APPENDIX I: PRIMERS

List of primers designed for use with the vectors in Chapter 4.

Name	bp	Oligo Sequence	Intended Purpose
35A LB seq	20	GTTTTAGGTCATGATTTTAG	Sequence vectors from CaMV35S polyA
35S *AleI* R	38	CATGCACACGCGGGTGCGACACTCTCGTCTACTCCAAG	Amplify 35S promoter and add *AleI* site
35S *BlpI* R	35	AGTACTGCTAAGCCGACACTCTCGTCTACTCCAAG	Amplify 35S promoter and add *BlpI* site
35S *BlpI* R2	35	AGTACTGCTAAGCAGCTTGCCAACATGGTGGAGCA	Amplify 35S promoter and add *BlpI* site
35S *SacI* R	33	ATGCAGAGCTCAGCTTGCCAACATGGTGGAGCA	Amplify 35S - polyA with *AleI* site
35S*PmeI* F	28	ATGTTTAAACAGTCAAAGATTCAAATAG	Amplify 35S promoter and add *PmeI* site
35S*PmeI* R	26	GGGTTTAAACCCTCTCCAAATGAAAT	Amplify 35S promoter and add *PmeI* site
35Sx2*PmeI* F	28	CCGTTTAAACGGGATCCTCTAGAGTCGA	Amplify 35S promoter and add *PmeI* site
35Sx2*PmeI* R	26	CGGTTTAAACGGTCAACATGGTGGAG	Amplify 35S promoter and add *PmeI* site
AlcA*PmeI* F	31	CCGTTTAAACGGATAGTTCCGACCTAGGATT	Amplify alcA promoter with *PmeI* site
AlcR1Fseq	20	ACAGAGCACAATTGTCCATA	Sequence vectors from Alc R promoter
AlcR2Rseq	20	ACTTATTCTTGCTGCCAGGT	Sequence vectors from Alc R promoter
AlcR3seq	20	ACACAAATCTCTCAGCCAGC	Sequence vectors from Alc R promoter
AtHKT R NoSt	25	GGAAGACGAGGGGTAAAGTATCCAT	Amplify *AtHKT1;1* with no stop codon
AVP1 F	26	CACCATGGTGGCGCCTGCTTTGTTAC	Amplify *AtAVP1*
AVP1 R	27	TTAGAAGTACTTGAAAAGGATACCACC	Amplify *AtAVP1*
AVPFwd	20	ATGGTGGCGCCTGCTTTGTT	Amplify *AtAVP1*
basta F	20	GACTTCAGCAGGTGGGTGTA	Amplify bar-nos fragment in pMDC123
ccdB F seq	20	AACAGGGACTGGTGAAATGC	Sequence vectors from ccdB gene

Name	bp	Oligo Sequence	Intended Purpose
chlor R seq	20	TGCTCATGGAAAACGGTGTA	Sequence vectors from chlor R gene
DT-A_XhoI_F	28	CGCTCGAGTACTTCTGCAGATGGATCCT	Amplify *DT-A* gene with XhoI site
DT-A_XhoI_R	28	CGCTCGAGTAGAGAGAGACTGGTGATTT	Amplify *DT-A* gene with XhoI site
Eth*AscI* R	27	CCGGCGCGCCGTCCTCTCCAAATGAAA	Amplify AlcR-nos-alcA fragment with *AscI* site
Eth*PmeI* F	28	TGGTTTAAACATGGCAGATACGCGCCGA	Amplify AlcR-nos-alcA fragment with *PmeI* site
GAL4Q F	20	CTTGTTGCTGCTCTCCTCCG	Genotype or Q-PCR on *GAL4*
GAL4Q R	20	GGACATCAAAGCCCTGCTCA	Genotype or Q-PCR on *GAL4*
GUS F	28	CACCATGGTACGTCCTGTAGAAACCCCA	Amplify *GUS*
GUS R	24	TCATTGTTTGCCTCCCTGCTGCGG	Amplify *GUS*
half kan F	30	TAAGTAATCCAATTCGGCTAAGCGGCTGTC	Amplify 35S-kanamycin fragments in pMDC vectors
HKT1 F	29	CACCATGGACAGAGTGGTGGCAAAAATAG	Amplify *AtHKT1;1*
HKT1 R	25	TTAGGAAGACGAGGGGTAAAGTATC	Amplify *AtHKT1;1*
HKT1Fwd	20	ATGGACAGAGTGGTGGCAAA	Amplify *AtHKT1;1*
HKTI	40	gatgtacaagcacttagatcccgtctctctttgtattcc	amiRNA (HKT) construction
HKTII	40	gacgggatctaagtgcttgtacatcaaagagaatcaatga	amiRNA (HKT) construction
HKTIII	40	gacgagatctaagtggttgtacttcacaggtcgtgatatg	amiRNA (HKT) construction
HKTIV	40	gaagtacaaccacttagatctcgtctacatatatattcct	amiRNA (HKT) construction
HKTA	25	ctgcaaggcgattaagttgggtaac	amiRNA construction
HKTB	28	gcggataacaatttcacacaggaaacag	amiRNA construction
hygromycinF	20	GTTTCCACTATCGGCGAGTA	Amplify hyg-nos fragment in pMDC99
INT-OCS F Al	24	CACCCGCGTGCAGCTTGGTAAGGA	Amplify intron to OCS term from pHELLSGATE8
INT-OCS R Al	24	CACGCGGGTGGCAGATTTAGGTGA	Amplify intron to OCS term from pHELLSGATE8
NHX1 F	25	CACCATGTTGGATTCTCTAGTGTCG	Amplify *AtNHX1*
NHX1 R	24	TTACTAAGATCAGGAGGGTTTCTC	Amplify *AtNHX1*
NHXFwd	20	ATGTTGGATTCTCTAGTGTC	Amplify *AtNHX1*

