

## New Phytologist Supporting Information

Article title: Dynamics in plant roots and shoots to minimise stress, save energy and maintain water and nutrient uptake

Authors: Borjana Arsova, Kylie J. Foster, Megan C. Shelden, Helen Bramley, Michelle Watt

Article acceptance date: 19 April 2019

**Supporting Information Table S1.** Details of ion ( $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Cl}^-$ ,  $\text{NO}_3^{-2}$ ,  $\text{NH}_4^+$ ) and water transporters positioned in roots and shown in Figure 2, main text. Complete set of publications presented. <sup>1</sup>Label used in Figure 2, main text; \*Protein function not fully understood or not characterized in cited study, \*\* protein localization as a result of promotor::GUS/LUC fusion.

Transp. Type	Fig. 2 <sup>1</sup>	Species	Ion	Transporter	Location in plant root and inside the cell	In planta Transcript (t), protein (p) localization in publication	Publication
HKT	1a	Barley	$\text{Na}^+$ , $\text{K}^+$	HvHKT1;1	Cell plasma membrane in root stele and epidermis radial root $\text{Na}^+$ transport	t, p	Han et al. (2018)
	1b	Wheat	$\text{Na}^+$	TmHKT1;5-A	Xylem, plasma membrane	t	Munns et al. (2012)
		Barley	$\text{Na}^+$	HvHKT1;5	Root epidermis and root stele (transcript data)	t	Zhu et al. (2017)
		Barley	$\text{Na}^+$ *	<i>HKT1;5 and HKT2.4</i> (rice ortholog)	Seminal roots (maturation zone)	t	Hill et al. (2016)
		Barley	$\text{Na}^+$ , $\text{K}^{+*}$	<i>HKT2.1</i> (rice ortholog)	Seminal roots (elongation and maturation zone)	t	Hill et al. (2016) (Mian et al., 2011)
		Barley	$\text{Na}^+$ , $\text{K}^{+*}$	<i>HKT2.3</i> (rice ortholog)	Seminal roots (elongation zone and maturation zone)	t	Hill et al. (2016)
SOS1	1c	Arabidopsis	$\text{Na}^+$	SOS 1	Young root cells, root tip	p	Shi, (2002)
		Barley	$\text{Na}/\text{H}$	SOS1	Root epidermis and root stele (transcript data) Seminal roots (apex, elongation maturation zone)	t	Zhu et al. (2017), Hill et al. (2016)
NHX		Arabidopsis	$\text{Na}/\text{H}$	NHX6	Root apical region (meristem) Lateral root development	p	Dragwidge et al. (2018)

NPL	2a	Maize	$\text{Cl}^-/\text{NO}_3^-$	NPL6.4 & 6.6	Also selective for $\text{NO}_3^{-2}$ , Plasma membrane localization.	p	Wen et al. (2017)
NPF		Arabidopsis	$\text{Cl}^-$	AtNPF2.4	Root stele, plasma membrane	t	Reviewed in Li et al. (2017)
	2b	Arabidopsis	$\text{Cl}^-$	AtNPF2.5	Plasma membrane, along the root length of 2 week old Arabidopsis and in root- to-shoot junction. In cortical cells.	p	Li et al. (2016)
		Arabidopsis	$\text{Cl}^-/\text{NO}_3^-$	AtNRT1.5/ AtNPF7.3 AtNRT1.8/ AtNPF7.2	Stele specific, root xylem	t	Reviewed in Li et al. (2017)
SLAH		Arabidopsis	$\text{Cl}^-$	AtSLAH1	Root pericycle	t, p	Reviewed in Li et al. (2017)
		Arabidopsis	$\text{Cl}^-/\text{NO}_3^-$	AtSLAH3	Root pericycle (and stomatal guard cells)	t, p	Reviewed in Li et al. (2017)
CCC		Arabidopsis	$\text{Cl}^-, \text{Na}^+,$ $\text{K}^+$	AtCCC	root vasculature, golgi and trans-golgi network	t, p	Reviewed in Li et al. (2017)
CLC		Arabidopsis	$\text{Cl}^-/\text{NO}_3^-$	AtCLC	Vacuolar tonoplast, root cells	p	Reviewed in Li et al. (2017)
KT- HAK- KUPs	3a	Arabidopsis	$\text{K}^+$	AtKUP7	Plasma membrane, ubiquitously expressed in mature root and stele of root tip.	p	Han et al. (2016)
	3b	Arabidopsis	$\text{K}^+$	AtHAK5	Strongly induced by $\text{K}^+$ starvation, expression strongest in epidermis and stele of primary root and epidermis of lateral roots.	t, p	Gierth et al. (2005)
		Rice	K	HAK5	root epidermis, parenchyma of stele tissue and primordial of the lateral root	t, p	Yang et al. (2014)
Shaker like channel s		Barley	$\text{K}^+$	SKOR	Root stele	t	Zhu et al. (2017)
		Arabidopsis	$\text{K}^+$	SKOR	Root Stele, xylem parenchyma, epidermis	t, p**	Gaymard (1998)
		Arabidopsis	K	AKT2	Root Phloem	t, p**	Lacombe et al. (2000)
		Arabidopsis	K	GORK	Root hairs	t, p	Ivashikina et al. (2001)

