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ARTICLE ADDENDUM

How DELLAs contribute to control potassium uptake under conditions of potassium scarcity? Hypotheses and uncertainties

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ABSTRACT

Maintenance of the inward transport of potassium (K) by roots is a critical step to ensure K-nutrition for all plant tissues. When plants are grown at low external K concentrations a strong enhancement of the activity of the AtHAK5 transporter takes place. In a recent work, we observed that the *gai-1* mutant of *Arabidopsis thaliana*, which bears an altered function version of a DELLA regulatory protein, displays reduced accumulation of *AtHAK5* transcripts and reduced uptake of Rubidium, an analog for K. In this Addendum we discuss some hypotheses and uncertainties regarding how DELLAs could contribute to the control of K uptake under those conditions. We advance the idea that, following K-restriction, there is a zone and tissue specific regulation of DELLAs by gibberellins through a pathway that likely involves ethylene. According to this model in the epidermis of non-apical zones, DELLAs repress transcription factors that promote *AtHAK5* accumulation.

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Higher plants need large quantities of potassium to fully accomplish their life cycles. As revealed by studies with *Arabidopsis thaliana*, the inward flux of this essential nutrient to the root symplasm from moderate or low external K concentrations requires the activity of two transport systems, the inward rectifier K-channel AKT1[1] and AtHAK5,[2] which is a member of the group I of the KT-HAK-KUP family of transporters.[3] While the first transport entity operates over a relatively wide range of external K concentrations, the second one is essentially involved in the transport of K from diluted concentrations and dominates this process at K-concentrations below 10 μM.[4] Given that maintenance of K-inward flux is a critical step in ensuring K-acquisition, it should not surprise that plants evolved admirable mechanisms that allow them to regulate the transport mediated by AKT1 and AtHAK5, whose activities increase following the perception of K scarcity.[2,5-7] Those regulatory mechanisms, to be effective in plant acclimation, need to be integrated within the complex network that connects the economy of carbon with that of all the elements necessary for plant growth. Current evidence indicates that a central point for the integration of signals is provided by the DELLAs proteins,[8] which are a group of plant regulatory elements modulated by gibberellins (GAs). Recent findings unveiled that the shoot of Arabidopsis plants bearing an altered function version of DELLA (gai-1) exhibits a major modification of its mineral composition[9] in addition to display the well-known restriction of plant growth.[10]

DELLAs proteins contain two main domains: a C-terminal GRAS domain and the N-terminal domain which plays a major role for the binding with the complex GAs/GAs-receptor which guides DELLAs to degradation at the proteasome, thus leading to reduced DELLAs accumulation.[8] Because of DELLAs are growth inhibitors, plants carrying versions of DELLAs containing either a deletion or specific amino acid substitutions in some motifs of the N terminus, display dwarf phenotypes, as mentioned to occur with the Arabidopsis *gai-1* mutant which is insensitive to the DELLAs degradation driven by GAs. The new findings indicated that, among the major essential elements differentially affected in *gai-1* relative to wild type (*WT*) plants, K occupies a prominent position both when plants are grown with or without an adequate supply of K.[9] The low concentration of this element in *gai-1* shoots proved to be associated with reduced K uptake by roots. A further analysis of the inward flux of the K-analog, Rubidium (Rb), showed that it was considerably lower in *gai-1* than in *WT* plants, particularly under conditions of K-deficiency, thus suggesting that one or both systems involved in the transport of K from low K concentrations cannot be upregulated in *gai-1* plants. Moreover, the induction of the expression of *AtHAK5* by K-deficiency was sharply diminished in *gai-1*, while no differential effects were evident on the expression of *AKT1* between *gai-1* and *WT* plants. These, and additional observations, along with knowledge already available on the action of DELLAs, led us to consider here which is the precise role of these proteins in the control of K-uptake mediated by AtHAK5 from diluted K-

