

Developmental pattern of aquaporin expression in barley (*Hordeum vulgare* L.) leaves

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SUPPLEMENTARY FILE S1

Details of primers used

Table S1. Primers

(a) qPCR expression analyses: barley MIP genes, HvPIP2;7 and HvSIP1;1 were annotated.

Aquaporins	Accession Number	Forward 5'-3'	Reverse 5'-3'	Length (bp)
HvPIP1;1	AB286964	ACTGCGTGTTCTGAGTGAATTTCT	CATACAAGTCCTCTGTCTGGGTTTC	153
HvPIP1;2	AB275278	CAAGAGCCCGGACTAGTATAC	CGCTCGCTCAAGAACAGTAC	174
HvPIP1;3	AB009308	AATAGTTCTCAAATCTGCCGTCTC	TACTGGTACAATGCAAAAGGTAGG	131
HvPIP1;4	AB275279	CGACTGTGGATGGAGTCTTC	CCTTATATGGGCACATAGCC	189
HvPIP1;5	AB009309	ATCGGAATAACGAGCCTGTTTC	ACCACAACACTGTCACACTGAATTAC	117
HvPIP2;1	AB219366	AGATATGTGCGAAGAAGAAGGCCG	ATATGCACAAGCCGAGGAACGGTA	123
HvPIP2;2	AB377269	TCCTTGTCGCCCTTAATGTTGTGCG	ATTGCAGCACTTGTCACTCACAGC	103
HvPIP2;3	AB275280	CGGATCGTGTATTATGCAAGCGTC	GGCATCCAAATTCCTCAACGATCC	106
HvPIP2;4	AB219525	TCGAGTGTGTCTTCGTCAGTCAGT	AATCAGCTCACGAACCGAACCGTA	151
HvPIP2;5	AB377270	GCAAGATTGAAGCAATGGCGACCT	CGAATTACAACACACGGCACGCAT	168
HvPIP2;7	AK248491	GCGCGAACCTAAATCACATA	GGACATACATCGCGAGAACA	102
HvTIP1;1	AB540221	TCCGTCCGTGTGGTCGAT	TCGTACAGGTTTCACAGCACCA	135
HvTIP1;2	AB540226	GATCATCCATGCGTCATCGCCATG	ACTGAGGACGTACACCAAGACAAG	169
HvTIP2;1	AB540222	GACCCGTCTCTGAATTTGCATCGT	CGTGACCGTGACACACCATTTGTA	96
HvTIP2;2	AB540223	CCTTGTGTGAATTTCCCTTCTC	CGTTCATGTAAGATCGAGTGGT	84
HvTIP2;3	AB540224	TAAGCCATCCATGTGAAGGGTTCGT	CTGGGCTTCGATCTGAACCAAACA	138
HvTIP3;1	AB540228	AGCTGCTGCTGCTTCAAATCCT	ACCATAGGGTCAACCATGCACA	144
HvTIP4;1	AB540225	TTTGCAATGTATACCGGTGTGT	AGCTTCTAACCCCATCCCTCTA	134
HvTIP5;1	AB540227	TTCAAGAACCAGGCCGTGTA	GAACACCAGGTTCTGATGCAC	81
HvNIP1;1	AB540230	AGTGGTCTTGGCCTAACTGAACT	TGCACACCTGTCTGTCTCCATCAA	142
HvNIP1;2	AB540231	TTGTGGGTAGTGAATTTTCGATG	GCAGGGCATAACCACTAGTTTC	159
HvNIP2;1	AB540229	ATAGCGTGAGTACATGGCTGGTGT	ACCTCAGACGCAGTACAAAGCGAA	114
HvSIP1;1	AK252830	ACGCGGCAAAGAATTCAGGCAA	TCAGCCACGATTGCTTTTCGAGT	137
HvSIP2;1	-	CCAAGAAGACCAAGGAGCAAGA	GACTTCAACCCAGGTGATGGTA	127

(b) Reference genes used (bold) and additionally tested for qPCR expression analyses.

Housekeeping genes	Accession Number	Forward 5'-3'	Reverse 5'-3'	Length (bp)
EF-1	Z50789	AAGGCTGCCATCAAGAAGAA	CAGAAGCATCCATGTTTTCCC	182
H⁺-ATPase	AJ344078	ACATCGACACCATCAACCAA	ACAAC TAGGGGCTGGTCAGA	163
GADPH	X60343	GTGAGGCTGGTGCTGATTACG	TGGTGCAGCTAGCATTGAGAC	198
HSP70	AK248694	CGACCAGGGCAACCGCACCAC	ACGGTGTGATGGGGTTCATG	108
α-Tubulin	U40042	AGTGTCCTGTCCACCCACTC	AGCATGAAGTGGATCCTTGG	248
Cyclophilin	AK253120	CCTGTCGTGTCGTCGGTCTAAA	ACGCAGATCCAGCAGCCTAAAG	122
Ubiquitin	X04133	AGGCGAAGATCCAGGACAAG	ACAACCAGACATGCTCCAACCT	244
HvSIP2;1	Contig19630 _at	ACCCAAGAAGACCAAGGAGCA AGA	AGGACTTCAACCCAGGTGATGGT A	127

(c) Up- and Down primers used for in-situ hybridisation experiments.

Aquaporins and Reference	Primers 5'-3'
HvPIP1;2-Up	CCGCCATCATCTACAACAAG
HvPIP1;2-Down	CGCTCGCTCAAGAACAGTAC
HvPIP2;2-Up	TACTGCTCCTCGTGCCATC
HvPIP2;2-Down	ATTGCAGCACTTGTCACACAGC
HvPIP2;5-Up	AGGCCTGGGATGATCAGTGG
HvPIP2;5-Down	CGAATTACAACACACGGCACGCAT
HvPIP2;7-Up	GTACAACCAGCACAAGGCATGGAA
HvPIP2;7-Down	GGACATACATCGCGAGAACA
HvTIP1;1-Up	GGGGTACCAGTGGGTGTACT
HvTIP1;1-Down	TCGTCACAGGTTTCACAGCACCA
HvTIP2;3-Up	CCTGTACATGTGCGACAACC
HvTIP2;3-Down	CTGGGCTTCGATCTGAACCAAACA
Rib-Up	CCGACCCTGATCTTCTGTGAAGGG
Rib-Down	CCAAGTCAGACGAACGATTTGCACG
E-T7	GCGAAATTAATACGACTCAC

(d) T7-Up- and T7-Down primers used for in-situ hybridisation experiments.

Aquaporins and reference gene	Primers 5'-3'
HvPIP1;2-T7-Up	GAAATTAATACGACTCACTATAGGGAGACCGCCATCATCTACAACAAG
HvPIP1;2-T7-Down	GAAATTAATACGACTCACTATAGGGAGACGCTCGCTCAAGAACAGTAC
HvPIP2;2-T7-Up	GAAATTAATACGACTCACTATAGGGAGATACTGCTCCTCGTGCCATC
HvPIP2;2-T7-Down	GAAATTAATACGACTCACTATAGGGAGATTGCAGCACTTGTCACACAGC
HvPIP2;5-T7-Up	GAAATTAATACGACTCACTATAGGGAGAGGCCTGGGATGATCAGTGG
HvPIP2;5-T7-Down	GAAATTAATACGACTCACTATAGGGAGACGAATTACAACACACGGCACGCAT
HvPIP2;7-T7-Up	GAAATTAATACGACTCACTATAGGGAGAGTACAACCAGCACAAGGCATGGAA
HvPIP2;7-T7-Down	GAAATTAATACGACTCACTATAGGGAGAGGACATACATCGCGAGAACA
HvTIP1;1-T7-Up	GAAATTAATACGACTCACTATAGGGAGAGGGGTACCAGTGGGTGTACT
HvTIP1;1-T7-Down	GAAATTAATACGACTCACTATAGGGAGACAGGTTTCACAGCACCA
HvTIP2;3-T7-Up	GAAATTAATACGACTCACTATAGGGAGACCTGTACATGTGCGACAAC
HvTIP2;3-T7-Down	GAAATTAATACGACTCACTATAGGGAGACTGGGCTTCGATCTGAACCA
Rib-T7-Up	GCGAAATTAATACGACTCACTATAGGGAGACCGACCCTGATCTTCTGTGAAGGG
Rib-T7-Down	GCGAAATTAATACGACTCACTATAGGGAGACCAAGTCAGACGAACGATTTGCACG