NRT	4a	Arabidopsis	$\text{NO}_3^-$	AtNRT1.1 (CHL1)	Root tip, elongation zone in primary and lateral roots. It seems at the tip uniform distribution , in mature roots epidermal plasma membrane and central lamella	t, p	Guo et al. (2001), Guo, (2002)
	4b	Arabidopsis	$\text{NO}_3^-$	AtNRT1.9	phloem	t, p	Wang and Tsay (2011)
	4c	Arabidopsis	$\text{NO}_3^-$	AtNRT2.1	Periphery of epidermal cells (?!)  Along the length of the primary root and at older regions of lateral roots	p  t, p**	Chopin et al. (2007)  (Remans et al., 2006)
CLC		Arabidopsis	$\text{NO}_3^-$	AtCLC-b	Vacuolar tonoplast, young roots, lateral roots	t, p	von der Fecht-Bartenbach et al. (2010)
AMT	5a	Arabidopsis	$\text{NH}_4^+$	AtAMT1;1	Epidermis, root hairs	t, p	Yuan et al. (2007)
	5b	Arabidopsis	$\text{NH}_4^+$	AtAMT1;2	Endodermis, Plasma membrane (root hair zone), Endodermis and cortex in mature region; Cortex in basal region of the root	t, p	Yuan et al. (2007)
	5c	Arabidopsis	$\text{NH}_4^+$	AtAMT1;5	Root tips; Root hair zone	t, p	Yuan et al. (2007)
	5d	Arabidopsis	$\text{NH}_4^+$	AtAMT1;3	Outer cortex (?!)	t, p	Yuan et al. (2007)
PIP	6a	Barley	Water	HvPIP2;1*	All cells near the tip, but epidermis and vascular bundles in maturing region.	t, p	Bramley et al. (2007)
		Arabidopsis	Water	PIP2;1	Epidermis, elongation zone (1 cm from apex). Located on PM but internalized to vacuoles upon NaCl treatment. Water permeability further increases when co-expressed with AtPIP1;2 or AtPIP1;5	p	Ueda et al. (2016), Prak et al. (2008), Byrt et al. (2017)

		Rice	Water*	OsPIP2;4, OsPIP2;5	Exodermis, cortex, endodermis PiP2s also in stelar tissue of Lateral root > crown root > primary root, Located on plasma membrane but become internalised upon NaCl or PEG treatment	p	Chu et al. (2018)
6b	Maize	Water*	ZmPIP1;2		Highest in mature compared to elongating tissue, xylem parenchyma	t	Reviewed in Bramley et al. (2007)
	Maize	Water*	ZmPIP2;4 ZmPIP1;5 ZmPIP2;5 ZmPIP1;1, ZmPIP2;1,Zm PIP2;5, ZmPIP1;5				Reviewed in Bramley et al. (2007)
	Maize	Urea/ Water	ZmPIP1;5		Preferentially expressed in roots. Stele and cortex		Reviewed in Bramley et al. (2007)
	Rice	Water*	OsPIP1;1		Exodermis, cortex, endodermis	p	Chu et al. (2018)
	Barley	Water*	HvPIP1;2, HvPIP2;2, HvPIP2;5		Ubiquitous in all major root tissues (epidermis, cortex, endodermis, stele – but images show not in epidermis and HvPIP2;2 low expression in transition zone)	t	Knipfer et al. (2011)
	Barley	Water*	HvPIP2;7		Epidermis	t	Knipfer et al. (2011)
TIP	6c	Maize	Water	ZmTIP1	Meristems of primary and lateral roots. Epidermis, endodermis, xylem parenchyma of elongation zone. In mature roots highest in xylem parenchyma.	t	Reviewed in Bramley et al. (2007)
	Barley	Water*	HvTIP1;1,		Ubiquitous in all major root tissues (epidermis, cortex, endodermis, stele – but images show not in epidermis and HvPIP2;2 low expression in transition zone)	t	Knipfer et al. (2011)