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concentrations. A first possible explanation is that the modification harboured by the gai-1 protein helps it to establish interactions with transcription factors (TFs) different to those that occur in *WT* plants. As the N-terminal region of DELLAs is intrinsically disordered,[11] this possibility should be not entirely ruled out. However, the ability to regulate a set of target genes appears to be a general property of DELLAs and not only of gai-1.[12] Therefore, the above mentioned results would likely indicate that when *WT* plants are grown under conditions of K-deficiency, DELLAs may be preferentially degraded, thus relieving the restriction imposed by them on the transport of Rb (or K) through the control of *AtHAK5* transcription. As gai-1 is a DELLA stable variant, that degradation would not take place in the plants harbouring the corresponding allele, and the transport of K is not further induced. However, this simple hypothesis does not take into account an additional observation: under conditions of K scarcity the accumulation of the DELLA protein RGA, which in vegetative tissues has functional similarity to GAI,[13-15] becomes increased in the Arabidopsis root apex of *WT* plants.[9]

How to reconcile these apparently opposite observations? A recent work by Wild et al[16] on the role of DELLAs in the regulation of iron transport provides some possible clues. According to the information collected by those authors, DELLAs have a spatially differential accumulation under conditions of Fe-deficiency and Fe-sufficiency. Remarkably, under conditions of Fe-deficiency they are excluded in the epidermis of the root differentiation zone but accumulated in the root apex. We would like to advance the hypothesis that a conceptually similar pattern could be found under conditions of K-deficiency and that it plays a major role in the control of *AtHAK5* transcription. It is then proposed that in *WT* plants grown under conditions of K-scarcity, the DELLAs proteins predominantly acting on vegetative growth are accumulated in the root apex but not in the epidermis of non-apical root zones where they are degraded. Localized degradation of these DELLAs relieves their potential interaction with a specific subset of TFs. As one, or more, of these TFs could positively modulate the accumulation of transcripts coding for AtHAK5, the relief of their interaction with DELLAs must enhance the transcription of *AtHAK5* and thereby the amount of the AtHAK5 protein at the plasma-membrane. Therefore, the capacity of roots to mediate K transport through AtHAK5 may increase. This model entails, as an initial step, identifying TFs acting on *AtHAK5* that are also potentially subjected to interaction with DELLAs. A survey of TFs that probably interact with *AtHAK5*[17] showed that one of them is ALC, which is potentially able to interact with DELLAs in some organs,[18] while other TFs -such as DDF2- should be also considered possible candidates.[17] In addition to this process, it is possible that DELLAs could also indirectly modify the accumulation and/or the activity of AtHAK5 (e.g. by controlling the membrane potential or proteins regulating AtHAK5-mediated transport). The true establishment of any of these interactions in roots under conditions of variable K supply as well as the actual occurrence of spatially separated processes here advocated, need to be fully assessed.

This model, nevertheless, would remain non-entirely satisfactory. It seems worth to note that simultaneous studies with a multiple DELLA mutant unveiled that it displays a similar inward flux of Rb and *AtHAK5* expression pattern to that observed in *WT* plants both when deprived of, and well supplied with, potassium. While these data are consistent with the notion that degradation of DELLAs are necessary for the induction of *AtHAK5* expression, and thereby for K-influx mediated by this transporter under conditions of K-deficiency, they also suggest that additional components could act in the de-repression of K-uptake. This leads to consider that another signaling component is necessary for the induction of the TF(s) above mentioned. According to this, under conditions of K-deficiency -in *WT* plants- the induction of TFs that potentially could act on *AtHAK5* is a consequence of the action of this second signal. The degree to which TF(s) are able to induce *AtHAK5* transcription would depend on the degradation of DELLAs in the epidermis. When DELLAs are degraded or absent (as in *WT* and the multiple mutant, respectively) the TFs are able to act on *AtHAK5*. However, when DELLAs are non-degraded the positive influence of TFs on *AtHAK5* cannot be fully exerted.

The speculative model above outlined (Fig. 1), if supported by further evidence, will necessarily pose additional questions. One of them relates to the nature of the signal(s) leading to the spatially localized degradation of DELLAs at the epidermis in non-apical root zones. A probable hypothesis is that it involves other elements of the GAs-GID-DELLAs module, namely the GAs and GAs-receptors (GID). In this regard, an early work suggested the possible involvement of GAs, when applied in shoots, in increasing Rb-uptake.[19] In addition, movement of GAs among root tissues could also play a role in root responses.[20] As DELLAs can be also modified through non-GAs routes[21] alternative hypotheses should be not discarded. A second important question is the nature of the second signaling component. A likely candidate for this role is ethylene which increases under conditions of K-deficiency while genetic and pharmacological evidence indicates that it modulates *AtHAK5* expression.[22] Moreover, this hormone could also play a major function in determining GAs distribution,[20] thus potentially affecting localized DELLAs degradation. Noticeably, other candidates could eventually contribute to the proposed second signaling component. In this regard it seems worth to mention that the working model here proposed must be considered just as a part of a complex signaling network induced by K-deficiency that includes several components already identified,[4,23] including some involved in jasmonate signaling,[24] and others -such as now DELLAs proteins- which await to be discovered. New knowledge will contribute to solve the uncertainties above outlined as well as to advance alternative models to that here discussed.

