plants, being it less responsive to K-deprivation than the last two genotypes.

### Effect of Altered and Loss of Function Mutations on Individual Elements

The results above described suggested that DELLAs activity globally affected the elemental composition of plants. It was next analyzed the presence of differences in individual elements for each growth condition among genotypes. Under conditions of adequate K-supply, it was observed that the presence of the *gai-1* altered function allele led to significant differences relative to *WT* plants in the shoot concentration of four of the elements analyzed; namely K, Zn, Cu, and Mn in addition to water according to post hoc comparisons performed with the Tukey test at $p < 0.01$ (Fig. 4; Table 1). For plants carrying a disruption in the five DELLAs encoding genes, only one element, P, was differentially accumulated relative to *WT* plants. A comparison of plants carrying stabilized DELLA (*gai-1*) with those lacking DELLAs activity (*5xdella*) showed significant differences in the accumulation of 7 elements: K, Ca, P, Zn, Cu, Mn, and Sr, in addition to water, indicating a higher degree of variation between them than between each one of them and *WT* plants. With the important exception of K, quantitative differences in the concentration of those elements between *gai-1* and *5xdella* plants tended to be higher for trace than for macro elements. The observation that, among the macronutrients, a major constitutive difference was found for K between *gai-1* and *5xdella* plants, points out a major role of DELLAs in the control of K accumulation.

In shoots of plants exposed to K-deprivation, it was observed that *gai-1* plants differed from *WT* plants in four elements, namely K, Cu, Zn, and Mn; whereas *5xdella* plants differed from *WT* for K, P, and S (Fig. 4). A clear difference in the degree of responsiveness to K-deprivation was observed among the genotypes studied. While for *gai-1* plants, the concentration of only two elements (K and Mg) was significantly different between +K and –K growth conditions, differences involved 5 elements for *WT* (K, Mg, Ca, P and S) and 8 elements for *5xdella* plants (K, Mg, Ca, P, S, N, Mn, and Mo) in addition to water for the last two genotypes (Fig. 4; Table 1). These differences suggest the view that there is a DELLAs-dependent sequence in the changes in the individual components during the response to K-deprivation, being K and Mg the elements always affected, followed by Ca, P, and S, and then, the remaining elements above mentioned. On the other hand, no major differences among genotypes were detected for C and N concentrations in the experiments designed to measure these elements within each growth condition, while as above-mentioned K-deprivation led to a significant reduction of N concentration in *5xdella* plants (Table 1). Because C and N play major structural roles in plants, we attempted to obtain an assessment on the way that genotypes here studied handle the relationship between them in shoots. In this regard, it was found that while the C/N quotient was not significantly affected by K-deprivation in *gai-1* plants, it significantly increased in *WT* (6.7%) and *5xdella* (9.9%) plants following K-deprivation. On the other hand, in these experiments, it was observed that during the transition from high to low K-supply, the quotient between C and $H_2O$ remained unaltered for *gai-1* plants (3.52% increase relative to +K plants), while it sharply increased for both *WT* (22.03%) and *5xdella* (23.61%) plants, suggesting a differential compromise of water relationships between *gai-1* and the pair *WT*/*5xdella*.

### DELLAs Mutations Affect the Efficiency of K-Utilization and K-Acquisition

Whereas the quotients *Wd*/*Wc* and *RGRd*/*RGRc* provide a way to estimate the tolerance to low K-supply, they do not inform on potential differences among genotypes in the internal efficiency of potassium utilization (EKU), that is, the capacity of plants to generate biomass per unit of potassium. Calculations of four EKU indicators were made both in experiments with plants deprived of K for 7 days and for 13 days, when growth was restricted for the three genotypes. In both experiments, it seemed clear that using any of the four EKU indicators, *5xdella* plants were less efficient than *WT* plants (Table 2; Supplementary Table 2). Interestingly, the static indicators EKUo and EKUu did not yield a similar pattern when *gai-1* and *WT*, or *gai-1* and *5xdella*, plants were compared within each experiment (Table 2; Supplementary Table 3), providing additional support for the need of using a dynamic approach as recently proposed (Santa-María and others 2015). Analysis of the data obtained in the experiments here shown does not allow to reach an unequivocal conclusion regarding the existence of differences in EKU between *WT* and *gai-1* plants.