	Barley	Water*	HvTIP2;3,	Epidermis	t	Knipfer et al. (2011)
	Bamboo	Water*	PeTIP4;1, PeTIP4;2	Epidermis, pith and vasculature, Expression correlated with root pressure	t	Sun et al. (2018)
	Arabidopsis	Water*	AtTIP1;2	Root cap	p	Gattolin et al. (2009)
	Arabidopsis	Water*	AtTIP1;1	Throughout root axis (especially endodermis and pericycle), but not root cap	p	Gattolin et al. (2009)
	Arabidopsis	Water*	AtTIP2;1	Lateral root primordium, Primary and lateral roots	p	Gattolin et al. (2009)
	Arabidopsis	Water*	AtTIP2;2	Cortex and epidermis, extends to pericycle as root matures	p	Gattolin et al. (2009)
	Arabidopsis	Water*	AtTIP2;3	Similar to TIP2;2 but initiates in pericycle; Primary and lateral roots	p	Gattolin et al. (2009)
	Arabidopsis	Water*	AtTIP4;1	Pericycle, especially xylem poles, absent from endodermis	p	Gattolin et al. (2009)
NIP	Arabidopsis	Water	AtNIP2;1	Most root cells, Most of young root, but higher expression in root elongation zone and stele. Mainly located in the ER membrane under ambient conditions. Low water channel activity – most likely functions as aquaglyceroporin	t, p	Mizutani et al. (2006)
	Arabidopsis	Lactic acid		Root cap and vascular cylinder. Zone of cell specialization in primary root, not in zone of cell division or elongation or in lateral roots	t, p	Choi and Roberts (2007)
	Rice	Boron	OsNIP3;1	Exodermis and stele. Root tip, elongation and root hair zones in B-deficient plants. Found in primary and lateral roots (but not root hairs) and especially regions of lateral root emergence	t, p**	Hanaoka et al. (2014)

