Information Fig S1 and Table S1.

To represent the evolutionary relation among the plant HXKs presented in the Fig S1, the evolutionary history was inferred by using the Maximum Likelihood method based on the JTT matrix-based model using 2000 bootstrap [29, 30, 31]. The tree with the highest log likelihood (-40548.09) is shown. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using a JTT model, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 114 amino acid sequences. There was a total of 807 positions in the final dataset.

All amino acid sequences were aligned using MEGA X [29]. Group designations were made from visual inspection of apparent clusters and according to literature information. The amino acids sequences were obtained from Uniprot[1] (https://www.uniprot.org/uniprot The UniProt Consortium. UniProt: the universal protein knowledgebase. Nucleic Acids Res. 2017;45:D158-9), PLAZA[2] (https://bioinformatics.psb.ugent.be/plaza/), EnsemblPlants[3] (http://plants.ensembl.org/index.html) and NCBI[4] (https://www.ncbi.nlm.nih.gov/), for the following species:

Sc: Saccharomyces cerevisiae; Mp: Marchantia polymorpha; Pp: Physcomitrella patens; Sm: Selaginella moellendorffii; Hv: Hordeum vulgare; Os: Oryza sativa ssp japonica; Sb: Sorghum bicolor; Zm: Zea mays; Ta: Triticum aestivum; At: Arabidopsis thaliana; Vv: Vitis vinifera; Sl: Solanum lycopersicum; St: Solanum tuberosum; Mt: Medicago truncatula; Nt: Nicotiana tabacum. The gene and ID number of the analyzed sequences are in Table S1.