Name	bp	Oligo Sequence	Intended Purpose
nos F *SacI*	28	GAGCTCGAATTTCCCCGATCGTTCAAAC	Amplify nos term with *SacI* site
nos R EcoRI	28	GAATTCCCGATCTAGTAACATAGATGAC	Amplify nos term with EcoRI site
nosR	20	CCGGTCTTGCGATGATTATC	Amplify nos term
nosR123	20	CCGGTCTTGCGATGATTATC	Amplify bar-nos fragment in pMDC123
nosR99	20	CGGTCTTGCGATGATTATCA	Amplify hyg-nos fragment in pMDC99
nosT F *AleI*	33	CCATGACACCGCGGTGAGTAACATAGATGACAC	Amplify nos term with *AleI* site
nosT R *PacI*	31	CGCGGTTAATTAAGAATTTCCCCGATCGTTC	Amplify nos term with *PacI* site
miRNAI	40	gatggtaactaagaacgaccgggtctctctttgtattcc	amiRNA (miRNA) construction
miRNAII	40	gacccggtcgttcttagttaccatcaaagagaatcaatga	amiRNA (miRNA) construction
miRNAIII	40	gaccaggtcgttctttgttaccttcacaggtcgtgatatg	amiRNA (miRNA) construction
miRNAIV	40	gaaggtaacaaagaacgacctggtctacatatatattcct	amiRNA (miRNA) construction
OCS Fseqin	20	TAGGCGTCTCGCATATCTCA	Sequence vectors from OCS term
OCS Rseqout	20	ATGCATATTCTATAGTGTCA	Sequence vectors from OCS term
PDK Fseqout	20	TAATTAACATCACTTAACTA	Sequence vectors from PDK intron
PDK Rseqin	20	GTGTTATCATTGATCTTACA	Sequence vectors from PDK intron
PDK-OCS F Al	32	GTCAGTCACCCGCGTGCAGCTTGGTAAGGAAA	Amplify PDK intron - OCS term
PDK-OCS R Al	32	TGACCACACGCGGGTGGCAGATTTAGGTGACA	Amplify PDK intron - OCS term
polyA LBseq	20	CCAGATAAGGGAATTAGGGT	Sequence vectors from CaMV35S polyA
polyA *SacI* F	34	TAGTCGAGCTCCTGAATTAACGCCGAATTAATTC	Amplify 35S - polyA with *SacI* site
PpENA1 F	25	CACCATGGAGGGCTCTGGGGACAAG	Amplify *PpENA1*
PpENA1-tr F	25	CACCATGCTCCTCGCATTTGCGCTG	Amplify truncated *PpENA1*
ScENA1 F	25	CACCATGGGCGAAGGAACTACTAAG	Amplify *ScENA1*
ScENA1 R	25	TCATTGTTTAATACCAATATTAACT	Amplify *ScENA1*
ScENAFwd	20	ATGGGCGAAGGAACTACTAA	Amplify *ScENA1*
ScNHA1 F	27	CACCATGGCTATCTGGGAGGCAACTAG	Amplify *ScNHA1*
ScNHA1 R	26	TTACTTATTGAGACCAAGCGTTTTTG	Amplify *ScNHA1*
ScNHAFwd	20	ATGGCTATCTGGGAGCAACT	Amplify *ScNHA1*

Name	bp	Oligo Sequence	Intended Purpose
SKOR1 F	24	CACCATGGGAGGTAGTAGCGGCGG	Amplify *AtSKOR*
SKOR1 R	24	TTATGTTTCAACAGCCAAATACAG	Amplify *AtSKOR*
SKOR1Fwd	20	ATGGGAGGTAGTAGCGGCGG	Amplify *AtSKOR*
SOS1 F	27	CACCATGACGACTGTAATCGACGCGAC	Amplify *AtSOS1*
SOS1 R	24	TCATAGATCGTTCCTGAAAACGAT	Amplify *AtSOS1*
SOS1-trFwd	20	ATGACGACTGTAATCGACGC	Amplify *AtSOS1* truncated
SOS1-trunc R	23	CTGATCAGAGCTTGAGCTACGTG	Amplify *AtSOS1* truncated
SOSFwd	20	ATGACGACTGTAATCGACGC	Amplify *AtSOS1*
UAS *AscI* R	26	GCGGCGCGCCGTCGACCTGCAGGTCG	Amplify UAS with *AscI* site
UASF EcoRI	28	GAATTCGCATGCCTGCAGGTCGGAGTAC	Amplify UAS with EcoRI site
UASF HindIII	22	AAGCTTGCATGCCTGCAGGTCG	Amplify UAS with HindIII site
UAS*KpnI* F	28	ACGGTACCGCATGCCTGCAGGTCGGAGT	Amplify UAS with *KpnI* site
UAS*PmeI* F	28	TTGTTTAAACGCATGCCTGCAGGTCGGA	Amplify UAS with *PmeI* site
UAS*PmeI* R	28	GCGTTTAAACGTCGACCTGCAGGTCGTC	Amplify UAS with *PmeI* site
UASR EcoRI	27	GAATTCGTCGACCTGCAGGTCGTCCTC	Amplify UAS with EcoRI site
UASR HindIII	23	CCGAGCTCAAGCTTGTCGACCTG	Amplify UAS with HindIII site