(e) Transient expression in onion epidermis: Cloning pSAT6-EYFP-N

Aquaporins	Cloning site	Primers
HvPIP2;2	NcoI	F: 5'-TATAAGCTTCAGCAGCCATGGCCAAGGAGG-3'
	ApaI	R: 5'-ATAGGGCCCGAAGGAGCCGAGCGCCTTG-3'
HvPIP2;7	NcoI	F: 5'-AGATACCATGGCCATGTCCAAGGAGG-3'
	ApaI	R: 5'-TATGGGCCCGGTGCTCCTGAACGA-3'
HvTIP1;1	NcoI	F: 5'-TTCTCCATGGCGAAAATGCCGGTCAAC-3'
	ApaI	R: 5'-TAAGGGCCCGTAGTCGGTGGTGGGGAG-3'

(f) Transient expression in onion epidermis: Cloning pSAT6-EYFP-C

Aquaporins	Cloning site	Primers
HvPIP2;2	HindIII	F: 5'-TATAAGCTTCAGCAGCCATGGCCAAGGAGG-3'
HvPIP2;7	HindIII	F: 5'-ATTAAGCTTCAGGTCGTCGAACCATGTCC-3'
HvTIP1;1	HindIII	F: 5'-AGTAAGCTTGAGCGAAAATGCCGGT-3'

(g) Expression yeast for swelling and uptake experiments

Aquaporins	Forward 5'-3'	Reverse 5'-3'
HvPIP1;2	GGATTAAUAATGGAGGGCAAGGAGGAG	GGGTAAUCTAGTCGCGGCTCTTGAAG
HvPIP2;2	GGATTAAUAATGGCCAAGGAGGTGAGCG	GGGTAAUTCAGTTGCTCCGGCTGCTCC
HvPIP2;5	GGATTAAUAATGGCGAAGGACGAGGTG	GGGTAAUTTACGCGTTGCTCCTGAAG
HvPIP2;7	GGATTAAUAATGTCCAAGGAGGAGGTG	GGGTAAUTTAGGTTTCGCGCGGTGG
HvTIP1;1	GGATTAAUAATGCCGGTCAGCAGGATCGC	GGGTAAUTTAGTAGTCGGTGGTGGGGAG
HvTIP1;2	GGATTAAUAATGCCGGTGAGCAGGATCGC	GGGTAAUTCAGTACTCGGCGGTGGGGA
HvTIP2;3	GGATTAAUAATGCCAGGCTCCATCGCCTTTG	GGGTAAUTTAGTAGTCATTGCTGGCGACG

(h) Cloning of barley MIPs

Aquaporins	Forward 5'-3'	Reverse 5'-3'
HvPIP1;2	TCTGCCTAACAAGCTAAGCAGCTC	CGCTCGCTCAAGAACAGTAC
HvPIP2;1	AGCACCGTTCAAGCTAGAGG	ATATGCACAAGCCGAGGAACGGTA
HvPIP2;2	TCCATCAGACAAGAGAGAGAGAGCG	ATTGCAGCACTTGTCACACTCACAGC
HvPIP2;5	AAACCACACACACTGATGGCGAAG	CGAATTACAACACACGGCACGCAT
HvPIP2;7	ACTAAAGTTTGCACCAGGTGTCGTCG	GGACATACATCGCGAGAACA
HvTIP1;1	CTTCTTCTCTAGCGAGCTAGGCTT	TCGTCACAGGTTTCACAGCACCA
HvTIP1;2	AACACTAGCAGCTACACAGCACAC	ACTGAGGACGTACACCAAGACAAG
HvTIP2;3	GCGTGTTCTCAGCGACAACATTTT	CTGGGCTTCGATCTGAACCAAACA
HvNIP1;1	TGGTTACTCCTGTGTTCCGC	TGCACACCTGTCTGTCTCCATCAA

SUPPLEMENTARY FILE S2

Annotation and protein sequences of barley MIPs and of MIPs of Arabidopsis, maize and rice used for construction of phylogenetic trees

Table S2. *Annotation and protein sequences of barley MIPs and of MIPs of Arabidopsis, maize and rice used for construction of phylogenetic trees.*

(a) Barley (*Hordeum vulgare* L.) MIPs. *, the barley PIP1 HvPIP1;6 (Wei et al. 2007, GU989200, ADG03686) is allelic to HvPIP1;1; **, not available

Barley MIPs	cDNA clone accession No	Accession No.		amino acid
	Affymetrix probe set ID (BarleyBase)	Nucleic acid	Protein	
HvPIP1;1*	Contig1237_s_at/Contig1235_at/ Contig1242_at/Contig1225_s_at	AB286964	BAF41978	288
HvPIP1;2	Contig1228_s_at	AB275278	BAF33067	292
HvPIP1;3	Contig1226_at/Contig1239_s_at	AB009308	BAA23745	292
HvPIP1;4	Contig1219_s_at	AB275279	BAF33068	292
HvPIP1;5	Contig1230_at	AB009309	BAA23746	290
HvPIP2;1	Hv08C12u_x_at	AB219366	BAE02729	286
HvPIP2;2	Contig1216_s_at	AB377269	BAG06230	284
HvPIP2;3	Contig1223_at	AB275280	BAF33069	290
HvPIP2;4	-	AB219525	BAE06148	290
HvPIP2;5	Contig1222_s_at/ Contig1240_at	AB377270	BAG06231	290
HvPIP2;7	Contig19393_at	AK248491	_**	291
HvTIP1;1	HS07J06u_x/s_at/ HS18A22u_at	AB540221	BAI66434	250
HvTIP1.2	HVSMEf0019H18r2_at/Contig1309_at	AB540226	BAI66439	252
HvTIP2;1	Contig1310_at	AB540222	BAI66435	249
HvTIP2;2	Contig1308_at	AB540223	BAI66436	249
HvTIP2;3	Contig1315_s_at	AB540224	BAI66437	248
HvTIP3;1	Contig3772_at/HT03K14r_(s)_at	AB540228	BAI66441	263
HvTIP4;1	Contig7377_at	AB540225	BAI66438	257
HvTIP5;1	AF254799_CDS-2_at	AB540227	BAI66440	262
HvNIP1;1	Contig14229_at	AB540230	BAI66443	278
HvNIP1;2	Contig19214_at	AB540231	BAI66444	333
HvNIP2;1 (HvLsi6)	Contig5632_at/Contig5634_at	AB540229	BAI66442	295
HvSIP1;1	Contig6340_at/Contig6339_s_at	AK252830	-	244
Partial HvSIP2;1	Contig19630_at	-	-	142

(b) *Arabidopsis* (*A. thaliana*) MIPs

<i>Arabidopsis</i> MIPs	Accession No.
AtPIP1;1	CAB71073
AtPIP1;2	AAC28529
AtPIP1;3	AAF81320
AtPIP1;4	AAF02782
AtPIP1;5	CAA20461
AtPIP2;1	CAB67649
AtPIP2;2	AAD18142
AtPIP2;3	AAD18141
AtPIP2;4	BAB09839
AtPIP2;5	CAB41102
AtPIP2;6	AAC79629
AtPIP2;7	CAA17774
AtPIP2;8	AAC64216
AtTIP1;1	AAD31569
AtTIP1;2	BAB01832
AtTIP1;3	AAC62778
AtTIP2;1	BAB01264
AtTIP2;2	CAB10515
AtTIP2;3	BAB09071
AtTIP3;1	AAG52132
AtTIP3;2	AAF97261
AtTIP4;1	AAC42249
AtTIP5;1	CAB51216
AtNIP1;1	CAA16760
AtNIP1;2	CAA16748
AtNIP2;1	AAC26712
AtNIP3;1	AAG50717
AtNIP4;1	BAB10360
AtNIP4;2	BAB10361
AtNIP5;1	CAB39791
AtNIP6;1	AAF14664
AtNIP7;1	AAF30303
AtSIP1;1	AAF26804
AtSIP1;2	BAB09487
AtSIP2;1	CAB72165