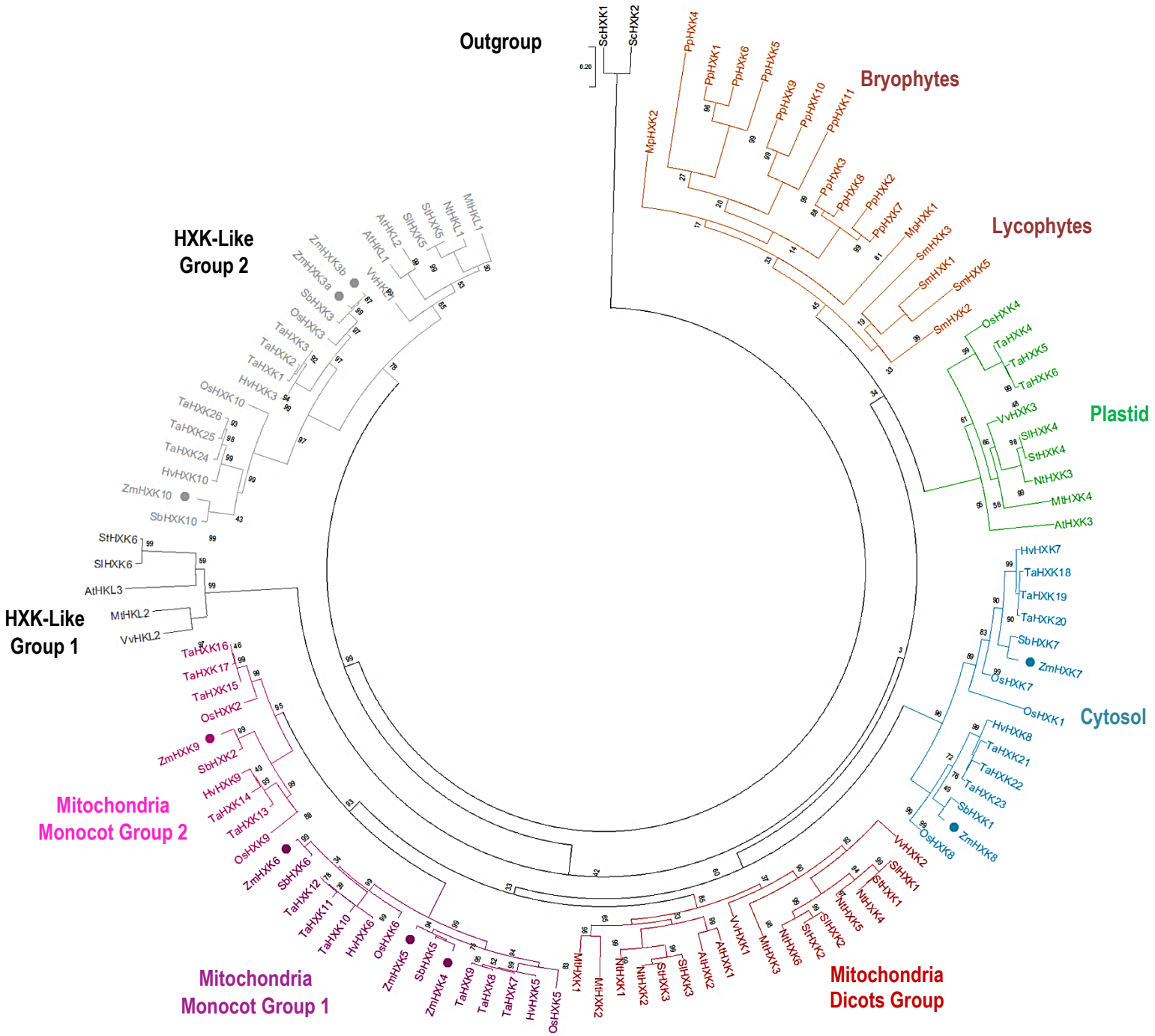

Fig S1. Molecular phylogenetic analysis for plant HXKs.

Table S1. ID numbers of the HXK gene families used for the phylogenetic analysis.

Gene Name	ID number	Gene Name	ID number
Saccharomyces cerevisiae			
ScHXK1	P04806[1]	*ScHXK2*	P04807[1]
Marchantia polymorpha			
MpHXK1	Mapoly0022s0069[2]	*MpHXK2*	Mapoly0056s0008[1]
Physcomitrella patens			
PpHXK1	Pp3c14_6150[2]	*PpHXK2*	Pp3c19_20120[2]
PpHXK3	Pp3c21_19280[2]	*PpHXK4*	Pp3c1_5000[2]
PpHXK5	Pp3c2_11350[2]	*PpHXK6*	Pp3c10_8650[2]
PpHXK7	Pp3c22_9450[2]	*PpHXK8*	Pp3c18_12510[2]
PpHXK9	Pp3c8_18980[2]	*PpHXK10*	Pp3c23_970[2]
PpHXK11	Pp3c15_12840[2]		
Selaginella moellendorffii			
SmHXK1	SMO134G0275[2]	*SmHXK2*	SMO147G0324[2]
SmHXK3	SMO141G0303[2]	*SmHXK5*	SMO230G0110[2]
Hordeum vulgare			
HvHXK3	HVU0038G3254[2]	*HvHXK5*	HVU0036G1776[2]
HvHXK6	HVU0038G0500[2]	*HvHXK7*	HVU0036G2577[2]
HvHXK8	HVU0038G2059[2]	*HvHXK9*	HVU0038G2827[2]
HvHXK10	HVU0036G1944[2]		
Oryza sativa ssp japonica			
OsHXK1	OS07G0446800[3]	*OsHXK2*	XP_015637797[4]
OsHXK3	XP_015621344[4]	*OsHXK4*	XP_015645316[4]
OsHXK5	OS05G0522500[3]	*OsHXK6*	OS01G0742500[3]
OsHXK7	OS05G0187100[3]	*OsHXK8*	OS01G0190400[3]
OsHXK9	XP_015614778[4]	*OsHXK10*	OS05G0375100[3]
Sorghum bicolor			
SbHXK1	Sobic.003G035500[2]	*SbHXK2*	Sobic.003G280400[2]

