Plant Signaling & Behavior 9, e27740; January; © 2014 Landes Bioscience

Upon bolting the GTR1 and GTR2 transporters mediate transport of glucosinolates to the inflorescence rather than roots

Tonni Grube Andersen and Barbara Ann Halkier*

DynaMo Centre of Excellence; Department of Plant and Environmental Sciences; Faculty of Science; University of Copenhagen; Frederiksberg C, Denmark

Keywords: glucosinolate, transport, roots, feeding, *Arabidopsis*, chemical defense distribution, specialized/secondary metabolites

We recently described the glucosinolate transporters GTR1 and GTR2 as actively contributing to the establishment of tissue-specific distribution of the defense compounds glucosinolates in vegetative *Arabidopsis* plants. Upon bolting and thereby development of the inflorescence and initiation of seed setting, the spatial distribution of glucosinolates does undergo major changes. Here we investigate the role of GTR1 and GTR2 in establishment of glucosinolate source-sink relationships in bolting plants. By in vivo feeding the exogenous *p*-hydroxybenzylglucosinolate to a rosette leaf or the roots of wildtype and a *gtr1 gtr2* mutant, we show that this glucosinolate can specifically translocate from the rosette and the roots to the inflorescence in a GTR1 – and GTR2-dependent manner. This marks that, upon bolting, the inflorescence rather than the roots constitute the strongest sink for leaf glucosinolates compared with plants in vegetative state.

A major component of the chemical defense system in *Arabidopsis* is the specialized metabolites glucosinolates (GLS).[1,2] GLS concentrations in leaves decrease with age until virtually absent upon senescence, where accumulation only occurs in the seeds.[3] Recently, we identified 2 vasculature-localized GLS transporters (GTR1 and GTR2) in *Arabidopsis*. These transporters facilitate import of GLS from maternal tissues into seeds.[4] By employing in vivo feeding and micrografting techniques, we further showed that GLS can translocate between rosettes and roots in a GTR1- and GTR2-dependent manner,[5] thereby providing evidence that the root can serve as a sink for GLS at this developmental stage. As the plant progresses from a vegetative stage into bolting and seed production, major changes are likely to occur in the source-sink relationships for GLS. Hence, in the current study, we investigate the source-sink relationships between root, rosette and inflorescence of exogenously fed *p*-hydroxybenzyl GLS (*p*OHB) in plants after the onset of bolting.

Results

*p*OHB was fed to leaves of hydroponically grown 5-week-old seed-setting plants. Roots, rosettes, and inflorescences were analyzed separately for *p*OHB content 48 and 72 h after feeding (HAF). In the wildtype, the *p*OHB content in the fed rosette leaf decreased significantly from constituting ~40% of the total plant content 48 HAF to ~15% 72 HAF($P < 0.01$) (**Fig. 1A**). This decrease was accompanied by a corresponding increase from ~50% to ~75% in the inflorescence ($P < 0.01$). No changes were observed in the remaining rosette leaves (**Fig. 1A**). In the *gtr1gtr2* mutant, no significant changes in *p*OHB distribution were observed in any of the analyzed tissues and ~70% of the total plant *p*OHB was retained in the fed leaf (**Fig. 1A**). Notably, pOHB was not detected in the roots of either wildtype or *gtr1gtr2*. We subsequently investigated the GLS distribution upward from the roots at this developmental stage, by incubating 5-week-old wildtype and *gtr1gtr2* plants in media containing *p*OHB. In the wildtype, the *p*OHB content in the fed roots decreased from ~32% of the total plant content 48 HAF to ~15% 72 HAF ($P < 0.01$) (**Fig. 1B**). This was accompanied by an increase to ~80% in the inflorescence ($P < 0.01$), while the content in the rosettes remained at ~5% (**Fig. 1B**). In the *gtr1gtr2* mutant, only minute amounts of *p*OHB were detected and no changes in the distribution were observed between 48 and 72 HAF (**Fig. 1B**). The total amount of *p*OHB recovered in leaf and root feeding experiments ranged from 2.43 +/– 1.26 to 35.7 +/– 26.1 nmol (**Table 1**).