(c) Rice (*Oryza sativa*) MIPs

Rice MIPs	Accession No.
OsPIP1;1	BAD28398
OsPIP1;2	Q7XSQ9
OsPIP1;3	BAD22920
OsPIP2;1	BAC15868
OsPIP2;2	BAD23735
OsPIP2;3	CAD41442
OsPIP2;4	BAC16113
OsPIP2;5	BAC16116
OsPIP2;6	CAE05002
OsPIP2;7	BAD46581
OsPIP2;8	AAP44741
OsTIP1;1	AAK98737
OsTIP1;2	BAB63833
OsTIP2;1	BAD25765
OsTIP2;2	BAD61899
OsTIP3;1	AAG13544
OsTIP3;2	CAE05657
OsTIP4;1	AAS98488
OsTIP4;2	BAA92993
OsTIP4;3	BAA92991
OsTIP5;1	BAG92052
OsNIP1;1	BAD27715
OsNIP1;2	BAD73177
OsNIP1;3	AAV44140
OsNIP1;4	BAD53665
OsNIP2;1	BAD16128
OsNIP2;2	BAD37471
OsNIP3;1	AAG13499
OsNIP3;2	BAC99758
OsNIP3;3	BAC65382
OsNIP4;1	BAB61180
OsSIP1;1	BAB32914
OsSIP2;1	Q10M80

(d) Maize (*Zea mays*) MIPs

Maize MIPs	Accession No.
ZmPIP1;1	Q41870
ZmPIP1;2	AAD29676
ZmPIP1;3	AAK26754
ZmPIP1;4	AAK26755
ZmPIP1;5	AAK26756
ZmPIP1;6	AAK26757
ZmPIP2;1	AAK26758
ZmPIP2;2	AAK26759
ZmPIP2;3	AAK26760
ZmPIP2;4	AAK26761
ZmPIP2;5	AAD28761
ZmPIP2;6	AAK26762
ZmPIP2;7	AAK26763
ZmTIP1;1	AAC09245
ZmTIP1;2	AAK26767
ZmTIP2;1	AAK26768
ZmTIP2;2	AAK26769
ZmTIP2;3	AAK26770
ZmTIP3;1	AAK26771
ZmTIP3;2	AAK26848
ZmTIP4;1	AAK26772
ZmTIP4;2	AAK26773
ZmTIP4;3	AAK26774
ZmTIP4;4	AAK26775
ZmTIP5;1	AAK26776
ZmNIP1;1	AAK26750
ZmNIP2;1	AAK26751
ZmNIP2;2	AAK26752
ZmNIP2;3	AAK26849
ZmNIP3;1	AAK26753
ZmSIP1;1	AAK26764
ZmSIP1;2	AAK26765
ZmSIP2;1	AAK26766

(e) Number of MIPs, identified so far, and members of PIP/TIP/NIP/SIP subgroups in selected plant species.

	<i>Arabidopsis</i>	<i>Rice</i>	<i>Maize</i>	<i>Wheat</i>	<i>Cotton</i>	<i>Barley</i>
PIP1s	5	3	6	12	15	5
PIP2s	8	8	7	12	13	6
TIPs	10	10	11	11	23	8
NIPs	9	10	4	-	12	4
SIPs	3	2	3	-	7	2
Total	36	33	31	35	70	25

(f) Protein sequences (order: barley, maize, rice, *Arabidopsis*)

PIP1s

>HvPIP1;1 (HvPIP1;6)

MEGKEEDVRLGANKYSERHAIGTAAQGSSEDKDYKEPPPAPLFEPEGELKSWSFYRAGIAEFMATFLFLYVTI
 LTVMGYSGAASKCATVGIQGIAWSFGGMIFALVYCTAGISGGHINPAVTFGLFLARKLSLTRAVFYIIMQC
 LGAICGAGVVKGFQQGLYMNGGGANVVASGYTKGSLGAEIIGTFVLVYTVFSATDAKRNARDSHVPILA
 PLPIGFAVFLVHLATIPITGTGINPARSLGAAI IYNREHAWSDDHWIFWVGPFIGAALAAIYHQVVIRAI
 PFKTS

>HvPIP1;2

MEGKEEDVRLGANRYSERQPIGTAAQGGGADEKDYKEPPPAPLFEAEELTSWSFYRAGIAEFLATFLFLYI
 SVLTVMGVGNPSPGSKCGTVGIQGIAWSFGGMIFVLVYCTAGISGGHINPAVTFGLFLARKLSLTRAVFYI
 VMQCLGAICGAGVVKGFQTTLYMNGGGANSVAPGYTKGDGLGAEIVGTFVLVYTVFSATDAKRSARDSHV
 PILAPLPIGFAVFLVHLATIPITGTGINPARSLGAAI IYNKKQSWDDHWIFWVGPFPGAALAAIYHVVIR
 AIPFKSRD

>HvPIP1;3

MEGKEEDVRLGANRYSERQPIGTAAQGGGADEKDYKEPPPAPLFEAEELTSWSFYRAGIAEFLATFLFLYI
 SVLTVMGVGNPSPGSKCGTVGIQGIAWSFGGMIFVLVYCTAGISGGHINPAVTFGLFLARKLSLTRAVFYI
 VMQCLGAICGAGVVKGFQTTLYQNGGGANSVAAGYTKGDGLGAEIVGTFVLVYTVFSATDAKRSARDSHV
 PILAPLPIGFAVFLVHLATIPITGTGINPARSLGAAI IYNKKQAWDDHWIFWVGPFIGAALAAIYHVVIR
 AIPFKSRG

>HvPIP1;4

MEGKEEDVRLGANRYSERQPIGTAAQGGGADEKDYKEPPPAPLFEAEELSSWSFYRAGIAEFLATFLFLYI
 SVLTVMGVGNPSPGSKCGTVGIQGIAWSFGGMIFVLVYCTAGISGGHINPAVTFGLFLARKLSLTRAVFYI
 VMQCLGAICGAGVVKGFQTTLYQNGGGANSVAAGYTKGDGLGAEIVGTFVLVYTVFSATDAKRSARDSHV
 PILAPLPIGFAVFLVHLATIPITGTGINPARSLGAAI IYNKKQAWDDHWIFWVGPFIGAALAAIYHVVIR
 AIPFKSRD

>HvPIP1;5

MEGKEEDVRLGANRYSERQPIGTAAQGGGDDKDYKEPPPAPLFEPEGELKSWSFYRAGIAEFVATFLFLYVT
 VLTVMGVSKAPSKCATVGVQGIAWSFGGMIFALVYCTAGISGGHINPAVTFGLFLARKLSLTRAIFYIIMQ
 CLGAICGAGVVKGFQQGLYMGNGGGANVVASGYTKGDGLGAEIIGTFVLVYTVFSATDAKRNARDSHVPIL
 APLPIGFAVFLVHLATIPITGTGINPARSLGAAI IYNRDHAWNDHWIFWVGPFVGAALAAVYHQVIIRAIP
 FNKSRS

>ZmPIP1;1

MEGKEEDVRLGANKFSERHAIGTAAQGTDDKDYKEPPPAPLFEPEGELKSWSFYRPGIAEFVATFLFLYISI
 LTVMGVSKSTSKCATVGIQGIAWSFGGMILALVYCTAGISGHINPAVTFGLFLARKLSLTRAVFYIIMQCL
 GAICGRGVVKGFQQGLYMGNGGRRNVVAPGYTKGDGLGAEIVGTFILVYTVFSATDAKRRARDSHVPI LAP
 LPIGFAVFLVHLATMGITGTGINPARSLGAAVIYNQHHAWADHWIFWVGPFIGAALAAIYHQVIIRAIPFK
 SRS

>ZmPIP1;2

MEGKEEDVRLGANKFSERQPIGTAAQGAADDKDYKEPPPAPLFEPEGELKSWSFYRAGIAEFVATFLFLYIT
 I LTVMGVSKSTSKCATVGIQGIAWSFGGMIFALVYCTAGISGGHINPAVTFGLFLARKLSLTRALFYIIMQ
 CLGAVCGAGVVKGFQQGLYMGNGGGANVVAPGYTKGDGLGAEIVGTFILVYTVFSATDAKRNARDSHVPIL
 APLPIGFAVFLVHLATIPITGTGINPARSLGAAI IYNRDHAWNDHWIFWVGPFIGAALAAIYHQVIIRAIP
 FKRS

>ZmPIP1;3

MEGKEEDVRLGANKFSERQPIGTAAQGAGAGDDDKDYKEPPPAPLFEPEGELKSWSFYRAGIAEFVATFLFL
 YITVLTVMGVSKSTSKCATVGIQGIAWSFGGMIFALVYCTAGISGGHINPAVTFGLFLARKLSLTRAIFYI
 IMQCLGAICGAGVVKGFQQGLYMGNGGGANVVAPGYTKGDGLGAEIVGTFILVYTVFSATDAKRNARDSHV
 PILAPLPIGFAVFLVHLATIPITGTGINPARSLGAAI IYNRDHAWSDHWIFWVGPFIGAALAAIYHQVIIR
 AIPFKSRS