SbHXK3	Sobic.003G421201[2]	*SbHXK5*	Sobic.009G203500[2]
SbHXK6	Sobic.003G291800[2]	*SbHXK7*	Sobic.009G069800[2]
SbHXK10	Sobic.009G119100[2]		
Zea mays			
ZmHXK3a	GRMZM2G068913[3]	*ZmHXK3b*	GRMZM2G467069[3]
ZmHXK4	GRMZM2G058745[3]	*ZmHXK5*	GRMZM2G432801[3]
ZmHXK6	GRMZM5G856653[3]	*ZmHXK7*	GRMZM2G051806[3]
ZmHXK8	GRMZM2G104081[3]	*ZmHXK9*	GRMZM2G171373[3]
ZmHXK10	GRMZM2G046686[3]		
Triticum aestivum			
TaHXK1	TAE02953G001[2]	*TaHXK2*	TAE54883G001[2]
TaHXK3	TAE52226G001[2]	*TaHXK4*	TAE05512G002[2]
TaHXK5	TAE56610G005[2]	*TaHXK6*	TAE57032G006[2]
TaHXK7	TAE37995G004[2]	*TaHXK8*	TAE47638G004[2]
TaHXK9	TAE40692G004[2]	*TaHXK10*	TAE57619G001[2]
TaHXK11	TAE13066G003[2]	*TaHXK12*	TAE40850G002[2]
TaHXK13	TAE40826G003[2]	*TaHXK14*	TAE19797G001[2]
TaHXK15	TAE41439G002[2]	*TaHXK16*	TAE21658G002[2]
TaHXK17	TAE05962G003[2]	*TaHXK18*	TAE02025G001[2]
TaHXK19	TAE30281G001[2]	*TaHXK20*	TAE51630G001[2]
TaHXK21	TAE08161G001[2]	*TaHXK22*	TAE51140G001[2]
TaHXK23	TAE53374G005[2]	*TaHXK24*	TAE08078G001[2]
TaHXK25	TAE49603G001[2]	*TaHXK26*	TAE51566G002[2]
Arabidopsis thaliana			
AtHXK1	AT4G29130[3]	*AtHXK2*	AT2G19860[3]
AtHXK3	AT1G47840[3]	*AtHKL1*	AT1G50460[3]
AtHKL2	AT3G20040[3]	*AHKL3*	AT4G37840[3]
Vitis vinifera			
VvHXK1	GSVIVG01015297001[2]	*VvHXK2*	GSVIVG01016971001[2]
VvHXK3	GSVIVG01009899001[2]	*VvHKL1*	GSVIVG01031551001[2]

VvHKL2	GSVIVG01002667001[2]		
Solanum lycopersicum			
SlHXK1	Solyc03g121070.2[2]	*SlHXK2*	Solyc06g066440.2[2]
SlHXK3	Solyc12g008510.1[2]	*SlHXK4*	Solyc04g081400.2[2]
SlHXK5	Solyc11g065220.1[2]	*SlHXK6*	Solyc02g091830.2[2]
Solanum tuberosum			
StHXK1	PGSC0003DMG400002525[2]	*StHXK2*	PGSC0003DMG400016521[2]
StHXK3	PGSC0003DMG400000295[2]	*StHXK4*	PGSC0003DMG400009861[2]
StHXK5	PGSC0003DMG400013187[2]	*StHXK6*	PGSC0003DMG400030624[2]
Medicago truncatula			
MtHXK1	Medtr8g102460[2]	*MtHXK2*	Medtr6g088795[2]
MtHXK3	Medtr8g014530[2]	*MtHXK4*	Medtr1g025140[2]
MtHKL1	Medtr5g009000[2]	*MtHKL2*	Medtr4g097900[2]
Nicotiana tabacum			
NtHXK1	AAS60195[1]	*NtHXK2*	AAS60197[1]
NtHXK3	Q6Q8A5[1]	*NtHXK4*	AAT77515[1]
NtHXK5	AAS60192[1]	*NtHXK6*	AAS60194[1]
NtHKL1	AAS60198[1]		

Table S2. Comparison of conserved amino acids at catalytic and substrate binding domains between ZmHXKs with AtHXK1 [32]. The amino acid changes are in bold.

Domain/ Residue	AtHXK1	ZmHXK 3a	ZmHXK 3b	ZmHXK 4	ZmHXK 5	ZmHXK 6	ZmHXK 7	ZmHXK 8	ZmHXK 9	ZmHXK 10
Hydrophobic channel	L 100	**I 100**	**I 100**	L 109	L 109	L 109	L 70	L 72	L 107	**V 102**
	L 102	L 102	L 102	L 111	L 111	L 111	L 72	L 74	L 109	L 104
	L 143	L 141	L 141	L 152	L 152	L 152	L 113	L 115	L 150	L 144
	I 147	**V 145**	**V 145**	I 156	I 156	I 156	I 117	I 119	I 154	I 148
	L 151	L 149	L 149	L 160	L 160	L 160	L 121	L 123	L 158	L 152
	V 155	V 153	V 153	V 164	V 164	V 164	V 125	V 127	**I 162**	**I 156**
	L 172	L 165	L 165	L 181	L 181	L 181	L 138	**I 144**	L 179	L 168
	F 174	F 167	F 167	F 183	F 183	F 183	F 140	F 146	F 181	F 170
	F 176	F 169	F 169	F 185	F 185	F 185	F 141	F 148	F 183	F 172
	F 197	F 190	F 190	F 206	F 206	F 206	F 163	F 169	F 204	F 193
	L 211	L 204	L 204	L 220	L 220	L 220	L 177	L 183	L 218	L 207
	L 215	L 208	L 208	**M 224**	**M 224**	**M 224**	**M 181**	**M 187**	**I 222**	L 211
Catalytic residues	K 195	K 188	K 188	K 204	K 204	K 204	K 161	K 167	K 202	K 191
	D 230	D 223	D 223	D 239	D 239	D 239	D 196	D 202	D 237	**N 226**
Glucose contacts	T 194	T 187	**N 187**	T 203	T 203	T 203	T 160	T 166	T 201	T 190
	K 195	K 188	K 188	K 204	K 204	K 204	K 161	K 167	K 202	K 191
	N 229	N 222	N 222	N 238	N 238	N 238	N 195	N 201	N 235	N 225
	D 230	D 223	D 223	D 239	D 239	D 239	D 196	D 202	D 236	**N 226**
	S 177	S 170	S 170	S 186	S 186	S 186	S 143	S 149	S 184	S 173
	N 256	N 249	N 249	N 265	N 265	N 265	N 222	N 228	N 263	N 252
	E 284	E 277	E 277	E 293	E 293	E 293	E 253	E 256	E 291	E 281
	E 315	E 308	E 308	E 324	E 324	E 324	E 284	E 287	E 322	E 312