## References:

- Bramley H, Turner DW, Tyerman SD, Turner NC** (2007) Water flow in the roots of crop species: The influence of root structure, aquaporin activity, and waterlogging. *Advances in Agronomy* **96**: 133-196
- Byrt CS, Zhao M, Kourghi M, Bose J, Henderson SW, Qiu J, Gilliam M, Schultz C, Schwarz M, Ramesh SA, Yool A, Tyerman S** (2017) Non-selective cation channel activity of aquaporin AtPIP2;1 regulated by Ca<sup>2+</sup> and pH. *Plant Cell Environ* **40**: 802-815
- Choi W-G, Roberts DM** (2007) ArabidopsisNIP2;1, a Major Intrinsic Protein Transporter of Lactic Acid Induced by Anoxic Stress. *Journal of Biological Chemistry* **282**: 24209-24218
- Chopin F, Wirth J, Dorbe MF, Lejay L, Krapp A, Gojon A, Daniel-Vedele F** (2007) The Arabidopsis nitrate transporter AtNRT2.1 is targeted to the root plasma membrane. *Plant Physiol Biochem* **45**: 630-635
- Chu TTH, Hoang TG, Trinh DC, Bureau C, Meynard D, Vernet A, Ingouff M, Do NV, Perin C, Guiderdoni E, Gantet P, Maurel C, Luu DT** (2018) Sub-cellular markers highlight intracellular dynamics of membrane proteins in response to abiotic treatments in rice. *Rice (N Y)* **11**: 23
- Dragwidge JM, Ford BA, Ashnest JR, Das P, Gendall AR** (2018) Two Endosomal NHX-Type Na<sup>+</sup>/H<sup>+</sup> Antiporters are Involved in Auxin-Mediated Development in Arabidopsis thaliana. *Plant Cell Physiol* **59**: 1660-1669
- Gattolin S, Sorieul M, Hunter PR, Khonsari RH, Frigerio L** (2009) In vivo imaging of the tonoplast intrinsic protein family in Arabidopsis roots. *BMC Plant Biology* **9**: 133
- Gaymard F, Pilot, G., Lacombe, B., Bouchez, D., Bruneau, D., Boucherez, J., Michaux-Ferrière, N., Thibaud, J. B., Sentenac, H.** (1998) Identification and Disruption of a Plant Shaker-like Outward Channel Involved in K<sup>+</sup> Release into the Xylem Sap. *Cell* **94**: 647-655
- Gierth M, Maser P, Schroeder JI** (2005) The potassium transporter AtHAK5 functions in K(+) deprivation-induced high-affinity K(+) uptake and AKT1 K(+) channel contribution to K(+) uptake kinetics in Arabidopsis roots. *Plant Physiol* **137**: 1105-1114
- Guo FQ, Wang RC, Chen MS, Crawford NM** (2001) The Arabidopsis dual-affinity nitrate transporter gene AtNRT1.1. (CHL1) is activated and functions in nascent organ development during vegetative and reproductive growth. *Plant Cell* **13**: 1761-1777
- Guo FQ, Wang, R. Crawford, N. M.** (2002) The Arabidopsis dual-affinity nitrate transporter gene AtNRT1.1 (CHL1) is regulated by auxin in both shoots and roots. *J Exp Bot* **53**: 835-844
- Han M, Wu W, Wu WH, Wang Y** (2016) Potassium Transporter KUP7 Is Involved in K(+) Acquisition and Translocation in Arabidopsis Root under K(+)-Limited Conditions. *Mol Plant* **9**: 437-446
- Han Y, Yin S, Huang L, Wu X, Zeng J, Liu X, Qiu L, Munns R, Chen ZH, Zhang G** (2018) A Sodium Transporter HvHKT1;1 Confers Salt Tolerance in Barley via Regulating Tissue and Cell Ion Homeostasis. *Plant Cell Physiol*
- Hanaoka H, Uraguchi S, Takano J, Tanaka M, Fujiwara T** (2014) OsNIP3;1, a rice boric acid channel, regulates boron distribution and is essential for growth under boron-deficient conditions. *Plant Journal* **78**: 890-902
- Hill CB, Cassin A, Keeble-Gagnere G, Doblin MS, Bacic A, Roessner U** (2016) De novo transcriptome assembly and analysis of differentially expressed genes of two barley genotypes reveal root-zone-specific responses to salt exposure. *Sci Rep* **6**: 31558
- Ivashikina N, Becker D, Ache P, Meyerhoff O, Felle HH, Hedrich R** (2001) K<sup>+</sup> channel profile and electrical properties of Arabidopsis root hairs. *Fefs Letters* **508**: 463-469
- Knipfer T, Besse M, Verdeil JL, Fricke W** (2011) Aquaporin-facilitated water uptake in barley (*Hordeum vulgare* L.) roots. *J Exp Bot* **62**: 4115-4126