>ZmPIP1;4

MEGKEEDVRLGANKFSERQPIGTAAQGAGAGDDDKDYKEPPPAPLFEPEGELKSWSFYRAGIAEFVATFLFL
 YITVLTVMGVSKSTSKCATVGIQGIAWSFGGMIFALVYCTAGISGGHINPAVTFGLFLARKLSLTRAIFYI
 IMQCLGAICGAGVVKGFQQGLYMGNGGGANVVAPGYTKGDGLGAEIVGTFILVYTVFSATDAKRNARDSHV
 PILAPLPIGFAVFLVHLATIPITGTGINPARSLGAAI IYNRDHAWSDHWIFWVGPFIGAALAAIYHQVIIR
 AIPFKSRS

>ZmPIP1;5

MEGKEEDVRLGANRYSERQPIGTAAQGTTEEKDYKEPPPAPLFEAEELTSWSFYRAGIAEFVATFLFLYISI
 LTVMGVSKSSSKCATVGIQGIAWSFGGMIFALVYCTAGISGGHINPAVTFGLFLARKLSLTRALFYMVMQC
 LGAICGAGVVKGFQEGLYMGAGGGANAVNPGYTKGDGLGAEIVGTFVLVYTVFSATDAKRSARDSHVPILA
 PLPIGFAVFLVHLATIPITGTGINPARSLGAAI IYVNRSHAWNDHWIFWVGPFIGAALAAIYHVVIIRALPF
 KSRD

>ZmPIP1;6

MAGGTLQDRSEEDVRVGVDRFPERQPIGTAADDLGRDYSEPPAAPLFEASELSSWSFYRAGIAEFVATFL
 FLYVTVLTVMGVSKSPSKCGTVGIQGIAWAFGGMIFALVYCTAGVSGGHINPAVTFGLLLARKLSLTRAVY
 YVVMQCLGAVCGAGVVKAFGSALYESAGGGANAVSPGYTKGDGLGAEVVGTFVLVYTVFSATDAKRTARDS
 HVPALAPLPIGFAVFLVHLATIPITGTGINPARSLGAAI IYDNPHGWGHWFVVGPFAGAALAAVYHQVV
 LRAIPFKSSAHY

>ZmPIP2;1

MKGDDVIESGAGGGEFAAKDYTDPPAPLIDAAELGSWSLYRAVIAEFVATLLFLYITVATVIGYKHQTD
 SASGADAACGGVGLGIAWAFGGMIFVLVYCTAGISGGHINPAVTFGLFLARKVSLVRALLYIVAQCLGAI
 CGVGLVKAFQSAFYDRYGGGANSLASGYSRGTGLGAEIIGTFVLVYTVFSATDPKRNARDSHVPVLA
 PLPIGFAVFMVHLATIPVTGTGINPARSLGAAVIYNKDKPWDDHWIFWVGPLVGAAIAAFYHQYILRAGAIKALG
 SFRSNA

>OsPIP1;1

Megkeedvrlganryserqpigtaaqqagddkdykepppaplfepgelkswsfyragiaefvatflflyit
iltvmgvskssskcatvgiqgiawsfggmifalvyctagisgghinpavtfglflarklsltraifyivmq
clgaicgagvkvqfqqglymgngganvvasgytkgdglgaeivgtfilvytvfsatdakrnardshvpil
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>ZmTIP2;2

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VVFEİVİITFALVYTVYATAADPKKGSİGTİAİPIAİGFİVGANİLAAGPFSGGSMNPARSFİGPAVAADDFAG
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>ZmTIP2;3

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VMEİVİITFALVYTVYATAADPKKGSİGTİAİPIAİGFİVGANİLAAGPFSGGSMNPARSFİGPAVAAGNFAGN
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>ZmTIP3;1

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VGDWHAVLİEAVMTFGLMYAYYATVİDPKRGHVGTİAİPLAVGFLLGANVLAGGPFİDGAGMNPARVFGPALV
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>ZmTIP3;2

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ALASGVGDGHAVLİEAVMTFGLVYAYYATVVDPKRGHLGTİAİPLAVGFLLGANVLAGGPFİDGAGMNPARVFG
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>ZmTIP4;1

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İSPMQGLVMEVİİLTFİSİLLFVTYAMİLDPRSQVRAİGİPLİTGLİVGANSLAGGNFTGASMNPARSFİGPALAT
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>ZmTIP4;2

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AGİRPMQGLVMEVİİLTFİSİLLFVTYAMİLDPRSQVRTİGİPLİTGLİVGANSLAGGNFTGASMNPARSFİGPAM
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>ZmTIP4;3

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VAEAVFTFİSİLLFVİYATİLDPRKİLLPGAGİPLİTGLİVGANSVAGAALSİGASMNPARSFİGPAVASGVWTHHW
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>ZmTIP4;4

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SAGİHVSGGHİNPAVTİGLAATGRİTİFRSALYVAAQLLGSTİLACİLLAFİLAVADSGVPVHALGAGVİGALR

GVLMEAVLTFSLFVAVYATVVDPRRAVGGMGPLLVLVVGANVLAGGPFSGASMNPARSFGPALVAGVWAD
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>ZmTIP5;1

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GGAVLEGLVTLFLLVYTVHVVGEREPRSRGGDGKREFAATALGALAVGLTQGAFLAAGALTGASMNPARSF
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>OsTIP1;1

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ALVLEIVMTFGLVYTVYATAVDPKKGSLGTI APIAIGFIVGANILVGGAFDGASMNPVAVSFGPALVSWSWE
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>OsTIP1;2

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>OsTIP2;1

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>OsTIP2;2

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>OsTIP3;1

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GVGDWHAVLLEATMTFGLMYAYYATVIDPKRGHVGTIAPLAVGFLLGANMLAGGPF DGAGMNP ARVFGPAL
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>OsTIP3;2

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GHRIHERHALLLEVMTFGLVYTVYATAVDRRS GGDIAPLAIGLVAGANILAGGPF DGAAMNPARAFGPA
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>OsTIP4;1

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QGLVMEIILTFSLLFVYATILDPRSSVPGFGPLL TGLIVGANTIAGGNFSGASMNPARSFGPALATGVWT
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>OsTIP4;2

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PVQGVAAEAVFTFTLLL VICATILDPRAAPP GTGPLLTGLLVGANTVAGGALTGASMNPARSFGPALATG
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>OsTIP4;3

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VAMEAVLTF SLLFAVYATVVD RRRRAVGALGPLLVGLVVGANILAGGPYSGASMNPARSFGPALAAGEWADH
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>OsTIP5;1

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ILEGLVTFM VVYTVHVAGDPRGGGFGRKGPAA TALGALVVGAVTGACVLAAGSLTGASMNPARSFGPAVV
SGHYSNQAVYWAGPMVGA AVALVHQALVFPTVPEPAPAPATNESARHGSVQTVVV

>AtTIP1;1

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>AtTIP1;2

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>AtTIP1;3

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>AtTIP2;1

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VVMEIIITFALVYTVYATAADPKKGS LGTIAPIAIGFIVGANILAAGPFSGGSMNPARSFGPAVAAGDFS
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>AtTIP2;2

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VVMEIVVTFALVYTVYATAADPKKGS LGTIAPIAIGFIVGANILAAGPFSGGSMNPARSFGPAVVS GDFSQ
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>AtTIP2;3

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>AtTIP3;1

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LASVGVAVNGLVLEIILTFGLVYVYSTLIDPKRGS LGTIAPLAIGLIVGANILVGGPFSGASMNPARAFG
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>AtTIP3;2

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VASGVSELHGLLMEIILTFALVYVVYSTAIDPKRGSIGIIAPLAIGLIVGANILVGGPFDGASMNPARAFG
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>AtTIP4;1

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>AtTIP5;1

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NIPs

>HvNIP1;1

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>HvNIP1;2

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>HvNIP2;1

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>HvNIP5634

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>ZmNIP1;1

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>ZmNIP2;1

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DHIQV

>ZmNIP2;2

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QLAADEFDTV

>ZmNIP2;3

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>ZmNIP3;1

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>OsNIP1;1

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>OsNIP1;2

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>OsNIP1;3

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>OsNIP1;4

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>OsNIP2;1

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IAADDVDEMENIQV

>OsNIP2;2

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>OsNIP3;1

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>OsNIP3;2

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>OsNIP3;3

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AHLPPYISSQILGAVAASFAVKGLYHPVNPVGTVEAFFVEFIIITFFLLFIITALATDPNAVKEL
IAVAVGATVMMNILVAGPSTGASMNPARTIGAAIATGRYTQIWWYLVATPLGAIAGTGAYVAIKL