ATP interaction	G 104	G 104	G 104	G 113	G 113	G 113	G 74	G 76	G 111	G 106
	T 105	T 105	T 105	T 114	T 114	T 114	T 75	T 77	T 112	T 107
	N 106	N 106	N 106	N 114	N 114	N 114	N 76	N 78	N 113	**S 108**
	S 177	S 170	S 170	S 186	S 186	S 186	S 143	S 149	S 184	S 173
	K 195	K 188	K 188	K 204	K 204	K 204	K 161	K 167	K 202	K 191
	D 230	D 223	D 223	D 239	D 239	D 239	D 196	D 202	D 236	**N 226**
	T 253	**A 246**	**A 246**	T 262	T 262	T 262	T 219	T 225	T 260	**A 249**
	G 254	G 247	G 247	G 263	G 263	G 263	G 220	G 226	G 261	G 250
	D 439	**E 438**	**E 438**	D 450	D 450	D 449	D 406	D 409	D 445	**E 444**
	G 440	G 439	G 439	G 451	G 451	G 450	G 407	G 410	G 446	G 445
	G 441	G 440	G 440	G 452	G 452	G 451	G 408	G 411	G 447	G 446
	S 478	S 477	S 477	S 489	S 489	S 486	S 445	S 448	S 484	S 483
Conserved Glycine	G 91	G 91	G 91	G 100	G 100	G 100	G 61	G 63	G 98	G 93
	G 95	G 95	G 95	G 104	G 104	G 104	G 65	G 67	G 102	G 97
	G 103	G 103	G 103	G 112	G 112	G 112	G 73	G 75	G 110	G 105
	G 173	G 166	G 166	G 182	G 182	G 182	G 139	G 145	G 180	G 169
	G 252	G 245	G 245	G 261	G 261	G 261	G 218	G 224	G 259	G 248
	G 254	G 247	G 247	G 263	G 263	G 263	G 220	G 226	G 261	G 250
	G 310	**N 303**	**N 303**	G 319	G 319	G 319	G 279	G 282	**D 317**	**Y 307**
	G 320	G 313	G 313	G 329	G 329	G 329	G 289	G 292	G 327	G 317
	G 440	G 439	G 439	G 451	G 451	G 450	G 407	G 410	G 446	G 445
	G 479	G 478	G 478	G 490	G 490	G 489	G 446	G 449	G 485	**V 484**