Comparisons of internal nutrient utilization efficiency among genotypes could be potentially affected by

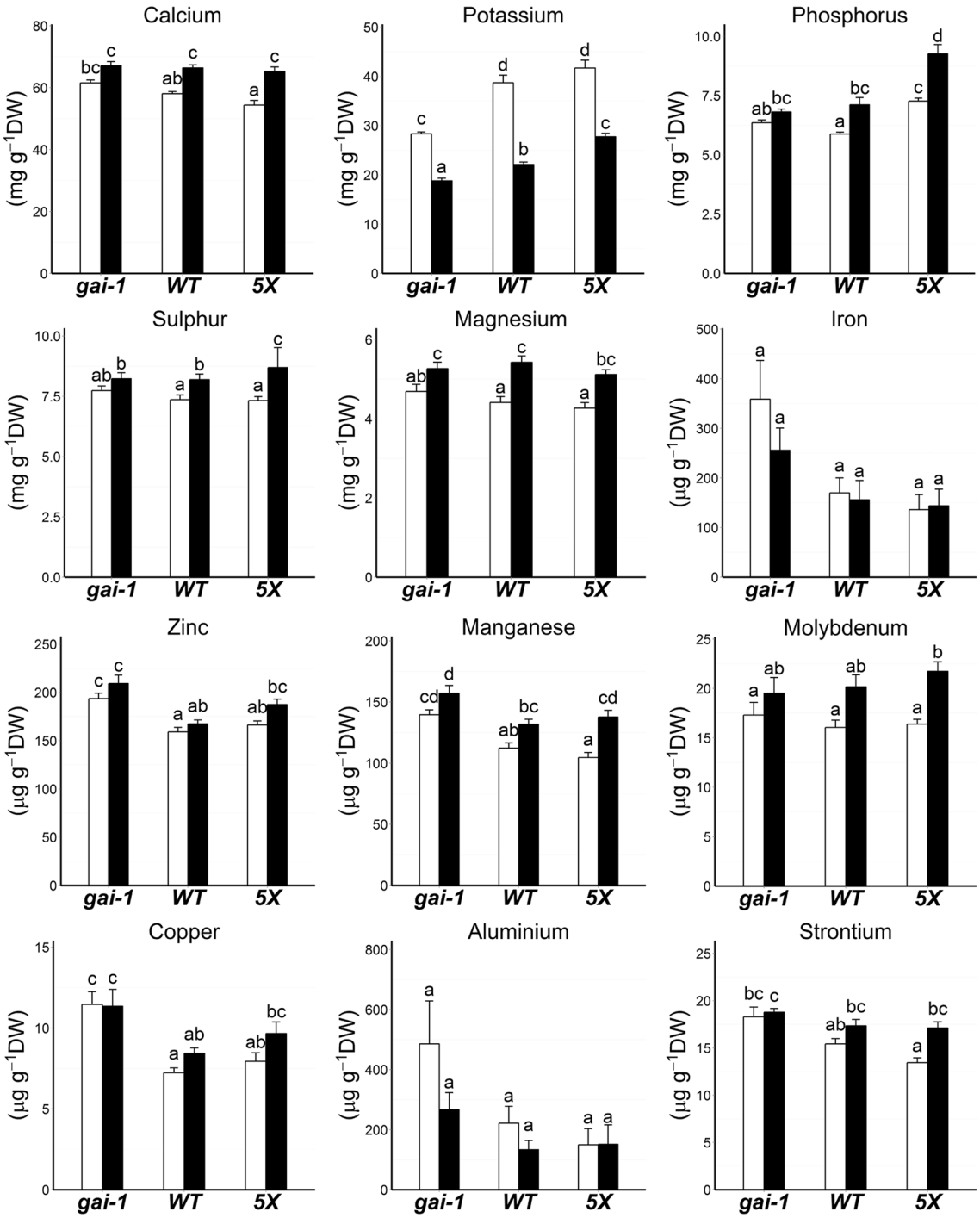


**Fig. 4** Concentration of individual elements in shoots of *WT, gai-1*, and *5xdella* plants grown either in the presence of 1.4 mM KCl or deprived of K during 7 days. *White columns* correspond to plants grown at 1.4 mM KCl, whereas *black columns* correspond to plants K-deprived, respectively. Results are the mean (±SE) values obtained in three experiments. Results labeled with the same letter are not significantly different (alpha = 0.01)

differences in element capture (Rose and Wissuwa 2012; Santa-María and others 2015). Although the K-deprivation procedure here used could theoretically lead to a nul K-capture in all genotypes (Moriconi and Santa-María 2013), in practice, the amount of K per plant increased for all genotypes at the end of 7- or 13-day K-deprivation periods, and it was significantly lower for *gai-1* than for *WT*, being similar for the *WT* and *5xdella* genotypes (Table 2;