>OsNIP4;1

MTTDHAGKKVDVVVGNVDGEHVGEQARHDLHEEAAAAAADHHATRGLAIGFLIREVMVEGLASFLVVF
WSCVAALMQEMYGTLTFPMVCLVAMTVAFVLSWLGP AHFNPAVTITFAAYRRFPVWPKLPLYVAAQLAGS
LLACL SVNAV MRPRHDH FYGTAPVVVHGTRL PFLMEFLASAVLMIVIATVATDGTAGKT VGGIAIGA AVGG
LGLVIGPVS GSGSMN PARTLGP AIVLGRYDGVWIYV VAPVAGMLV GALCNRAVRLSHRIVAF LCGTSVGIAG
SP

>AtNIP1;1

MADISGNGYGNAREEVVMVNLKDEVEHQEMEDIHNPRLKKQDSLLSVSVPFLQKLI AEFLGTYFLVFTG
CASVVNMQNDNVVTLPGIAI VWGLTIMVLIYSLGHI SGAHINPAVTIAFASCGRFPLKQVPAYVISQVIG
STLAAATLRLFLGLDHDVCSGKHDFVIGSSPVGSDLQAF TMEFIVTFYLMFIIISGVATDNRAIGELAGLAI
GSTVLLNVLIAAPVSSASMNPGRSLGPALVYG CYKIWIYLVAPT LGAIAGAWVYNTVRYTDKPLREITKS
GSFLKTVRIGST

>AtNIP1;2

MAEISGNGGDARDGAVVVNLKEEDEQQQQQQA IHKPLKKQDSLLSISVPFLQKLMAEVLGTYFLIFAGCAA
VAVNTQHDKAVTLPGIAI VWGLTVMVLVYSLGHI SGAHFNPAVTIAFASCGRFPLKQVPAYVISQVIGSTL
AAATLRLFLGLDQDVCSGKHDFVGTLP SGNLQSFVIEFIIITFYLMFVISGVATDNRAIGELAGLAVGST
VLLNVI IAGPVS GASMNPGRSLGPAMVYSCYRGLWIIYVSPIVGAVSGAWVYNMVRYTDKPLREITKSGSF
LKTVRNGSSR

>AtNIP2;1

MDDISVSKSNHGNVVVLNIKASSLADTSLPSNKHESSPPLLSVHFLQKLLAELVGTYYLIFAGCAAIAVN
AQHNHVTVLGVIAVWVGI VIMVLVYCLGHL SAHFNPAVTLALASSQRFPLNQVPAYITVQVIGSTLASATL
RLLFDLNDVCSKKHDFVFLGSSPSGSDLQAFVMEFIIITGFLMLVVCVTTTKRTTEELEGLIIGATVTLNV
IFAGEVSGASMNPARSIGPALVWGCYKIWIYLLAPT LGAVSGALIHKMLPSIQNAEPEFSKTS SHKRVT
DLPL

>AtNIP3;1

MIFAGCSAIVVNETYKPVTLPGIALVWGLVVTVMIIYSIGHVSGAHFNPAVSI AFASSKKFPFNQVPGYIA
AQLLGSTLAAAVLRLVFLHDDDDVCSLKG DVYVGTYP SNSNTTSFVMEFIATFNLMFVISAVATDKRATGSF
AGIAIGATIVLDILFSGPISGASMNPARSLGPALIWGCYKDLWLYIVSPVIGALS GAWTYGLLRSTKKSYS
EII RPNCKVSSRDRQEASQDEICVLRVVD PANQNYFICSSPTDINGKCNVTCKLA

>AtNIP4;1

MSSHSDIEIEEQISRIEKGKGDQCQGGIETVICTSPSIVCLTQKLI AEMIGTYFIVFSGCGVVVVNVLYGG
TITFPGICVTWGLIVMVMIIYSTGHISGAHFNPAVTVTFAIFRRFPWHQVPLYIGA QFAGSLLASLTLRLMF
KVTPEAFFGTT PADSPARALVAEIIISFLLMFVISGVATDNRAV GELAGIAVGMTIMVNVFVAGPISGASM
NPARSLGPALVMGVYKHIWVYIVGPVLGVISGGFVYNLIRFTDKPLRELTKSASF LRAVSPSHKGS SSKT

>AtNIP4;2

MTSHGEEIEDEQISRIEKGKCKDSQGGMETAICSSPSIVCLTQKLI AEMIGTYFIIIFSGCGVVVVNVLYGG
TITFPGICVTWGLIVMVMIIYSTGHISGAHFNPAVTVTFAVFRFPWYQVPLYIGA QLTGSLASLTLRLMF
NVTPKAFFGTTPTDSSGQALVAEIIISFLLMFVISGVATDSRATGELAGIAVGMTIILNVFVAGPISGASM
NPARSLGPALVIMGRYKIWIWYIVGPFVGFIFAGGFVYNFMRF TDKPLRELTKSASF LRSVAQKDNASKSDG

>AtNIP5;1

MAPPEAEVAVMVMAPPTPGTPTGGPLITGMRVDSMSFDHRKPTPRCKCLPVMGSTWQHDTCFTDFPS
 PDVSLTRKLGAEFVGTFLIFTATAGPIVNQKYDGAETLIGNAACAGLAVMI I I LSTGHI SGAHLNPSLTI
 AFAALRHFPWAHVPAIYIAAQVSASICASFALKGVFHFFMSGGVTIPSVSLGQAFALEF I I T F I L L F V V T A V
 ATDTRAVGELAGI AVGATVMLN I L V A G P S T G G S M N P V R T L G P A V A S G N Y R S L W V Y L V A P T L G A I S G A A V Y T
 GVKLND SVTD P P R P V R S F R R

>AtNIP6;1

MDHEEIPSTPSTPATTPTGAPLFGGFEGKRNGHNGRYTPKSLLKSCCKCF SVDNEWALEDGRLPPVTC SL
 PPPNVSLYRKLGAEFVGTFLIFAGTATAIVNQKT DGAETLIGCAASAGLAVMIVILSTGHI SGAHLNPAV
 TIAFAALKHFPWKHVPIYIGAQVMASVSAAFALKAVFEPTMSGGVTVP TVGLSQAFALEF I I S F N L M F V V T
 AVATDTRAVGELAGI AVGATVMLN I L I A G P A T S A S M N P V R T L G P A I A A N N Y R A I W V Y L T A P I L G A L I G A T
 Y T I V K L P E E D E A P K E R R S F R R

>AtNIP7;1

MNGEARSRVVDQEAGSTPSTLRDEDHPSRQRLFGLPYDIDLNPLRIVMAELVGTFILMFSVCGVISSTQL
 SGGHVGLLEYAVTAGLSVVVVVYSIGHISGAHLNPSITIAFAVFGGF PWSQVPLYITAQTLGATAATLVGV
 SVYGVNADIMATKPALSCVSAFFVELIATSIVVFLASALHCGPHQNLGNLTGFVIGTVISLGLVITGPI SG
 GSMNPARSLGPAVVAWDFEDLWIYMTAPVIGAIIGVLTYSISLKRPCSPVSPSVSSLLR

SIPs

>HvSIP1;1 (contig6340)

MAMGAAVREAAADGVVTF LWVLCVSTLGASTAAVT TYLSLHEGIHYALLVTVSILALLLFAFNLLCDALGG
 ASFNPTGVAAFYAAGLTSPSLFSIALRLPAQAAGAVGGALAI SELMPEQYKHM LGGPSLKVDPHTGAAAEG
 VLT F V I T F A V L C I I V K G P R N P I V K T A M L S V S T V S L V L T G A A Y T G P S M N P A N A F G W A Y V N N Q H N T W E Q L Y V Y
 W I C P F I G A I L A A W T F R A V F P P P A P K P K T K K A

>HvSIP2;1 (contig19630) [partial sequence]

GGPALYLF TVFVRVPAQVIGAVIGVMLMRFAFPKVGKGAALNVGVHGHALTEGLATLMVVMVSLTLKKKEQ
 GFFVKTWIASIWKMTIHILSSDITGGIMNPASAF AWAYARGDHTSFDHLLVYWLAPLQATLVGVVVVTF LT