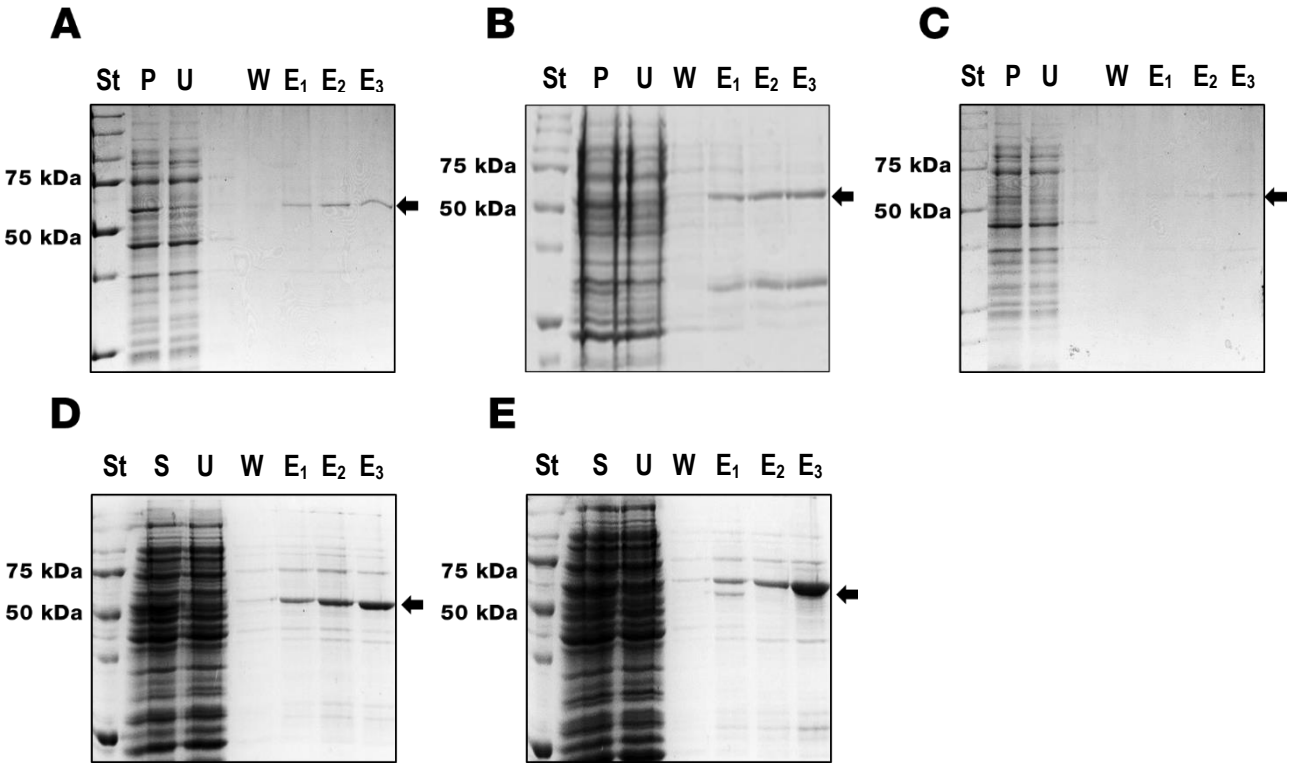

Fig S2. SDS-PAGE of full versions of recombinant ZmHXKs. Purification process of recombinant: **(A)** ZmHXK4, **(B)** ZmHXK5, **(C)** ZmHXK6, **(D)** ZmHXK7, and **(E)** ZmHXK8. St: Molecular weight standard, P: Pellet clarified with urea, S: Soluble supernatant, U: Unbound, W: Wash, E_X: Elution.