**Table 1** Concentration of carbon (C), nitrogen (N), and water in shoots of *gai-1, WT*, and *5xdella* plants grown either in the presence of 1.4 mM KCl or deprived of K during 7 days

| | +K | | | −K | | |
|---|---|---|---|---|---|---|
| | *gai-1* | *WT* | *5X* | *gai-1* | *WT* | *5X* |
| C | 35.03 ± 0.25a | 34.07 ± 0.21a | 34.00 ± 0.17a | 35.16 ± 0.15a | 34.88 ± 0.34a | 34.33 ± 0.25a |
| N | 7.41 ± 0.07c | 7.08 ± 0.08abc | 7.22 ± 0.12bc | 7.19 ± 0.09abc | 6.79 ± 0.11ab | 6.68 ± 0.12a |
| $H_2O$ | 11.19 ± 0.10a | 12.79 ± 0.20b | 12.33 ± 0.16bc | 10.78 ± 0.12a | 10.72 ± 0.25a | 10.41 ± 0.29a |
| C/N | 4.73 ± 0.04a | 4.82 ± 0.08a | 4.69 ± 0.09a | 4.83 ± 0.04a | 5.14 ± 0.08b | 5.16 ± 0.12b |
| C/$H_2O$ | 3.09 ± 0.04b | 2.77 ± 0.05a | 2.81 ± 0.07a | 3.21 ± 0.03bc | 3.38 ± 0.05 cd | 3.48 ± 0.07d |

Data for water concentration correspond to five experiments (4 replicates each one), while data for the remaining parameters were obtained in two experiments (4 replicates each one). The quotients C/N and C/$H_2O$ derived from these two experiments are also shown. Bars correspond to SE. Results labelled with the same letter are not significantly different (alpha = 0.01). Values for C and N concentration as well as the ratio C/$H_2O$ are expressed as percentage. Units for water concentration and C/N ratio are g($H_2O$) $g^{-1}$(DW) and g(C) $g^{-1}$(N), respectively

**Table 2** Internal efficiency of potassium utilization (EKU) of *gai-1, WT*, and *5xdella* plants exposed to 13 days of K-deprivation starting on day 21 from sowing, as calculated by four different estimators

| | *gai-1* | *WT* | *5X* | *P* values |
|---|---|---|---|---|
| EKUo | 56.88 ± 2.03b | 61.37 ± 1.97b | 43.80 ± 1.25a | 0.0002 |
| EKUu | 0.42 ± 0.02a | 1.01 ± 0.04b | 0.49 ± 0.03a | <0.0001 |
| EKUi | 3.51 ± 0.13b | 4.03 ± 0.10c | 2.74 ± 0.09a | 0.0001 |
| EKUe | 7.07 ± 0.29b | 9.08 ± 0.21c | 5.68 ± 0.28a | <0.0001 |
| K-capture | 0.05 ± 0.01a | 0.13 ± 0.02b | 0.11 ± 0.02ab | 0.0244 |

EKUo corresponded to the quotient between $W$ and $QK$, EKUu to the quotient between $W^2$ and $QK$, EKUi was calculated as $(W_f - W_i)/[(t_f - t_i)\ QK]$, and EKUe as $[\ln(W_f) - \ln(W_i)]/[(t_f - t_i)\ QK\ W^{-1}]$. $W$, corresponds to the whole plant dry weight, $QK$ to the amount of K in the whole plant, and $t$ to the time elapsed between the initial ($i$) and final ($f$) harvests, respectively. In turn, K-capture corresponds to the amount of K ($QK_f - QK_i$) accumulated by each plant over the 13-day period of K-deprivation. Results correspond to the mean (±SE) value of 4 replicates. Results labelled with the same letter are not significantly different (alpha = 0.05). Units for EKUo are mg(DW) $mg^{-1}$(K); for EKUu are $mg^2$ $10^{-3}$(DW) $mg^{-1}$(K), for EKUi are mg(DW) $mg^{-1}$(K) $d^{-1}$, for EKUe are mg(DW) $mg^{-1}$(K) $d^{-1}$, and for K-capture are mg (K)