APPENDIX II: EXTRA VECTORS

Other vectors used in construction or built, but not mentioned in chapter 4.

Vector	Source	Species	Plant	Bact.	Intended Purpose	Gateway
pGreen229-UAS-NHX1-nos	Inge Moller - University of Cambridge	Plants	basta	kan	Empty vector for cell-specific expression in *Arabidopsis*	No
pGreen229-UAS-SOS1-nos	Inge Moller - University of Cambridge	Plants	basta	kan	Cell-specific expression of *AtSOS1* in *Arabidopsis*	No
pGreen229-UAS-SOS1tr-nos	Inge Moller - University of Cambridge	Plants	basta	kan	Cell-specific expression of *AtSOS1tr* in *Arabidopsis*	No
pGreen229-UAS-AVP1-nos	Inge Moller - University of Cambridge	Plants	basta	kan	Cell-specific expression of *AtAVP1* in *Arabidopsis*	No
pGreen229-UAS-ScNHA1-nos	Inge Moller - University of Cambridge	Plants	basta	kan	Cell-specific expression of *ScNHA1* in *Arabidopsis*	No
pGreen229-UAS-SKOR1-nos	Inge Moller - University of Cambridge	Plants	basta	kan	Cell-specific expression of *AtSKOR1* in *Arabidopsis*	No
ScENA1 plasmid	Alonso Rodriguez-Navarro - Spain					
DT-A plasmid	Olivier Cotsaftis - ACPFG					
pYES-DEST52	Invitrogen	Yeast	URA3	amp	OEX in Yeast	Yes
pCR8/GW/TOPO TA + TaHKT1;5A	Caitlin Byrt - ACPFG	*E. coli*	n/a	spec	Gateway entry vector for *TaHKT1;5A*	Yes
pCR8/GW/TOPO TA + TaHKT1;5D	Caitlin Byrt - ACPFG	*E. coli*	n/a	spec	Gateway entry vector for *TaHKT1;5D*	Yes
pCR8/GW/TOPO TA + OsHKT1;5N	Olivier Cotsaftis - ACPFG	*E. coli*	n/a	spec	Gateway entry vector for *OsHKT1;5N*	Yes
pCR8/GW/TOPO TA + OsHKT1;5P	Olivier Cotsaftis - ACPFG	*E. coli*	n/a	spec	Gateway entry vector for *OsHKT1;5P*	Yes

Vector	Source	Species	Plant	Bact.	Intended Purpose	Gateway
pCR8/GW/TOPO TA + HKT1 (no stop)	Darren Plett - ACPFG	*E. coli*	n/a	spec	Gateway entry vector for *AtHKT1;1* (for pYES-DEST52)	Yes
pCR8/GW/TOPO TA + ScENA1	Darren Plett - ACPFG	*E. coli*	n/a	spec	Gateway entry vector for *ScENA1*	Yes
pCR8/GW/TOPO TA + AVP1	Darren Plett - ACPFG	*E. coli*	n/a	spec	Gateway entry vector for *AtAVP1*	Yes
pCR8/GW/TOPO TA + SOS1	Darren Plett - ACPFG	*E. coli*	n/a	spec	Gateway entry vector for *AtSOS1*	Yes
pCR8/GW/TOPO TA + SOS1tr	Darren Plett - ACPFG	*E. coli*	n/a	spec	Gateway entry vector for *AtSOS1tr*	Yes
pCR8/GW/TOPO TA + NHX1	Darren Plett - ACPFG	*E. coli*	n/a	spec	Gateway entry vector for *AtNHX1*	Yes
pCR8/GW/TOPO TA + SKOR1	Darren Plett - ACPFG	*E. coli*	n/a	spec	Gateway entry vector for *AtSKOR1*	Yes
pCR8/GW/TOPO TA + ScNHA1	Darren Plett - ACPFG	*E. coli*	n/a	spec	Gateway entry vector for *ScNHA1*	Yes
pCR8/GW/TOPO TA + DT-A	Darren Plett - ACPFG	*E. coli*	n/a	spec	Gateway entry vector for *DT-A*	Yes
pCR8/GW/TOPO TA + UAS *AscI*/*KpnI*	Darren Plett - ACPFG	*E. coli*	n/a	spec	Cloning UAS fragment w/ *AscI* and *KpnI* restriction sites	Yes
pCR8/GW/TOPO TA + UAS *PmeI*	Darren Plett - ACPFG	*E. coli*	n/a	spec	Cloning UAS fragment w/ *PmeI* restriction sites	Yes
pCR8/GW/TOPO TA + INT-OCS fragment	Darren Plett - ACPFG	*E. coli*	n/a	spec	Cloning INT-OCS fragment from pHELLSGATE8	Yes
pCR8/GW/TOPO TA + *PpENA1*trR10	Darren Plett - ACPFG	*E. coli*	n/a	spec	Gateway entry vector for *PpENA1*trR10	Yes
pMDC32 + HKT1	Darren Plett - ACPFG	Plants	hyg	kan	OEX of *AtHKT1;1*	Yes
pMDC32 + AVP1	Darren Plett - ACPFG	Plants	hyg	kan	OEX of *AtAVP1*	Yes
pMDC32 + SKOR1	Darren Plett - ACPFG	Plants	hyg	kan	OEX of *AtSKOR1*	Yes
pMDC32 + SOS1	Darren Plett - ACPFG	Plants	hyg	kan	OEX of *AtSOS1*	Yes
pMDC32 + SOS1tr	Darren Plett - ACPFG	Plants	hyg	kan	OEX of *AtSOS1tr*	Yes