Discussion

Prior studies indicate that transport of glucosinolates occur from the rosette to reproductive tissue in *Arabidopsis*.[5,7] We have previously shown that *p*OHB is a substrate for GTR1 and GTR2 expressed in *Xenopus laevis* oocytes[4] and that it is distributed identically to the aliphatic 2-propenyl GLS upon feeding to both leaves and roots.[5] Hence, we utilized *p*OHB as a model GLS to investigate source/sink relationship for endogenous GLS in bolting *Arabidopsis* plants. In contrast to the previously observed

*Correspondence to: Barbara Ann Halkier; Email: bah@plen.ku.dk
Submitted: 12/11/2013; Revised: 01/06/2014; Accepted: 01/06/2014; Published Online: 01/30/2014
Citation: Andersen TG, Halkier BA. Upon bolting the GTR1 and GTR2 transporters mediate transport of glucosinolates to the inflorescence rather than roots. Plant Signaling & Behavior 2014; 9:e27740; PMID: 24481282; http://dx.doi.org/10.4161/psb.27740

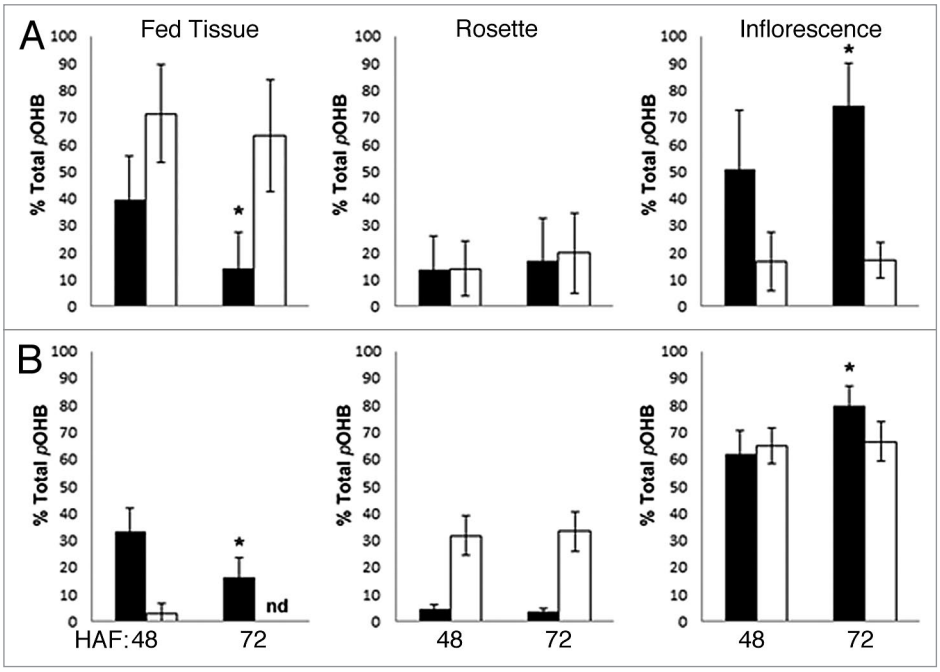

Figure 1. Distribution of leaf- and root-fed *p*-hydroxybenzylglucosinolate. Hydroponically grown 5-week-old wild-type (Col-0) and *gtr1gtr2* plants were fed with the exogenous *p*-hydroxybenzylglucosinolate (*p*OHB) for a total timespan of 72 h. The content of *p*OHB was analyzed in the separate tissues by HPLC and normalized to the total content of the plant. (**A**) Infiltration of *p*OHB to a rosette leaf. (**B**) Feeding of *p*OHB to roots by addition of *p*OHB to the growth media. Total amount of recovered *p*OHB can be found in **Table 1**. HAF; hours after feeding, nd; none detected. Solid bars represent wild-type plants and open bars represent the *gtr1gtr2* mutant. * $P < 0.01$ (Student *t*-test vs corresponding tissue at 48 h. Bars are average, ± SD, n = 10).