**Table 3** Specific absorption rate (*SARK*) and specific translocation rate (*STRK*) of K in *gai-1, WT*, and *5xdella* plants grown in the presence of 1.4-mM KCl from germination

| | *gai-1* | *WT* | *5X* | *P* values |
|---|---|---|---|---|
| SARK | 26.03 ± 1.70a | 46.52 ± 2.20b | 50.94 ± 3.18b | <0.0001 |
| STRK | 20.62 ± 0.89a | 38.33 ± 2.04b | 43.72 ± 2.45b | <0.0001 |

*SARK*: $(QK_f - QK_i)/[(t_f - t_i) \times (Wroot_f + Wroot_i)/2]$, $QK$ corresponds to the amount of K in the whole plant, and $t$ to the time elapsed between the initial ($i$) and final ($f$) harvests and *Wroot* to the root dry weight. For calculation of *STRK*, amount of K in shoot was used instead of that of the whole plant. Time elapsed between harvests was 7 days. Results correspond to the mean (±SE) value of 12 replicates obtained in three experiments. Results labelled with the same letter are not significantly different (alpha = 0.05). Units are mg(K) $g^{-1}$ RDW $d^{-1}$, where RDW corresponds to root dry weight

Supplementary Table 3). Therefore, differences in EKU between *WT* and *5xdella* plants above shown do not involve differences in K-capture. On the other hand, the observation that K-capture per plant differed between *gai-1* and *WT* plants suggested the possibility that the presence of a DELLA stabilized version could affect the acquisition of K. For plants grown at 1.4 mM K, the mean net uptake rate per unit of root weight (SARK), as estimated by successive harvests, was significantly lower for *gai-1* than for *WT* and *5xdella* plants, being similar between the last two (Table 3). Furthermore, an examination of the pattern of the specific translocation of K to shoot (STRK) per unit of root weight unveiled that it was significantly lower in *gai-1* than in *WT* and *5xdella* plants.

Although estimates of K-uptake through successive harvests could yield accurate values for plants grown at adequate K-supply levels, that kind of measurements cannot be properly used to estimate the potential capacity of roots to absorb K under conditions of K-deprivation. A complementary study showed that Rb-depletion from 50 µM Rb was significantly lower for *gai-1* than for *WT* K-deprived plants (Supplemental Fig. 5), providing support to the idea that *gai-1* plants had a reduced capacity to deplete K from diluted K-solutions.

## Unidirectional Rb-Fluxes are Differentially Affected in *gai-1* Plants

To further advance in the identification of the unidirectional K-fluxes differentially affected in the lines under examination, studies above were accompanied with estimates of the inward flux and the translocation of the K-analogue, Rb, on plants grown at 1.4 mM KCl and subsequently exposed to RbCl 1.4 mM. Under those conditions, Rb-uptake increased with the Rb-loading time for the three genotypes (Fig. 5a). The initial slope (0–15 min), which estimates Rb-influx, was approximately a 30% lower for *gai-1* than for *WT* (10.1 and 14.5 µmol $g^{-1}$ RFW $h^{-1}$, respectively), being