Vector	Source	Species	Plant	Bact.	Intended Purpose	Gateway
pMDC32 + ScENA1	Darren Plett - ACPFG	Plants	hyg	kan	OEX of *ScENA1*	Yes
pMDC32 + ScNHA1	Darren Plett - ACPFG	Plants	hyg	kan	OEX of *ScNHA1*	Yes
pMDC32 + NHX1	Darren Plett - ACPFG	Plants	hyg	kan	OEX of *AtNHX1*	Yes
pTOOL2 + HKT1	Darren Plett - ACPFG	Plants	basta	amp	OEX of *AtHKT1;1*	Yes
pTOOL2 + NHX1	Darren Plett - ACPFG	Plants	basta	amp	OEX of *AtNHX1*	Yes
pTOOL2 + AVP1	Darren Plett - ACPFG	Plants	basta	amp	OEX of *AtAVP1*	Yes
pTOOL2 + SKOR1	Darren Plett - ACPFG	Plants	basta	amp	OEX of *AtSKOR1*	Yes
pYES-DEST52 + *AtHKT1;1* (no stop codon)	Darren Plett - ACPFG	Yeast	URA3	amp	OEX of *AtHKT1;1* in Yeast	Yes
pYES-DEST52 + TaHKT1;5A	Darren Plett - ACPFG	Yeast	URA4	amp	OEX of *TaHKT1;5A* in Yeast	Yes
pYES-DEST52 + TaHKT1;5D	Darren Plett - ACPFG	Yeast	URA5	amp	OEX of *TaHKT1;5D* in Yeast	Yes
pYES-DEST52 + OsHKT1;5N	Darren Plett - ACPFG	Yeast	URA6	amp	OEX of *OsHKT1;5N* in Yeast	Yes
pYES-DEST52 + OsHKT1;5P	Darren Plett - ACPFG	Yeast	URA7	amp	OEX of *OsHKT1;5P* in Yeast	Yes

APPENDIX III: MEDIA

Media

NB Medium

NB medium	1 l
N6 Macroelements	50 ml
FeEDTA (Base NB)	10 ml
B5 Microelements	10 ml
B5 Vitamins	10 ml
Myo-inositol	100 mg
Proline	500 mg
Glutamine	500 mg
Casein hydrosylate	300 mg
Sucrose (Double-crystallized)	30 g
2,4-D	2.5 mg
Phytagel (Sigma P-8169)	2.6 g
pH	5.8

Autoclave half volume (500 ml) with just the phytagel. Prepare the rest in half volume, pH, and then filter-sterilise into the bottle of phytagel solution. Ensure the phtygel stays hot, and heat the other half before adding, because phytagel solidifies rapidly.

25 ml into sterile, deep petri dishes. 10 seeds per dish, or 50-100 embryonic units per dish.

NBS Medium

NBS medium	1 l
N6 Macroelements	50 ml
FeEDTA (Base NB)	10 ml
B5 Microelements	10 ml
B5 Vitamins	10 ml
Myo-inositol	100 mg
Proline	500 mg
Glutamine	500 mg
Casein hydrosylate	300 mg
Sucrose	30 g
2,4-D	2.5 mg
Cefotaxime	400 mg
Vancomycine	100 mg
Geneticin (G418)	200 mg
Agarose type I: low EEO	7 g
pH	6

Autoclave half volume (500 ml) with just the agarose. Prepare the rest in half volume, pH, and then filter-sterilise into the bottle of agarose solution when it has reached a temperature that it can be touched.

25 ml into sterile, deep petri dishes. 7 calli per dish.