>ZmSIP1;1

MAMGATVRAAAADAVVTF LWVLCASALGASTAAVTSYLG VQEGAGHYALLVTTSLLSVLLFTFDLLCGALG
 GASFNPTDFAASYAAGLDSPSLFSVALRFPQAAGAVGGALAI SELMPAQYKHTLAGPSLKVDPHTGALAE
 GVLTFVITLTVLWVIVKGRNVILKTLTLLSTSIVSVILAGAEYTGPSMNPANAFGWAYVNNWHNTWEQLYV
 YWICPFIGAMLAGWIFRVVFLPPAPKPKTKKA

>ZmSIP1;2

MAMGEALRAAAADAVVTF LWVLCVSTLGASTTAVTSYLR LQGVHFALLVTVSLLSVLLFVFNILCDALGGA
 SFNPTGVAAFYAAGVTSPSLFSIALRLPAQAAGAVGGALAI SELMPAQYRHMLGGPSLKVDPHTGAGAELV
 LTFVITLAVLLIIVKGRNPIIKTWMISICTLCLVLSGAAYTGPSMNPANAFGWAYVNNRHNTWEQFYVY
 ICPFIGAILAAWIFRAMFLT PPPKPKAKKA

>ZmSIP2;1

MSPAPSRPRIRPWL VVGDLALAAAWVCAGALVKLLVYGG LGLGGRPEAEAVKVSLSLVYMF LFAWLEAASG
 GASYNPLTVLAAALASHGGPAVYLF TAFARIPAQVIGAVLGVKLIQVTFPNVGKGARLSVGAHHGALAEGL
 ATFMVVMVSVTLKKKEMKSF FMKTWITSIWKN TIHLLSSDITGGIMNPASAF AWAYARGDHTTFDHL L V Y W
 LAPLQATLLGVWAVTFFT KPKKIKEQKV DENKIKKE

>OsSIP1;1

MAVAAVRAAAADA VVTF LWVLCVSTLGASTAAVTSYLR IHEGIHYALLVTVSLLSVLLF AFNLLCDALGGA
 SFNPTALAAFHAAGLSSPRHSSLFPLALRFPQAAGAVGGAMA I SELMPEQYKHM LGGPSLKV DLHTGAAA
 ELVLT F V I T L A V L W I I V K G P R N P I V K T W M L S I S T V C L V L T G A A Y T G P S M N P A N A F G W A Y V N N R H N T W E Q F Y
 V Y W I C P F V G A V L A A W V F R A V F P P P A P K P K A K K A

>OsSIP2;1

MSPAPPPSRGRIRPWLVVGD LVVAAMWVCAGALVKLAVYGV LGLGGRPEADAVKVALSLVYMFFF AWLEGF
TGGASYNPLTVLAGALASRAGPSLYLFAAFVRMPAQVFGSILGVKLIRAALPKVGKGAPLSVGVHHGALAE
GLATFMVVIVSVTLKKKEMKGF FMKTISSIWKMTFHLLSSDITGGVMNPASAF AWAYARGDHTTTFDHL LV
YWLAPLQATLLGVVVVTL LTKPKKIEEEEADES KTKKE

>AtSIP1;1

MMGVLKSAIGDMLMTFSWVVL SATFGIQTAAIISAGDFQAITWAPLVILTSLIFVYVSIFTVIFGSASFNP
TGSAAFYVAGVPGDTLFLSLAIRLPAQAIGAAGGALAIMEFIPEKYKHMIGGPSLQVDVHTGAI AETILSFG
ITFAVLLIILRGPRLLAKTFLLALATISFVVAGSKYTG PAMNPAIAFGWAYMYSSHNTWDHIYVYWISS F
VGALSAALLFRSIFPPRPQKKKQKKA

>AtSIP1;2

MSAVKSALGDMVITFLWVILSATFGIQTAAIVSAVGFHGITWAPLVISTLVV FVSISIFTVIGNVLGGASF
NPCGNAAFYTAGVSSDSLFLAIRSPAQAIGAAGGAITIMEM IPEKYKTRIGGKPSLQFGAHNGAISEVVL
SFSVTFLVLLIILRGPRLLAKTFLLALATVSVFVVGSKFTRPFMNPAIAFGWAYIYKSHNTWDHFYVYWI
SSYTGAILSAMLFRIIFPAPPLVQKKQKKA

>AtSIP2;1

MGRIGLVVTDLVLSFMWIWAGVLVNILVHGVLGFSRTDPSGEIVRYLFSIISMFI FAYLQQATKGGLYNPL
TALAAGVSGGFSSFIFSVFVRIPVEVIGSILAVKHIIHV FPEIGKGPKNVAIHHGALTEGILTFFIVLLS
MGLTRKIPGSFFMKTWIGSLAKLTLHILGSDLTGGCMNPAAVMGWAYARGEHITKEHLLVYWLGPVKATLL
AVWFFKVVFKPLTEEQEKPKAKSE

SUPPLEMENTARY FILE S3

Differential expression of membrane intrinsic proteins (MIPs) between developmental regions of leaf three of barley as revealed by microarray analysis.

Table S3. *Differential expression of membrane intrinsic proteins (MIPs) between developmental regions of leaf three of barley as revealed by microarray analysis.*

Probe	MIP	EZ<EB	EZ>EB	EZ<NE	EZ>NE	EB>NE	EB<NE
Contig1226	PIP						
Contig1239_s_at							
Contig1219_s_at							
Contig1228_s_at							
Contig1230_at							
Contig1235_at							
Contig1242_at							
Contig1237_s_at							
Contig1225_s_at							
Contig1223_at							
HV_CEb0007N06r2_at							
Contig1222_s_at							
Contig1240_at							
Hv08C12u_x_at							
Contig1216_s_at							
Contig19393_at							
HS07J06u_x/s_at	TIP						
HS18A22u_at							
Contig1309_at							
HVSMEf0019H18r2_at							
Contig1310_at							
Contig1308_at							
Contig1315_s_at							
HT03K14r_(s)_at							
Contig3772_at							
Contig7377_at							
HF03B07r_at							
AF254799_CDS-2_at							
Contig14229_at	NIP						
Contig19214							
Contig5632_at							
Contig5634_at							
Contig6339_s_at	SIP						
Contig19630_at							

Differences in expression between leaf regions were analysed by the RankProduct method, which ranks genes according to being the most (RankProduct 1) differentially expressed gene of all >21,000 sequences on the chip. To simplify presentation of data, Rank Products are classified into three groups: RankProduct < 50 (red); 50-700; (dark pink); >700 (light pink); EZ, NE, EB, elongation zone, non-elongation zone and emerged-blade; '<', '>', expression lower (<) or larger (>) in leaf region listed first.

SUPPLEMENTARY FILE S4

qPCR data of the expression of candidate barley MIPs in different leaf regions, together with their statistical analysis

Table S4a. *Expression of PIP family members in different leaf regions of barley.*

Gene	Leaf region									
	EZ		NEZ		EmBL		L2		Sheath	
	Means	SD	Means	SD	Means	SD	Means	SD	Means	SD
HvPIP1;1	7.30	1.42	3.55	0.91	1.14	0.56	2.02	0.34	3.37	0.31
HvPIP1;2	0.003	0.005	0.004	0.004	0.010	0.016	0.005	0.005	0.152	0.167
HvPIP1;3	0.49	0.11	1.66	0.03	2.93	1.21	7.54	1.57	5.73	1.22
HvPIP1;4	0.000	0.000	0.013	0.019	0.026	0.032	0.267	0.290	0.158	0.141
HvPIP1;5	0.106	0.008	0.036	0.002	0.007	0.000	0.005	0.000	0.056	0.017
TOTAL	7.90	1.38	5.26	0.93	4.11	1.70	9.84	2.09	9.47	1.52
HvPIP2;1	0.009	0.004	0.077	0.029	0.064	0.040	0.245	0.103	0.069	0.010
HvPIP2;2	1.40	0.24	0.27	0.05	0.02	0.01	0.01	0.00	0.32	0.12
HvPIP2;3	0.018	0.020	0.096	0.048	0.115	0.076	0.233	0.095	0.052	0.011
HvPIP2;4	0.007	0.002	0.190	0.016	0.458	0.096	0.496	0.058	0.450	0.022
HvPIP2;5	13.27	2.34	4.61	0.69	1.31	0.11	1.71	0.26	8.29	0.45
HvPIP2;7	0.0014	0.0016	0.086	0.027	0.339	0.098	0.231	0.033	0.010	0.003
TOTAL	14.70	2.60	5.32	0.70	2.31	0.26	2.92	0.34	9.18	0.33

Expression was analysed by qPCR, and average $2^{-(\Delta Ct)}$ values, compared to the average expression of three reference genes, for (n=) three batches of plants are shown, together with standard deviations (SD); LD, limit of detection or not detectable; EZ, NEZ, EmBL, elongation zone, non-elongation zone and emerged-blade portion of the growing leaf three; L2, Sheath, mature blade and sheath of leaf two; LD, limit of detection or not detectable.