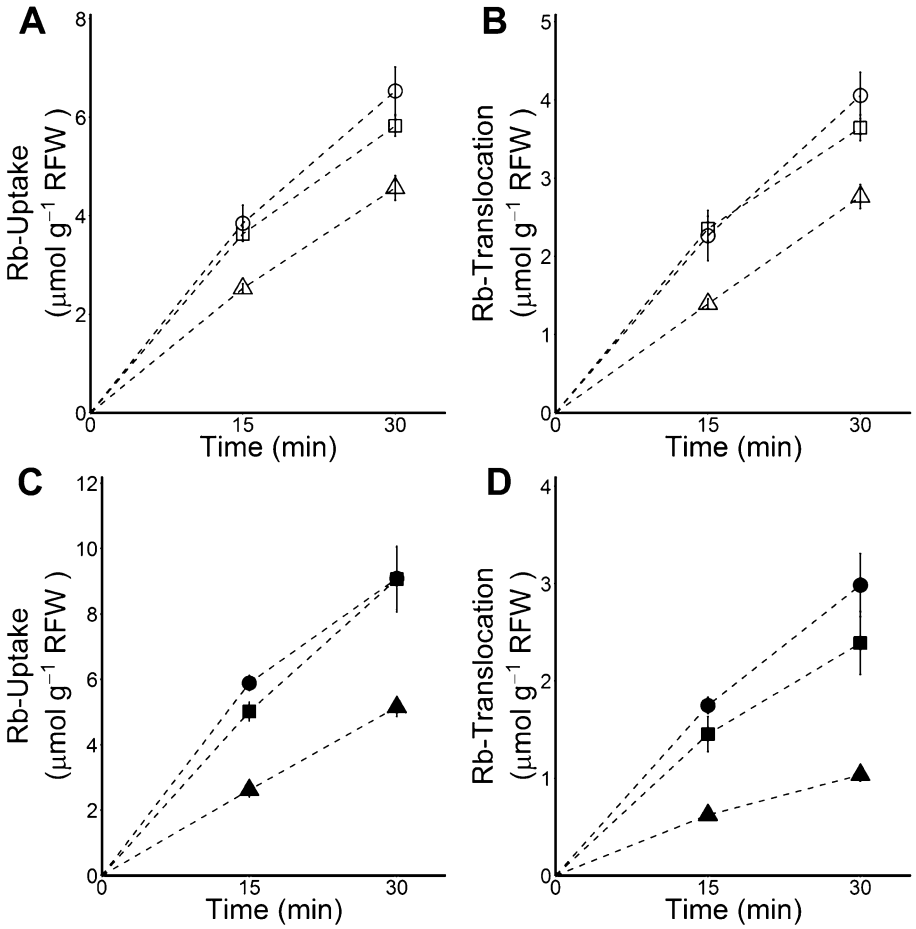


**Fig. 5** Time course for the uptake and translocation of the K-analogue, Rubidium, in *WT, gai-1* and *5xdella* plants. **a, b** Results corresponding to plants grown in 1.4 mM K and then transferred to a complete solution without K-addition but containing 1.4 mM RbCl are shown. *Symbols* are as follows: *squares* correspond to *WT, triangles* to *gai-1*, and *circles* to *5xdella* plants, respectively. **c, d** Results obtained for plants starved of K for a week and then transferred to a complete solution without K-addition but containing 0.1 mM RbCl are shown. *Panels A* and *C* correspond to Rb-uptake, whereas *panels B* and *D* correspond to Rb-translocation. Results correspond to the mean value of 4 replicates, *error bars* correspond to standard error

similar for *WT* and *5xdella* plants (15.4 µmol $g^{-1}$ RFW $h^{-1}$ for the later). In turn, long-distance transport of Rb from roots to shoots (Fig. 5b) experienced a similar reduction to that observed for Rb-uptake in *gai-1* relative to *WT* and *5xdella*. The pattern of uptake and long-distance transport of Rb observed in 7-day K-deprived plants exposed to a 0.1-mM Rb solution resembled that observed for K-well-supplied plants (Fig. 5c, d). In this case, *gai-1* plants displayed approximately a 50% lower potential influx of Rb (10.5 µmol $g^{-1}$ RFW $h^{-1}$) than that observed in *WT* (20.1 µmol $g^{-1}$ RFW $h^{-1}$) and *5xdella* (23.6 µmol $g^{-1}$ RFW $h^{-1}$) plants. These results indicated that differences in K-capture (Table 2) and Rb-depletion (Supplemental Fig. 5) from diluted solutions in K-deprived plants can also be explained, at least partially, by differences in influx. Again, the pattern of long-distance Rb-transport resembled that observed for Rb-uptake (Fig. 5d).