PR-AG Medium

PR-AG medium	1 l
N6 Macroelements	50 ml
FeEDTA (Base NB)	10 ml
B5 Microelements	10 ml
B5 Vitamins	10 ml
Myo-inositol	100 mg
Proline	500 mg
Glutamine	500 mg
Casein hydrosylate	300 mg
Sucrose	30 g
ABA	5 mg
BAP	2 mg
NAA	1 mg
Cefotaxime	100 mg
Vancomycine	100 mg
Geneticin (G418)	200 mg
Agarose type I: low EEO	7 g
pH	5.8

Autoclave half volume (500 ml) with just the agarose. Prepare the rest in half volume, pH, and then filter-sterilise into the bottle of agarose solution when it has reached a temperature that it can be touched. 25 ml into sterile, deep petri dishes. Put between 7-21 calli per line, around 10 lines, per dish. Draw separation between lines on bottom of dish with marker.

RN Medium

RN medium	1 l
N6 Macroelements	50 ml
FeEDTA (Base NB)	10 ml
B5 Microelements	10 ml
B5 Vitamins	10 ml
Myo-inositol	100 mg
Proline	500 mg
Glutamine	500 mg
Casein hydrosylate	300 mg
Sucrose	30 g
BAP	3 mg
NAA	0.5 mg
Phytagel	3.5 g
pH	5.8

The entire medium can be sterilised by autoclaving.
30 ml into sterile, deep petri dishes. 7 calli per dish.

R2-CL Medium

R2-CL liquid medium	1 l
Macroelements R2-I	100 ml
Macroelements R2-II	100 ml
FeEDTA (Base R2)	10 ml
Microelements R2	1 ml
Vitamins R2	25 ml
Glucose	10 g
2,4-D	2.5 mg
Acetosyringone	100 ul of stock
pH	5.2

Sterilise by filtration. Suspend Agrobacterium on day of transformation in 30 ml of R2-CL (in a sterile 50 ml tube). Measure the O.D. (optical density) at 600 nm, and adjust to 1.

R2-CS Medium

R2-CS medium	1 l
Macroelements R2-I	100 ml
Macroelements R2-II	100 ml
FeEDTA (Base R2)	10 ml
Microelements R2	1 ml
Vitamins R2	25 ml
Glucose	10 g
2,4-D	2.5 mg
Acetosyringone	100 ul of stock
Agarose type I: low EEO (Sigma A-6013)	7 g
pH	5.2

Autoclave half volume (500 ml) with just the agarose. Prepare the rest in half volume, pH, and then filter-sterilise into the bottle of agarose solution when it has reached a temperature that it can be touched. 20 ml into sterile, deep petri dishes. 10 calli per dish.

R2-S Medium

R2-S medium	1 l
Macroelements R2-I	100 ml
Macroelements R2-II	100 ml
FeEDTA (Base R2)	10 ml
Microelements R2	1 ml
Vitamins R2	25 ml
Sucrose	30 g
2,4-D	2.5 mg
Cefotaxime	400 mg
Vancomycin	100 mg
Geneticin (G418)	200 mg
Agarose type I: low EEO	7 g
pH	6

Autoclave half volume (500 ml) with just the agarose. Prepare the rest in half volume, pH, and then filter-sterilise into the bottle of agarose solution when it has reached a temperature that it can be touched. 25 ml into sterile, deep petri dishes. 10 calli per dish.

P Medium

P medium	1 l
Murashige & Skoog Medium Basal Salt Mixture	4.3 g
Murashige & Skoog Vitamin Mixture	103 mg
Sucrose	50 g
Phytagel (Sigma)	2.6 g
pH	5.8
The entire medium can be sterilised by autoclaving.	
40 ml per jar (1 L = 25 jars)	

AB Medium

AB medium	1 l
AB MEDIUM STOCK (1L)	
K_2HPO_4	60 g
NaH_2PO_4	20 g
AB SALTS STOCK (1L)	
NH_4Cl	20 g
$MgSO_4 \times 7H_2O$	6 g
KCl	3 g
$CaCl_2$	200 mg
$FeSO_4 \times 7H_2O$	50 mg
Autoclave both of the above stocks for sterilisation. For 1 L of AB medium: add 5 g glucose and 15 g Difco bacto agar to 900 ml dH2O, and autoclave. After, add 50 ml of AB medium stock and 50 ml of AB salts stock, as well as appropriate antibiotics (Rif 50 + Kan 50).	
20 ml into sterile, thin petri dishes.	

Stock Solutions

N6 Macroelements

N6 Macroelements	1 l
KNO_3	56.6 g
$(NH_4)_2SO_4$	9.26 g
KH_2PO_4	8.0 g
$CaCl_2 \times 2H_2O$	3.3 g
$MgSO_4 \times 7H_2O$	3.7 g

FeEDTA (Base NB)

FeEDTA (Base NB)	1 l
$FeSO_4 \times 7H_2O$	2.78 g
Na_2 EDTA	3.72 g
First heat up water (half-volume) by microwave, then put stir bar and place on stir plate. Add the EDTA and let dissolve. Add the $FeSO_4$ and let dissolve. Fill up to 1 L with warm water.	
Let solution cool to room temperature before placing into fridge.	