Table 1. Total amount of *p*OHB (nmol) in plants 48 and 72 h after feeding (HAF). Numbers are average (n = 10), in parentheses are SD

	Leaf feeding		Rootfeeding	
	48HAF	72HAF	48HAF	72HAF
WT	13.7	6.54	6.5	9.32
	(11.7)	(7.0)	(2.67)	(4.73)
gtr1gtr2	35.7	29.8	2.43	5.75
	(26.1)	(17.3)	(1.26)	(2.91)

GTR-dependent transport of leaf-fed *p*OHB from rosette to root in vegetative plants,[5] we solely detected GTR-dependent upward transport of leaf-fed *p*OHB to the inflorescence (**Fig. 1A**). Besides illustrating that GTR1 and GTR2 also mediate retention of GLS in the roots at this developmental stage, our data demonstrates a shift in GLS sink strength between the root and inflorescence upon bolting compared with vegetative plants. The results suggest that, similar to in vegetative plants, GLS can move between tissues GTR1- and GTR2-dependently in the bolting plant, and that endogenous GLS may be transported from both the rosette and roots to the inflorescence in a GTR1- and GTR2-dependent manner when the plant changes its life strategy from the vegetative stage to development of inflorescence with seeds that represent a strong sink for GLS. Glucosinolate movement to the inflorescences upon bolting is likely to reflect a need of the plant to protect plant parts with high fitness value, i.e., flowers, fruits, and seeds, from herbivore and pathogen enemies.

Methods

GLS feeding and analysis

GLS were analyzed as desulfo GLS as previously described.[5] To evaluate root uptake *p*OHB was added to growth media to a final concentration of 200 μM. Feeding to leaves was done by infiltrating max 4 μL Murashige and Skoog (MS) media adjusted to pH 5,6 containing 10 mM *p*-OHB directly to a leaf using a 1 mL syringe with a 10 μL pipette tip. After 48 and 72 h incubation, plant organs from 10 individual plants were harvested, washed 3 times in water, and analyzed for GLS content as described elsewhere.

Plant growth conditions

Arabidopsis thaliana (Col-0) plants were grown in a hydroponic system as previously described.[5]

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References

1. D'Auria JC, Gershenzon J. The secondary metabolism of Arabidopsis thaliana: growing like a weed. Curr Opin Plant Biol 2005; 8:308-16; PMID:15860428; http://dx.doi.org/10.1016/j.pbi.2005.03.012
2. Halkier BA, Gershenzon J. Biology and biochemistry of glucosinolates. Annu Rev Plant Biol 2006; 57:303-33; PMID:16669764; http://dx.doi.org/10.1146/annurev.arplant.57.032905.105228
3. Brown PD, Tokuhisa JG, Reichelt M, Gershenzon J. Variation of glucosinolate accumulation among different organs and developmental stages of *Arabidopsis thaliana*. Phytochemistry 2003; 62:471-81; PMID:12620360; http://dx.doi.org/10.1016/S0031-9422(02)00549-6
4. Nour-Eldin HH, Andersen TG, Burow M, Madsen SR, Jørgensen ME, Olsen CE, Dreyer I, Hedrich R, Geiger D, Halkier BA. NRT/PTR transporters are essential for translocation of glucosinolate defence compounds to seeds. Nature 2012; 488:531-4; PMID:22864417; http://dx.doi.org/10.1038/nature11285
5. Andersen TG, Nour-Eldin HH, Fuller VL, Olsen CE, Burow M, Halkier BA. Integration of biosynthesis and long-distance transport establish organ-specific glucosinolate profiles in vegetative Arabidopsis. Plant Cell 2013; 25:3133-45; PMID:23995084; http://dx.doi.org/10.1105/tpc.113.110890
6. Petersen BL, Chen S, Hansen CH, Olsen CE, Halkier BA. Composition and content of glucosinolates in developing Arabidopsis thaliana. Planta 2002; 214:562-71; PMID:11925040; http://dx.doi.org/10.1007/s004250100659
7. Ellerbrock BLj, Kim JH, Jander G. Contribution of glucosinolate transport to Arabidopsis defense responses. Plant Signal Behav 2007; 2:282-3; PMID:19704682; http://dx.doi.org/10.4161/psb.2.4.4014