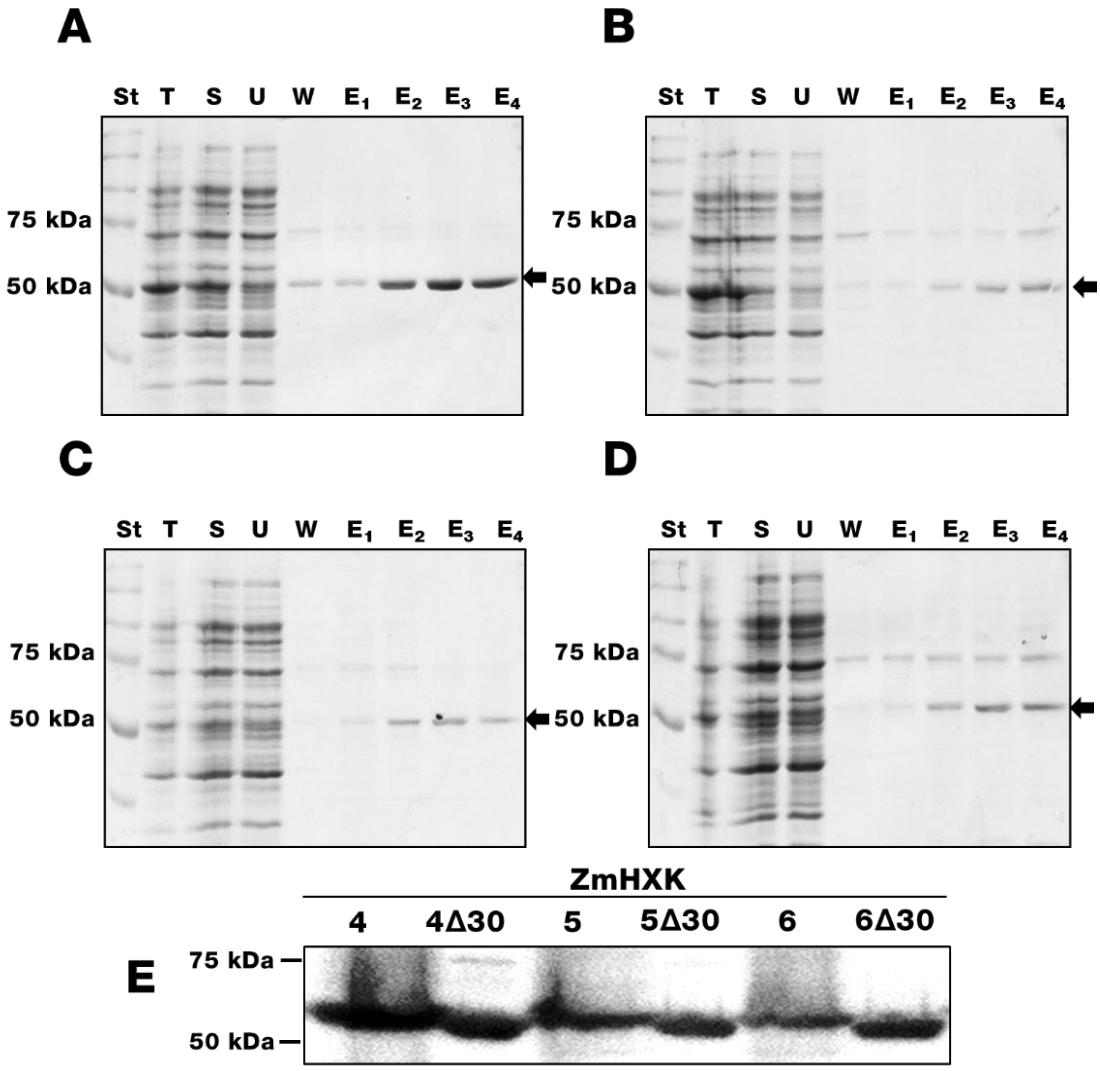

Fig S3. Purification of truncated versions of recombinant ZmHXKs**.** Purification process of recombinant: **(A)** ZmHXK4Δ30, **(B)** ZmHXK5Δ30, **(C)** ZmHXK6Δ30 and **(D)** ZmHXK9Δ30. All fractions were separated by SDS-PAGE and stained with Coomasie blue. **(E)** Western Blot of full and truncated versions of ZmHXK4-6**.** The proteins were detected using anti-V5-HRP. St: Molecular weight standard, T: Total cell extract S: Soluble supernatant, U: Unbound, W: Wash, E_X: Elution.