B5 Microelements

B5 Microelements	1 l
H_3BO_3	300 mg
$MnSO_4$ x H_2O	758 mg
$ZnSO_4$ x $7H_2O$	200 mg
KI	75 mg
Na2MoO4 x $2H_2O$	25 mg
$CuSO_4$ x $5H_2O$	2.5 mg
$CoCl_2$ x $6H_2O$	2.5 mg

B5 Vitamins

B5 Vitamins	1 l
Nicotinic acid	100 mg
Pyroxidine HCl	100 mg
Thiamine HCl	1 g

Macroelements R2-II

Macroelements R2-II	1 l
$CaCl_2$	1.46 g

Macroelements R2-I

Macroelements R2-I	1 l
KNO_3	40 g
$(NH_4)_2SO_4$	3.3 g
NaH_2PO_4 x H_2O	3.12 g
$MgSO_4$ x $7H_2O$	2.46 g

Note: Calcium is not added here to avoid precipitation of the macroelements.

FeEDTA (Base R2)

FeEDTA (Base R2)	1 l
$FeSO_4$ x $7H_2O$	1.25 g
Na_2 EDTA	177 mg

First heat up water (half-volume) by microwave, then put stir bar and place on stir plate. Add the EDTA and let dissolve. Add the $FeSO_4$ and let dissolve. Fill up to 1 L with warm water.

Let solution cool to room temperature before placing into fridge.

Microelements R2

Microelements R2	1 l
$MnS0_4$ x H_2O	1.6 g
$ZnSO_4$ x $7H_2O$	2.2 g
H_3BO_3 (Boric Acid)	2.83 g
$CuSO_4$ x $5H_2O$	195 mg
Na_2MoO_4 x $2H_2O$	125 mg

Vitamins R2

Vitamins R2	1 l
Thiamine HCl	40 mg

Vancomycin

Vancomycin

For 100 mg/ml stock: add 1 g vancomycin to 10 ml dH2O. Protect from light.
Aliquot 1 ml portions into 1.5 ml eppendorf tubes and freeze

Cefotaxime

Cefotaxime

Cefotaxime sodium Distributed by Duchefa
For 400 mg/ml stock: add 1 g of cefotaxime to 2.5 ml dH_2O. Protect from light.
Aliquot 1 ml portions into 1.5 ml eppendorf tubes and freeze.

Acetosyringone

Acetosyringone

For 100 mM stock: add 19.62 mg of acetosyringone to 1 ml of DMSO (dimethyl sulphoxide). Protect from light.

Hygromycin

Hygromycin

Hygromycin B Sigma catalog number H-7772
For 50 mg/ml stock: dissolve 1g in 20 ml dH_2O, protect from light.
Aliquot 1 ml portions into 1.5 ml eppendorf tubes and freeze.

Geneticin

Geneticin

For 200 mg/ml stock: dissolve 1g in 5 ml dH_2O, protect from light.
Aliquot 1 ml portions into 1.5 ml eppendorf tubes and freeze.

NAA

Napthaleneacetic acid

alpha-Napthaleneacetic acid Sigma catalog number N1641
For 1mg/ml stock of NAA: dissolve 100 mg in 1N NaOH (may need several ml), add dH_2O to final volume of 100 ml.

ABA

Abscisic acid

Abscisic acid Sigma catalog number A-1049
For 5 mg/ml stock of ABA: dissolve 100 mg in 1N NaOH, add dH_2O to final volume of 20 ml. Sterilise by filtration, and protect from light

BAP

6-Benzylaminopurine

6-Benzylaminopurine Sigma catalog number B-3408
For 1mg/ml stock of BAP: dissolve 100 mg of powder in drops of 1N NaOH, add dH_2O to final volume of 100 ml.

2,4-D
2,4-D
2,4-Dichlorophenoxyacetic acid Sigma catalog number D7299
For 2 mg/ml stock: dissolve 100 mg in 50 ml absolute ethanol, then dH_2O to 100 ml.