### Expression of Genes Coding for Root K-Transporters

The above-described results indicated that the inward flux is a critical point for the differences observed among lines. Because DELLAs play a key role in the regulation of transcription factors, we next explored to what extent the accumulation of transcripts coding for transport proteins involved in the influx (AKT1 and AtHAK5), as well as in the outward flux from the root epidermis (GORK), could be differentially affected among *WT, gai-1* and *5xdella* plants. It was found that accumulation of transcripts coding for the inward rectifier K-channel AKT1 was within the same range for all genotypes when grown at adequate K-supply as well as when deprived of K (Fig. 6). In turn, expression of *AtHAK5* tended to increase under conditions of K-deficiency, but the accumulation of transcripts was much lower in *gai-1* than in *WT* and *5xdella* K-deprived plants (Fig. 6). On the other hand, accumulation of transcripts coding for the outward rectifier K-channel GORK was within the same range at high K-supply. It tended to slightly increase under conditions of K-deficiency for *WT* and *5dellas* plants but not for *gai-1* plants (Fig. 6). Although differences in translocation appear to be, at least partially, a consequence of differences in Rb-uptake, the possibility that the lines here studied may also differ in the pattern of expression of the stelar outward rectifier K-channel SKOR, which contributes to long-distance transport of K from roots to shoots, was analyzed in a complementary experiment (Supplementary

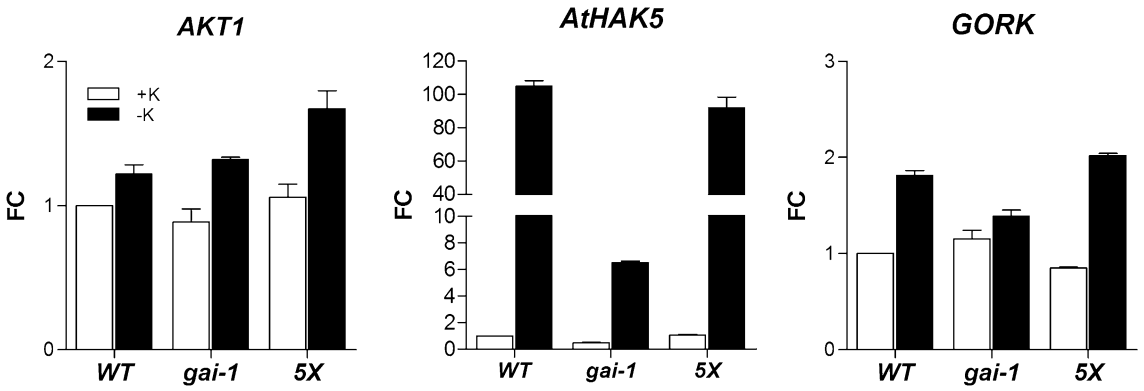


**Fig. 6** Accumulation of transcripts coding for root expressed K-transporters in *WT, gai-1* and *5xdella* plants grown under conditions of adequate K-supply (1.4 mM) or starved of K for 7 days. *White columns* correspond to plants grown at 1.4-mM KCl, whereas *black columns* correspond to plants K-deprived, respectively. Results shown correspond to the fold change relative to *WT* plants grown in the presence of K and are the mean of 3 replicates. *Bars* indicate standard error

Fig. 6). No differences were detected in the accumulation of SKOR transcripts either at high or at low K-supplies.

## Discussion

The modification of DELLAs activity has been of paramount importance for the increase of plant yield that took place during the green revolution (Hedden 2003). The further identification and characterization of these proteins, apart to establish their pivotal role in plant growth restriction (Harberd and others 2009; Hauvermale and others 2012), helped to disclose that they influence plant responses to multiple environmental stresses, including salinity (Achard and others 2006), low phosphorus supply (Jiang and others 2007), and pathogen attack (Achard and others 2008), being also necessary in symbiotic plant/fungal interactions (Floss and others 2013). The possibility that DELLAs are involved in plant responses to low potassium supply was first advanced by studies comparing wheat near isogenic lines carrying either "wild-type" or altered function alleles of the *Rht-1B*/*Rht-1D* genes (Moriconi and others 2012). That study showed that wheat dwarf lines, with reduced sensitivity to GAs, exhibit reduced senescence symptoms and could contribute to enhance the tolerance to K-deprivation. The present work illustrates that potassium deprivation in *Arabidopsis* promotes DELLAs accumulation in root cells close to the root tip and that relative plant performance under this stress condition increases in the GAs-insensitive mutant *gai-1*. In addition, the results also show that lack of DELLAs leads to decreased tolerance to K-deprivation; thus, providing conclusive evidence for a major role of DELLAs in plant acclimation to low K-supply. Furthermore, a role of DELLAs in plant responses to this adverse condition is also inferred from the observation that variations in the relative performance among *gai-1, WT, and 5Xdellas* plants were companied by important differences in the elemental composition and that internal efficiency of K-utilization or K-capture was also differentially affected in those lines.