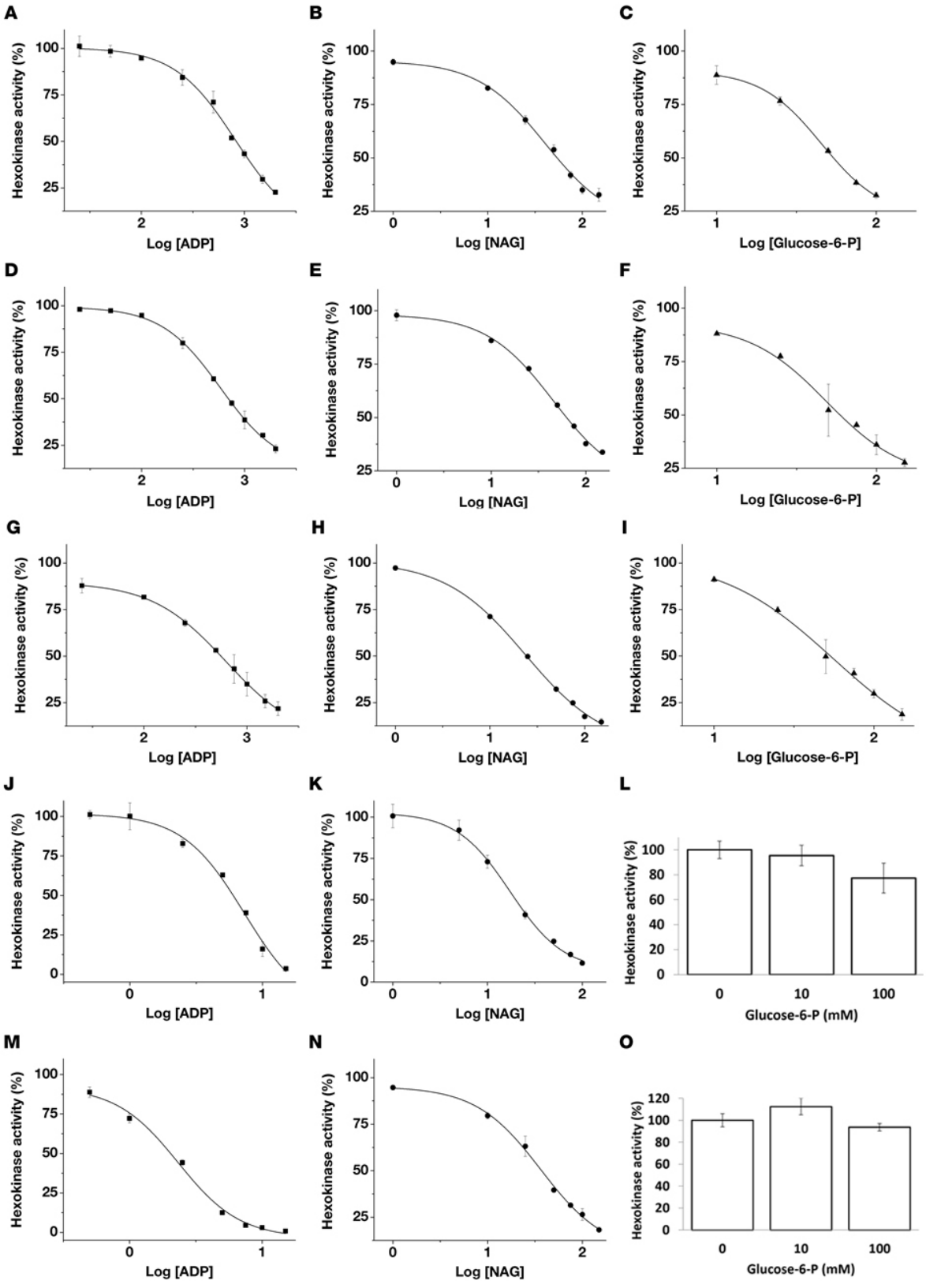

Fig S4. Inhibitory effects of ADP, NAG and G6P on ZmHXKs. (**A, B, C**) ZmHXKΔ4, (**D, E, F**) ZmHXKΔ5, (**G, H, I**) ZmHXKΔ6, (**J, K, L**) ZmHXK7, and (**M, N, O**) ZmHXK8.

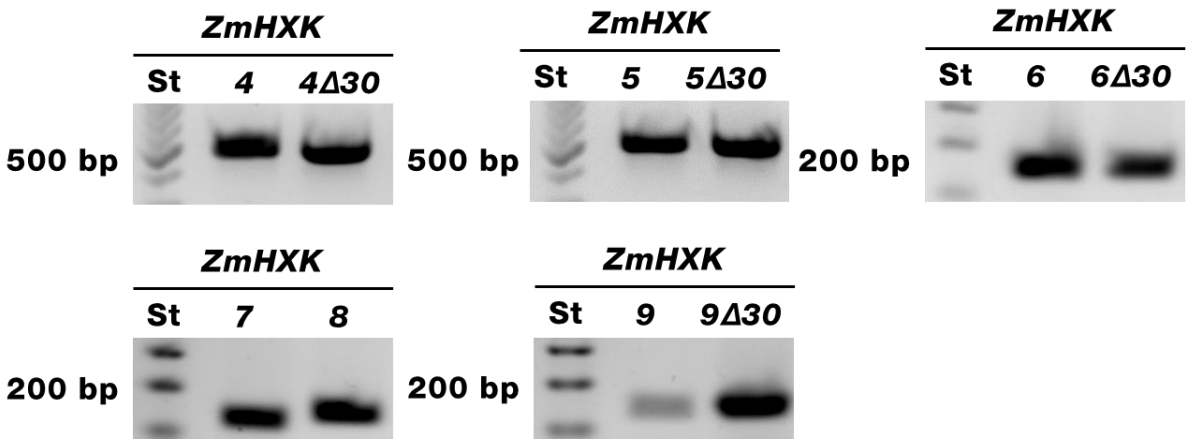

Fig S5. Expression profile of full and truncated versions of ZmHXKs in the JT 20088 yeast mutant. St: Molecular weight standard.

Table S3. Subcellular prediction of ZmHXKs.