Table S4b. *Percent contribution to total expression of PIP1s and PIP2s of individual PIP isoforms in different leaf regions of barley.*

Gene	Leaf region									
	EZ		NEZ		EmBL		L2		Sheath	
	Means	SD	Means	SD	Means	SD	Means	SD	Means	SD
HvPIP1;1	92.2	2.4	66.8	5.5	27.0	4.3	20.6	1.0	35.9	3.0
HvPIP1;2	0.04	0.06	0.08	0.09	0.4	0.8	0.1	0.0	1.5	1.5
HvPIP1;3	6.4	2.2	32.2	5.6	71.3	3.0	76.7	2.5	60.2	3.8
HvPIP1;4	0.0	0.0	0.21	0.30	1.1	1.6	2.5	2.4	1.9	1.8
HvPIP1;5	1.4	0.3	0.7	0.1	0.2	0.1	0.05	0.01	0.6	0.1
TOTAL	100	0	100	0	100	0	100	0	100	0
HvPIP2;1	0.1	0.0	1.4	0.4	2.7	1.6	8.2	3.0	0.8	0.1
HvPIP2;2	9.5	0.3	5.0	0.9	0.6	0.3	0.30	0.17	3.5	1.4
HvPIP2;3	0.11	0.11	1.9	1.2	4.8	2.6	8.0	3.3	0.6	0.1
HvPIP2;4	0.05	0.01	3.6	0.8	19.8	2.7	17.0	1.0	4.9	0.2
HvPIP2;5	90.3	0.3	86.4	2.0	57.6	9.2	58.5	5.5	90.2	1.6
HvPIP2;7	0.0	0.0	1.6	0.5	14.5	2.9	8.0	1.9	0.11	0.03
TOTAL	100	0	100	0	100	0	100	0	100	0

Percentages were calculated from individual values (n=3 experiments) used to calculate means shown in Table S4a. Those PIPs which make the highest contribution to the total expression of each PIP subfamily are highlighted in red; those PIPs which make the second, or, or joint second highest contribution are highlighted in pink; LD, limit of detection or not detectable; EZ, NEZ, EmBL, elongation zone, non-elongation zone and emerged-blade portion of the growing leaf three; L2, Sheath, mature blade and sheath of leaf two.

Table S4c. *Expression of TIP family members in different leaf regions of barley.*

Gene	Leaf region									
	EZ		NEZ		EmBL		L2		Sheath	
	Means	SD	Means	SD	Means	SD	Means	SD	Means	SD
HvTIP1;1	54.72	3.00	23.65	3.30	3.96	0.25	2.68	1.17	37.29	11.77
HvTIP1;2	0.01	0.00	0.58	0.11	0.92	0.39	1.77	0.45	0.46	0.10
HvTIP2;1	LD		LD		LD		LD		LD	
HvTIP2;2	LD		LD		LD		LD		LD	
HvTIP2;3	1.34	0.36	0.80	0.32	0.02	0.02	0.12	0.18	3.29	0.48
HvTIP3;1	LD		LD		LD		LD		LD	
HvTIP4;1	0.02	0.01	1.55	0.26	2.93	0.95	3.15	1.19	4.71	2.06
HvTIP5;1	LD		LD		LD		LD		LD	
TOTAL	56.09	3.23	26.58	2.87	7.84	1.14	7.71	1.53	45.75	11.18

Expression was analysed by qPCR, and average $2^{-(\Delta Ct)}$ values, compared to the average expression of three reference genes, for (n=) three batches of plants are shown, together with standard deviations (SD); LD, limit of detection or not detectable; EZ, NEZ, EmBL, elongation zone, non-elongation zone and emerged-blade portion of the growing leaf three; L2, Sheath, mature blade and sheath of leaf two.

Table S4d. *Percent contribution to total expression of TIPs of individual TIP isoforms in different leaf regions of barley.*

Gene	Leaf region									
	EZ		NEZ		EmBL		L2		Sheath	
	Means	SD	Means	SD	Means	SD	Means	SD	Means	SD
HvTIP1;1	97.6	0.6	88.8	3.0	51.5	10.6	34.1	11.6	80.5	8.1
HvTIP1;2	0.01	0.01	2.19	0.49	11.46	3.78	24.08	9.32	1.10	0.55
HvTIP2;1	LD		LD		LD		LD		LD	
HvTIP2;2	LD		LD		LD		LD		LD	
HvTIP2;3	2.4	0.6	3.1	1.3	0.3	0.2	1.3	1.8	7.6	2.9
HvTIP3;1	LD		LD		LD		LD		LD	
HvTIP4;1	0.038	0.014	5.94	1.6	36.7	7.04	40.6	12.7	10.8	5.05
HvTIP5;1	LD		LD		LD		LD		LD	
TOTAL	100	0	100	0	100	0	100	0	100	0

Percentages were calculated from individual values (n=3 experiments) used to calculate means shown in Table S4c. Those TIP isoforms which make the highest contribution to the total expression of TIPs are highlighted in red; those TIP isoforms which make the second, or, or joint second highest contribution are highlighted in pink; LD, limit of detection or not detectable; EZ, NEZ, EmBL, elongation zone, non-elongation zone and emerged-blade portion of the growing leaf three; L2, Sheath, mature blade and sheath of leaf two.

Table S4e. Statistical analysis of differences in expression of barley PIPs between leaf regions

Gene	Leaf regions						
	EZ:EmBL	EZ:L2	EZ:Sh	EmBL:L2	Sh:L2	EZ:NEZ	NEZ:EmBL
HvPIP1;1	*	**	*		*	**	*
HvPIP1;2							
HvPIP1;3	*	**	**	(*)		***	
HvPIP1;4							
HvPIP1;5	**	**	**	*	*	**	***
ΣPIP1s		*				*	
HvPIP2;1		*	**			*	
HvPIP2;2	**	**	**		*	**	**
HvPIP2;3	*	*	*	(*)	*	*	
HvPIP2;4	**	**	***			**	*
HvPIP2;5	**	**	*	(*)	***	*	**
HvPIP2;7	*	**	*		**	*	*
ΣPIP2s	**	**	*		***	*	*

Averaged (across reference genes) $2^{-(\Delta Ct)}$ values for three batches of plants were used to carry out paired t-test (Excel) of differences in expression between any two leaf regions. EZ, NEZ, EmBL, elongation zone, non-elongation zone and emerged blade portion of the growing leaf three; L2, mature blade of leaf two; Sh, sheath (mature) of leaf two; ΣPIP1s and ΣPIP2s, sum of expression of PIP1s and PIP2s, respectively. Expression of HvPIP1;4 in EZ was near the limit of detection; expression of HvPIP1;2 was mostly close to zero. No statistics were performed for these two genes. Two-factor ANOVA analysis, with replication, showed that leaf regions differed highly significantly ($p=6.41E-21$) in expression.

*, **, *** statistically significant difference between leaf regions at $p < 0.05$, $p < 0.01$ and $p < 0.001$; (*), just below 0.05 (> 0.046); boxes in pink: significantly higher expression in leaf region listed first; boxes in green: significantly higher expression in leaf region listed second.

Table S4f. Statistical analysis of differences in expression of TIPs between elongation zone and other leaf regions analysed.

Gene	Leaf regions			
	EZ:NEZ	EZ:EmBL	EZ:L2	EZ:Sheath
HvTIP1;1	**	***	***	
HvTIP1;2	**	*	*	**
HvTIP2;3	*	*	**	***
HvTIP4;1	**	*	*	*
Σ-TIPs	**	***	***	

Averaged (across reference genes) $2^{-(\Delta Ct)}$ values for three batches of plants were used to carry out paired t-test (Excel) of differences in expression between any two leaf regions. EZ, NEZ, EmBL, elongation zone, non-elongation zone and emerged blade portion of the growing leaf three; L2, mature blade of leaf two; Sh, sheath (mature) of leaf two; ΣTIPs, sum of expression of TIP1s. Those TIPs, which had expression close to the limit of detection, were not considered. Two-factor ANOVA analysis, with replication, showed that leaf regions differed highly significantly ($p=2.32E-08$) in expression.