Several works have shown that disruption of specific genes could lead to significant changes in plant elemental composition (that is, Lahner and others 2003; Baxter and others 2009; Chao and others 2011; McDowell and others 2013), whereas the modifications derived from gain of function mutations on elemental composition have been less frequently pursued (Punshon and others 2012). In spite of the central role of DELLAs in the integration of signals (Achard and others 2006), no previous studies have examined the possible role of loss and gain of function mutations of DELLAs in controlling plant mineral composition. Here, both PCA as well as analysis of individual elements performed on shoots unveiled that disruption of the five DELLAs coding genes exerted a minor effect on the shoot elemental composition when compared with the strong effect exerted by the *gai-1* mutation relative to the constitutive elemental composition of *WT* plants. Considering that *5xdella* plants lack DELLAs activity, whereas in *gai-1* plants, GAI-1 is stabilized relative to GAI in *WT*, the observation of a more pronounced difference between *5xdella* and *gai-1* plants than between each one of them and *WT* plants, as revealed by analysis of individual elements, suggest a progressive effect of DELLAs activity on shoot elemental composition. In addition, when just the individual elements constitutively modified in the *5xdella* mutant are considered, a major effect on shoot P-concentration became evident. This observation differed from that made by Jiang and others (2007), who did not find changes in the concentration of this element when a quadruple DELLAs mutant was used. This discrepancy can be explained

in several ways as the quadruple DELLA mutation used in those studies was built-up in a *ga1-3* background and plants were grown on a medium supplemented with sucrose. Overall, the existence of constitutive differences between the ionomes of *gai-1* and *WT* plants discloses a new and interesting role of GAI-1 stabilization.

The idea that changes in the elemental composition reflect the adaptation of plants to their environments (Baxter and Dilkes 2012) has been advanced, because, under conditions of low Fe or P supply, those changes can be used to predict plant physiological status (Baxter and others 2008), with this concept potentially applied to other nutrients, including K (Prinzenberg and others 2010). Results here obtained with PCA as well as those obtained by considering the number and identity of the elements that significantly changed their concentration during the transition from adequate to low K-supply, for each genotype, indicate that the degree of responsiveness of the elemental composition to K-deprivation was negatively modulated by DELLAs. In turn, reduced effects on the elemental composition (*gai-1* < *WT* < *5xdellas*) seem to be inversely connected with the order observed for the relative effect exerted by K-deprivation on relative plant performance (*5xdella* > *WT* > *gai-1*). This suggested that changes in the elemental composition inversely associated with DELLAs are an important component of the injury exerted by K-deficiency. It should be mentioned that because of the pivotal effect of DELLAs on plant elongation, a major question in studies on their role in acclimation responses to stress conditions is whether acclimation differences are just a consequence of intrinsic differences in growth rate. Certainly, an effect of intrinsic growth differences on the above-mentioned pattern should not be excluded, particularly when comparing *gai-1* and *WT* plants. However, ordering genotypes by relative performance as well as by the degree of response of the elemental composition to K-deprivation, as above done, did not show a simple association with the sequence followed by constitutive differences in biomass accumulation or RGR (*gai* < *5xdella* ≤ *WT*). This suggested that differences in acclimation to low K-supply in *Arabidopsis* should not be only attributed to differences in growth habit. Therefore, the data here introduced suggest that DELLAs are important components in setting the changes suffered by the shoot mineral composition as well as the tolerance during plant acclimation to K-scarcity.