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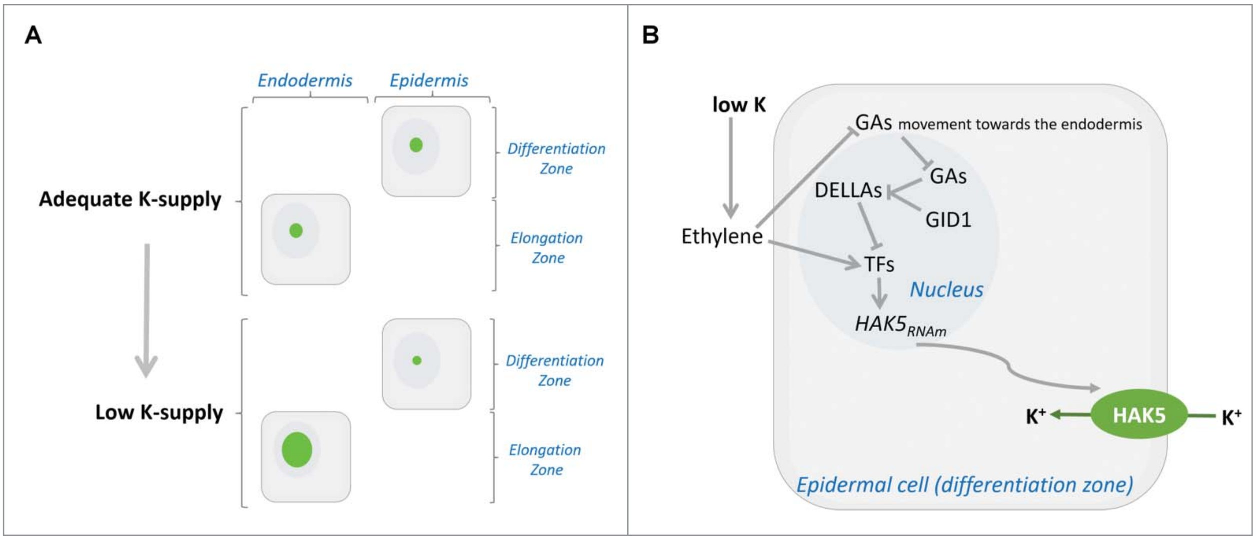

Figure 1. Hypothetical model for the regulation of AtHAK5 as influenced by DELLAs in roots. (A) Proposed scheme for the spatial accumulation of DELLAs in roots as affected by the supply of K during the growth of *WT* plants. In roots exposed to adequate K-supply, levels of DELLAs (green circles) are low in the endodermis of the elongation zone; while they are accumulated to some extent in the epidermis of the differentiation zone. Under conditions of low K supply, DELLAs are preferentially accumulated in the endodermis of the root elongation zone while their accumulation diminished in the epidermis of the differentiation zone. (B) Hypothetical model for the action of DELLAs on AtHAK5 in the epidermis of the differentiation zone at low K-supply. Perception of low K-supply leads to enhanced accumulation of ethylene, which leads to accumulation of transcription factors (TFs) with the capacity to enhance *AtHAK5* transcription. In turn, ethylene or other unknown signals could decrease the movement of GAs toward the endodermis, thereby increasing GAs accumulation in the epidermal cells. GAs in that zone, through their interaction with GAs-receptors (GID), promote DELLAs degradation, thus relieving the restriction imposed by these proteins on the activity of the TFs acting on *AtHAK5*.

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Abbreviations

AtHAK5	*Arabidopsis thaliana* high-affinity potassium transporter 5
AKT1	Arabidopsis potassium transporter 1
GAas	gibberellins
GAI	GA-insensitive
gai-1	GA insensitive mutant 1
RGA	Repressor of GA1–3
TFs	transcription factors
WT	wild type

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