*, **, *** statistically significant difference between leaf regions at $p < 0.05$, $p < 0.01$ and $p < 0.001$; (*), just below 0.05 (> 0.046); boxes in pink: significantly higher expression in leaf region listed 1st; green boxes: significantly higher expression in leaf region listed 2nd.

Table S4g. Statistical analysis of differences in expression of TIPs between non-elongation zone, emerged blade, mature blade and sheath.

Gene	Leaf regions					
	NEZ:EmBL	NEZ:L2	NEZ:Sh	EmBL:L2	EmBL:Sh	L2:Sh
HvTIP1;1	**	**			*	*
HvTIP1;2		*	*			*
HvTIP2;3	*	**	**		**	**
HvTIP4;1			*			
Σ-TIPs	**	**			*	*

Averaged (across reference genes) $2^{-(\Delta Ct)}$ values for three batches of plants were used to carry out paired t-test (Excel) of differences in expression between any two leaf regions. EZ, NEZ, EmBL, elongation zone, non-elongation zone and emerged blade portion of the growing leaf three; L2, mature blade of leaf two; Sh, sheath (mature) of leaf two; ΣTIPs, sum of expression of TIP1s. Those TIPs, which had expression close to the limit of detection, were not considered. Two-factor ANOVA analysis, with replication, showed that leaf regions differed highly significantly ($p=2.32E-08$) in expression.

*, **, *** statistically significant difference between leaf regions at $p < 0.05$, $p < 0.01$ and $p < 0.001$; (*), just below 0.05 (> 0.046); boxes in pink: significantly higher expression in leaf region listed first; boxes in green: significantly higher expression in leaf region listed second.

Table S4h. Comparison of total expression of PIP1s and PIP2s between leaf regions.

Compared	Leaf region						
	EZ:NEZ	EZ:EmBL	EZ:L2	EZ:Sh	EmBL:L2	L2:Sh	NEZ:EmBL
PIP1s	*		*				
PIP2s	*	**	**	**		***	*
PIP2:PIP1	*	*	**	*		*	

Pair-wise t-test. *, **, *** statistically significant (paired t-test) different between leaf regions at $p < 0.05$, $p < 0.01$ and $p < 0.001$; (*); boxes in pink: significantly higher expression in leaf region listed first; boxes in green: significantly higher expression in leaf region listed second, EZ, NEZ, EmBL, elongation zone, non-elongation zone and emerged blade portion of the growing leaf three; L2, mature blade of leaf two; Sh, sheath (mature) of leaf two.

Table S4i. Comparison of total expression of PIP1s and PIP2s within leaf regions.

Compared	Leaf region				
	EZ	NEZ	EmBL	L2	Sheath
PIP1s>PIP2s				*	
PIP2s>PIP1s	*				

Pair-wise t-test. *, **, *** statistically significant (paired t-test) different between PIP1s and PIP2s at $p < 0.05$, $p < 0.01$ and $p < 0.001$; EZ, NEZ, EmBL, elongation zone, non-elongation zone and emerged blade portion of the growing leaf three; L2, mature blade of leaf two; Sh, sheath (mature) of leaf two.

Table S4j. Summary of ANOVA analyses of qPCR data, where expression of MIPs was determined in five leaf regions of barley, in three independent experiments.

ANOVA	What was compared	P-value	Conclusion
Two-factor with replication	$2^{(-\Delta Ct)}$ values of PIPs (sample) from three experiments (n=3) against leaf region (column)	Sample: 2.6E-68 Column: 6.4E-21 Interaction: 1.1E-48	Individual PIP isoforms differ in expression between leaf regions, as do leaf regions in their PIP expression. Clear interaction between leaf region and PIP expression profile
Two-factor with replication	$2^{(-\Delta Ct)}$ values of total expression of PIP1s and PIP2s (sample) from three experiments (n=3) against leaf region (column)	Sample: 0.42 Column: 2.3E-08 Interaction: 1.9E-06	No basic difference between total expression of PIP1s and PIP2s throughout all leaf regions. Leaf regions differ in total expression of PIP1s and PIP2s. Clear interaction between leaf region and total PIP1 or PIP2 expression
Two-factor with replication	$2^{(-\Delta Ct)}$ values of expression of TIP1s analysed (sample) from three experiments (n=3) against leaf region (column). Only HvTIP1;1/1;2/2;3/4;1 considered since other TIPs with expression close to limit of detection in at least one leaf region	Sample: 1.5E-25 Column: 5.5E-14 Interaction: 2.0E-19	Individual TIP isoforms differ in expression between leaf regions, as do leaf regions in their TIP expression. Clear interaction between leaf region and TIP expression profile
Single factor	$2^{(-\Delta Ct)}$ values of HvPIPX;X expression from three experiments (n=3) against leaf region (groups)	Between groups: 1;1: 3.6E-05 1;3: 4.5E-05 1;5: 2.1E-07 2;1: 2.3E-03 2;2: 3.6E-07 2;3: 1.1E-02	Significant difference in expression of particular PIP between leaf regions. HvPIP1;2 and HvPIP1;4 not tested since expression at

		2;4: 1.3E-06 2;5: 5.9E-07 2;7: 1.9E-05	limit of detection in at least one leaf region
Single factor	Leaf region against $2^{(-\Delta Ct)}$ expression values of all members of the PIP1 or PIP2 family from three experiments (n=3) (groups)	Between groups: EZ/PIP1: 1.9E-07 EZ/PIP2: 3.8E-09 NEZ/PIP1: 2.5E-06 NEZ/PIP2: 7.2E-10 EB/PIP1: 4.7E-04 EB/PIP2: 1.1E-09 L2/PIP1: 6.5E-07 L2/PIP2: 1.2 E-08 Sh/PIP1: 5.7E-07 Sh/PIP2: 4.3E-15	In all leaf regions tested, there is a significant difference in expression among individual members of the PIP1 and PIP2 family

EZ, NEZ, EB, elongation zone, non-elongation zone and emerged blade portion of the growing leaf three; L2, mature blade of leaf two; Sh, sheath (mature) of leaf two.

Supplementary File S5

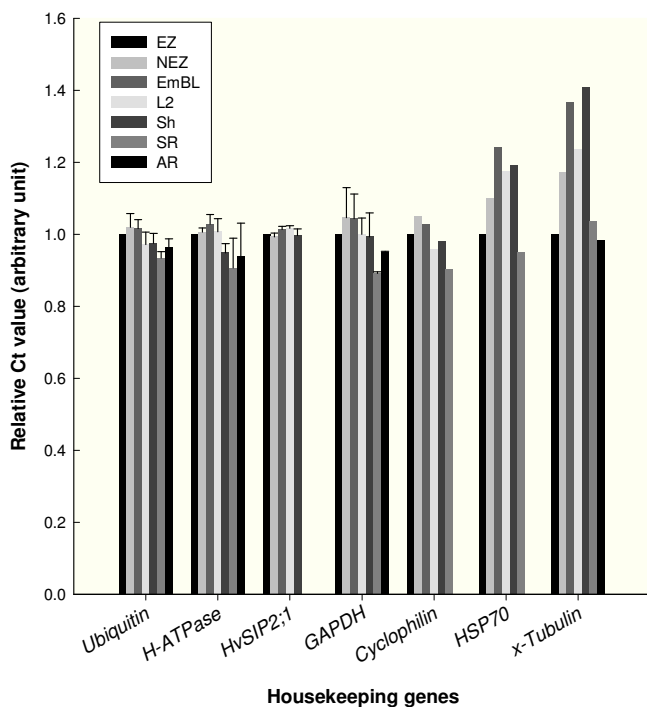


Fig. S5. Real-time (qPCR) expression analyses of housekeeping genes in leaf and root regions of barley. The elongation zone (EZ), adjacent non-elongation zone (NEZ) and emerged-blade portion (EmBL) of the growing leaf three was analysed, together with the blade and sheath of the mature leaf two (L2 and Sh, respectively). In addition, entire seminal roots (SR) and adventitious roots (AR) were analysed. Expression (Ct values) of genes in a particular leaf region or type of root was related to the expression in the leaf elongation zone (set to 1.0). Results for ubiquitin, H-ATPase, HvSIP2;1 and GAPDH are averages \pm SD (error bars) of the analyses of three batches of plants. For cyclophilin, HSP70 and alpha-tubulin only two batches of plants were analysed (no SD).