APPENDIX IV: Cl^-, Mg^{2+} AND Ca^{2+} XRMA RESULTS

Chloride

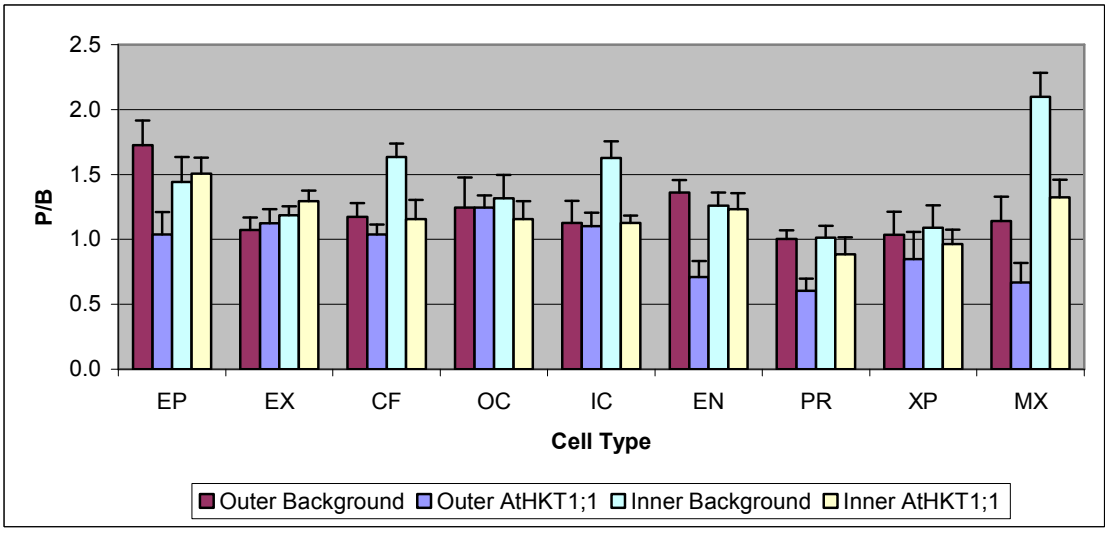

Figure 1: Cl^- content (P/B) measured by x-ray microanalysis in 9 root cell-types of Outer Background, Outer *AtHKT1;1*, Inner Background and Inner *AtHKT1;1* lines treated for 5 d with 50 mM Na^+ in ACPFG solution (see Chapter 6: Methods and Materials for details). Cell-types analysed were epidermis (EP), exodermis (EX), cortical fibers (CF), outer cortex (OC), inner cortex (IC), endodermis (EN), pericycle (PR), xylem parenchyma (XP) and metaxylem (MX). Each bar represents an average of 9 individual cell measurements (3 cells from each of 3 plants) with standard errors.

The Cl^- content of the inner background line appears to be slightly higher than the outer background line, especially in the cortical fibres (CF), inner cortex (IC) and metaxylem (MX) cells (Figure 1). However, the metaxylem (MX) difference may be due to contamination.

The outer *AtHKT1;1* line appears to have slightly lower Cl^- content than the outer background line in the epidermis (EP), endodermis (EN), pericycle (PR) and metaxylem (MX) cells (Figure 1). Interestingly, there is no difference in the cortical fibres (CF), outer cortex (OC) and inner cortex (IC) cells which contain more Na^+ in the outer *AtHKT1;1* line. The outer *AtHKT1;1* line contains more Na^+ in the root in general and it would be expected that Cl^- content would be similarly high to balance the electrical charge, but perhaps the decreased K^+ content sufficiently balances this difference. It may be the NO_3^- content is higher in the outer *AtHKT1;1* line. Also, the outer *AtHKT1;1* line appears to be lower in Cl^- in the inner cells of the stele in general,

which is odd considering the Na^+ profile has been altered in the outer cells of the root. Chloride has not been measured on the whole plant level and would be interesting to examine further to see if the *AtHKT1;1* expression has altered Cl^- accumulation in the shoot as well.

The inner *AtHKT1;1* line also has lower Cl^- content than the inner background line with the largest differences observed in the cortical fibres (CF), inner cortex (IC) and metaxylem (MX) cells (Figure 1). If the metaxylem (MX) difference is due to contamination, it means the cell-types showing different Cl^- accumulation are in outer cells of the root, despite the Na^+ accumulation increase in the xylem parenchyma cells in the inner part of the root (where the transgene was expressed).