It has recently been argued that tolerance could not be necessarily coupled to enhance efficiency of nutrient utilization (Santa-María and others 2015). The necessity of adequate measurements of EKU is particularly relevant to consider the potential involvement of specific genes in setting the balance between these two key nutritional traits. In this context, it should be noted that the use of static indicators of internal nutrient utilization efficiency has recently been criticized, as they do not necessarily reflect actual phenotypic differences in this parameter (Moriconi and Santa-María 2013; Santa-María and others 2015). Differences here found between EKUo and EKUu when used to compare EKU between *gai-1* and *WT* (or *5xdella*) plants are consistent with the previous reports indicating that those indicators do not always show coincident patterns (Siddiqi and Glass 1981; Gurley and others 1994). Using the most reliable dynamic estimators EKUi and EKUe, it was observed that ENU, considering it as the amount of biomass generated by unit of potassium accumulated in tissues, was maximized in *WT*, relative to *5xdella* plants. This result indicates that the lack of DELLAs (*5xdella* plants) leads to both decreased tolerance and decreased internal utilization efficiency of K at sub-optimum levels of K-supply, which suggests that DELLAs activity is required to hold both traits.

The observation that *gai-1* plants consistently display a lower specific absorption rate of K under conditions of adequate K-supply as well as a lower capacity to deplete Rb when deprived of K, combined with reduced root size of these plants relative to *WT* and *5xdella* plants, points out that the altered function version of DELLAs negatively contributes to regulate K-acquisition, at least under the hydroponic conditions assayed in this work. Although the ways by which DELLAs can influence *Arabidopsis* root growth have been the subject of some studies (Fu and Harberd 2003; Wild and others 2016), no previous information has linked DELLAs with K-uptake. Our results uncover that influx of the K-analogue, Rb, is negatively modulated in *gai-1* relative to *WT* plants under conditions of both adequate and insufficient K-supply. K-influx in *Arabidopsis* over a wide range of external K concentrations is driven by the inward rectifier AKT1 channel being the role of the HAK5 transporter increased as the external concentration of K during growth decreases (Nieves-Cordones and others 2014). The regulation of AKT1 and HAK5 involves several mechanisms, with HAK5 subjected to strong transcriptional control (Nieves-Cordones and others 2014). A remarkable finding obtained in this work was that the induction of *AtHAK5* expression under conditions of K-deprivation was sharply reduced in *gai-1* relative to *WT* plants, uncovering a critical role of the altered DELLA in this process. The mode by which the altered DELLA influences *AtHAK5* expression could be related to the interaction of GAI with transcription factors that modulate *AtHAK5*. In this regard, at least one transcription factor, ALCATRAZ (ALC), which likely interacts with DELLAs (Arnaud and others 2010), has been reported to be potentially involved in the control of *AtHAK5* expression (Hong and others 2013). Other transcription factors, such as DDF2, are also promising candidates (Hong and others 2013). In this context, it is

interesting to mention the model recently proposed for the action of DELLAs in plants suffering iron deficiency (Wild and others 2016). According to it, under iron deficiency conditions, DELLAs accumulate in the root meristem, while their accumulation at the root epidermis is gradually prevented, leading to relief of inhibition on the FIT transcription factor and thus accumulation of the iron transporter IRT1 as Fe-deficiency advances. The possibility that a similar model could help to explain the effect of *gai-1* on *AtHAK5* expression under conditions of K-deprivation should be taken into consideration. On the other hand, it is necessary to consider that influx in plants grown at external concentrations as high as 1.4-mM KCl, in which K-uptake is essentially mediated by AKT1, was also lower in *gai-1* than in *WT* plants. Because the levels of *AKT1* transcripts did not differ between these two genotypes, differences in Rb-influx observed under those conditions could be likely related with a modulation of AKT1 activity that does not involve transcriptional control of the coding gene.

Taken together, the results obtained along this work indicate that DELLAs contribute to set several plant responses to K-deprivation. In this regard, plants with reduced degradation of one of the DELLAs proteins (*gai-1* plants) display increased tolerance and low ionome responsiveness. On the other hand, lack of DELLAs activity (*5xdella* plants) leads to both low tolerance, high ionome responsiveness, and low EKU. For *gai-1* plants, those traits are companied by reduced root size and diminished capacity for K-uptake coupled to diminished influx, which under conditions of K-deprivation can be related, at least partially, to modulation of the expression of *AtHAK5*. In summary, the results here introduced unveil that DELLAs are major players in determining tolerance to low K-supply, K-utilization efficiency, and K-acquisition, as well as the elemental composition of *Arabidopsis* plants.

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**Compliance with Ethical Standards**

**Conflict of interest** The authors declare that they have no conflict of interest.

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