- Lacombe B, Pilot G, Michard E, Gaymard F, Sentenac H, Thibaud J-B** (2000) A Shaker-like K<sup>+</sup>-channel with Weak Rectification Is Expressed in Both Source and Sink Phloem Tissues of Arabidopsis. *The Plant Cell* **12**: 837-851
- Li B, Qiu J, Jayakannan M, Xu B, Li Y, Mayo GM, Tester M, Gillham M, Roy SJ** (2016) AtNPF2.5 Modulates Chloride (Cl(-)) Efflux from Roots of Arabidopsis thaliana. *Front Plant Sci* **7**: 2013
- Li B, Tester M, Gillham M** (2017) Chloride on the Move. *Trends Plant Sci* **22**: 236-248
- Mian A, Oomen RJ, Isayenkova S, Sentenac H, Maathuis FJ, Very AA** (2011) Over-expression of an Na<sup>+</sup>- and K<sup>+</sup>-permeable HKT transporter in barley improves salt tolerance. *Plant J* **68**: 468-479
- Mizutani M, Watanabe S, Nakagawa T, Maeshima M** (2006) Aquaporin NIP2;1 is mainly localized to the ER membrane and shows root-specific accumulation in Arabidopsis thaliana. *Plant Cell Physiol* **47**: 1420-1426
- Munns R, James RA, Xu B, Athman A, Conn SJ, Jordans C, Byrt CS, Hare RA, Tyerman SD, Tester M, Plett D, Gillham M** (2012) Wheat grain yield on saline soils is improved by an ancestral Na(+) transporter gene. *Nat Biotechnol* **30**: 360-364
- Prak S, Hem S, Boudet J, Viennois G, Sommerer N, Rossignol M, Maurel C, Santoni V** (2008) Multiple phosphorylations in the C-terminal tail of plant plasma membrane aquaporins: role in subcellular trafficking of AtPIP2;1 in response to salt stress. *Mol Cell Proteomics* **7**: 1019-1030
- Remans T, Nacry P, Pervent M, Girin T, Tillard P, Lepetit M, Gojon A** (2006) A central role for the nitrate transporter NRT2.1 in the integrated morphological and physiological responses of the root system to nitrogen limitation in Arabidopsis. *Plant Physiol* **140**: 909-921
- Shi H** (2002) The Putative Plasma Membrane Na<sup>+</sup>/H<sup>+</sup> Antiporter SOS1 Controls Long-Distance Na<sup>+</sup> Transport in Plants. *The Plant Cell Online* **14**: 465-477
- Sun H, Wang S, Lou Y, Zhu C, Zhao H, Li Y, Li X, Gao Z** (2018) Whole-Genome and Expression Analyses of Bamboo Aquaporin Genes Reveal Their Functions Involved in Maintaining Diurnal Water Balance in Bamboo Shoots. *Cells* **7**(11): 195
- Ueda M, Tsutsumi N, Fujimoto M** (2016) Salt stress induces internalization of plasma membrane aquaporin into the vacuole in Arabidopsis thaliana. *Biochem Biophys Res Commun* **474**: 742-746
- von der Fecht-Bartenbach J, Bogner M, Dynowski M, Ludewig U** (2010) CLC-b-mediated NO<sub>3</sub><sup>-</sup>/H<sup>+</sup> exchange across the tonoplast of Arabidopsis vacuoles. *Plant Cell Physiol* **51**: 960-968
- Wang YY, Tsay YF** (2011) Arabidopsis nitrate transporter NRT1.9 is important in phloem nitrate transport. *Plant Cell* **23**: 1945-1957
- Watt M, Kirkegaard JA, Rebetzke GJ** (2005) A wheat genotype developed for rapid leaf growth copes well with the physical and biological constraints of unploughed soil. *Functional Plant Biology* **32**: 695
- Wen Z, Tyerman SD, Dechorganat J, Ovchinnikova E, Dhugga KS, Kaiser BN** (2017) Maize NPF6 Proteins Are Homologs of Arabidopsis CHL1 That Are Selective for Both Nitrate and Chloride. *Plant Cell* **29**: 2581-2596
- Yang T, Zhang S, Hu Y, Wu F, Hu Q, Chen G, Cai J, Wu T, Moran N, Yu L, Xu G** (2014) The role of a potassium transporter OsHAK5 in potassium acquisition and transport from roots to shoots in rice at low potassium supply levels. *Plant Physiol* **166**: 945-959
- Yuan L, Loque D, Kojima S, Rauch S, Ishiyama K, Inoue E, Takahashi H, von Wieren N** (2007) The organization of high-affinity ammonium uptake in Arabidopsis roots depends on the spatial arrangement and biochemical properties of AMT1-type transporters. *Plant Cell* **19**: 2636-2652
- Zhu M, Zhou M, Shabala L, Shabala S** (2017) Physiological and molecular mechanisms mediating xylem Na<sup>+</sup> loading in barley in the context of salinity stress tolerance. *Plant Cell Environ* **40**: 1009-1020

**Supporting Information Table S2.** Wheat root geometry used for model simulations in Figure 3, based on the micrographs shown in Figure 1.

Tissue Type	Seminal Root		Branch Root	
	Cells per tissue type	Tissue thickness (μm)	Cells per tissue type	Tissue thickness (μm)
Epidermis	43	6.2	38	2.5
Cortex layer 1	36	7.7	17	6.4
Cortex layer 2	29	7.0	9	7.0
Cortex layer 3	19	7.5	9	6.8
Cortex layer 4	19	5.2	-	-
Cortex layer 5	8	3.1	-	-
Endodermis	30	1.7	10	2.9
Stele	142	4.7	15	1.2

**References:**

**Watt M, Magee LJ, McCully ME. 2008.** Types, structure and potential for axial water flow in the deepest roots of field-grown cereals. *New Phytologist* **178**:135-146.