Magnesium

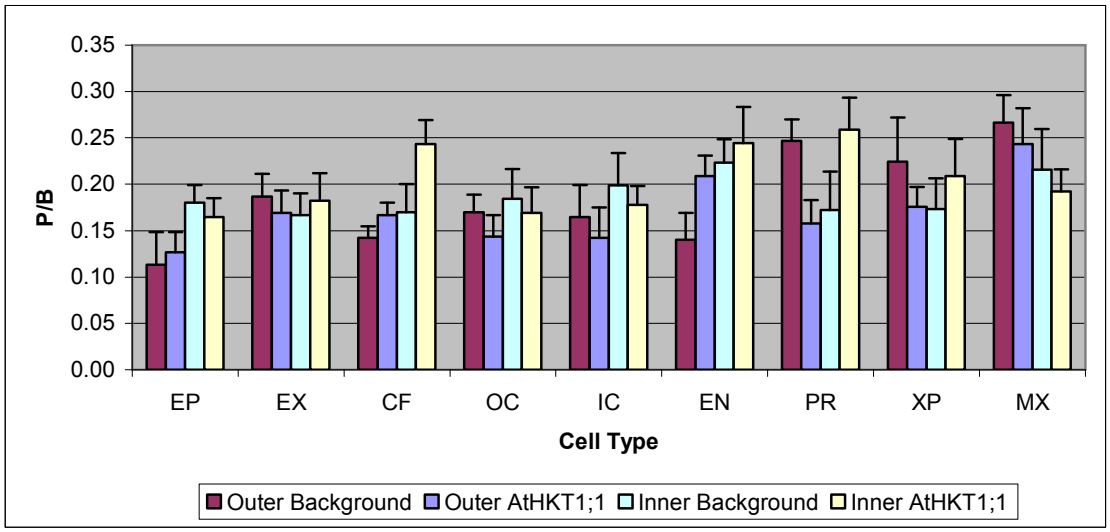

Figure 2: Mg^{2+} content (P/B) measured by x-ray microanalysis in 9 root cell-types of Outer Background, Outer *AtHKT1;1*, Inner Background and Inner *AtHKT1;1* lines treated for 5 d with 50 mM Na^+ in ACPFG solution (see Chapter 6: Methods and Materials for details). Cell-types analysed were epidermis (EP), exodermis (EX), cortical fibers (CF), outer cortex (OC), inner cortex (IC), endodermis (EN), pericycle (PR), xylem parenchyma (XP) and metaxylem (MX). Each bar represents an average of 9 individual cell measurements (3 cells from each of 3 plants) with standard errors.

Minor differences in Mg^{2+} accumulation were observed between the inner and outer background lines (Figure 2). A general trend appears to be lower Mg^{2+}

accumulation in the outer cells of the inner background and lower accumulation in the inner cells of the outer background.

The outer *AtHKT1;1* line had higher Mg^{2+} in the endodermis (EN) cells and lower Mg^{2+} accumulation in the pericycle (PR) cells than the outer background line (Figure 2).

The inner *AtHKT1;1* line had higher Mg^{2+} in the cortical fibres (CF) and pericycle (PR) cells than the inner background line (Figure 2). There was slightly higher Mg^{2+} accumulation in general in the inner *AtHKT1;1* line than in inner background.

Calcium

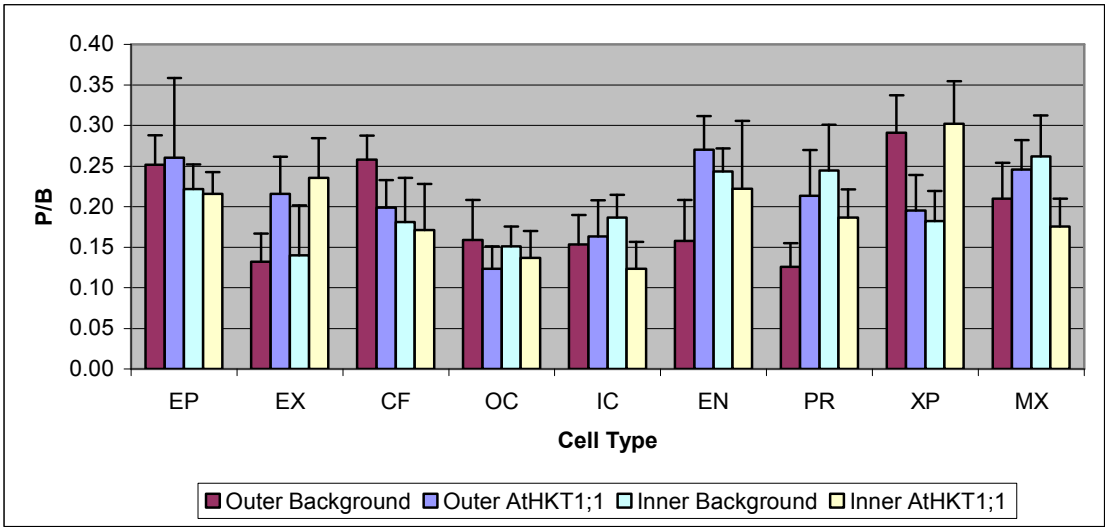

Figure 3: Ca^{2+} content (P/B) measured by x-ray microanalysis in 9 root cell-types of Outer Background, Outer *AtHKT1;1*, Inner Background and Inner *AtHKT1;1* lines treated for 5 d with 50 mM Na^{+} in ACPFG solution (see Chapter 6: Methods and Materials for details). Cell-types analysed were epidermis (EP), exodermis (EX), cortical fibers (CF), outer cortex (OC), inner cortex (IC), endodermis (EN), pericycle (PR), xylem parenchyma (XP) and metaxylem (MX). Each bar represents an average of 9 individual cell measurements (3 cells from each of 3 plants) with standard errors.

The endodermis (EN) and pericycle (PR) cells of the inner background accumulated more Ca^{2+} than the outer background line, while the outer background accumulated more Ca^{2+} in the cortical fibres (CF) and xylem parenchyma (XP) cells (Figure 3).

The differences between the outer *AtHKT1;1* line and the outer background line in Ca^{2+} accumulation were complex (Figure 3). The outer *AtHKT1;1* line accumulated more Ca^{2+} in the exodermis (EX), endodermis (EN) and pericycle (PR), but accumulated less Ca^{2+} in the cortical fibres (CF) and xylem parenchyma (XP) cells.

The differences between the inner *AtHKT1;1* line and the inner background line in Ca^{2+} accumulation were equally complex (Figure 3). The inner *AtHKT1;1* line accumulated more Ca^{2+} in the exodermis (EX) and xylem parenchyma (XP, but accumulated less Ca^{2+} in the inner cortex (IC), pericycle (PR) and metaxylem (MX) cells.

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