Protein	Transmembrane domain	Transit peptide	Subcellular localization
ZmHXK3a	No	5-25	Mitochondrion
ZmHXK3b	No	5-25	Mitochondrion
ZmHXK4	4-24	No	Secretory pathway
ZmHXK5	4-24	No	Secretory pathway
ZmHXK6	9-29	No	Secretory pathway
ZmHXK7	No	No	Other
ZmHXK8	No	No	Chloroplast
ZmHXK9	4-24	No	Secretory pathway
ZmHXK10	6-26, 339-359	No	Secretory pathway

The prediction of transmembrane domains and transit peptide was made with the full amino acid sequence of each HXK with the aid of Toppred 1.10 software. The first 60 amino acids of each HXK protein sequence was used to predict the subcellular localization with the TargetP 1.1 software [32].

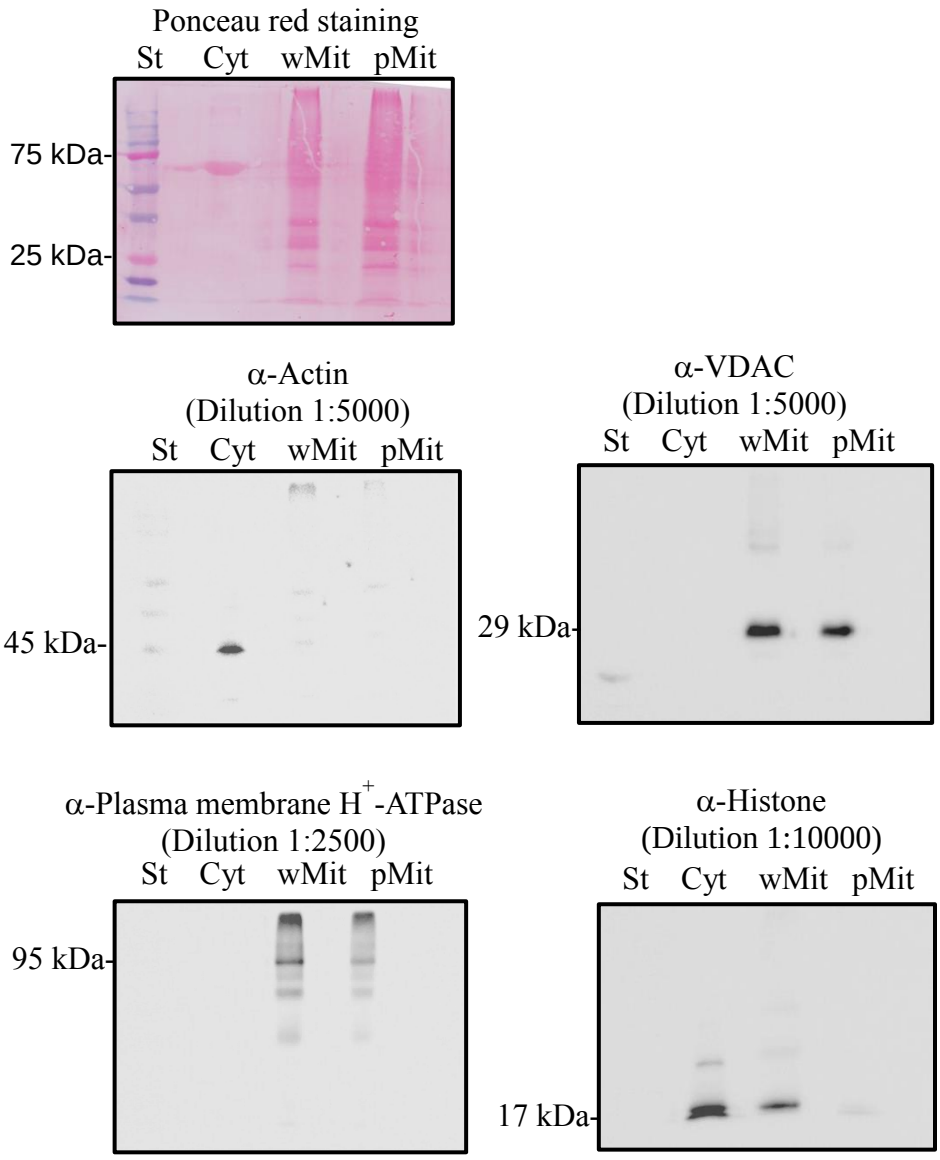

Fig S6. Evaluation of cytosolic and mitochondrial purity using specific antibodies. The purity of the cytosolic (Cyt), mitochondrial washed (wMit) and mitochondrial Percoll purified (pMit) fractions (10 µg) was evaluated by Western blot using Agrisera (Vännäs, Sweden) antibodies. These are representative membranes of at least three replicates.

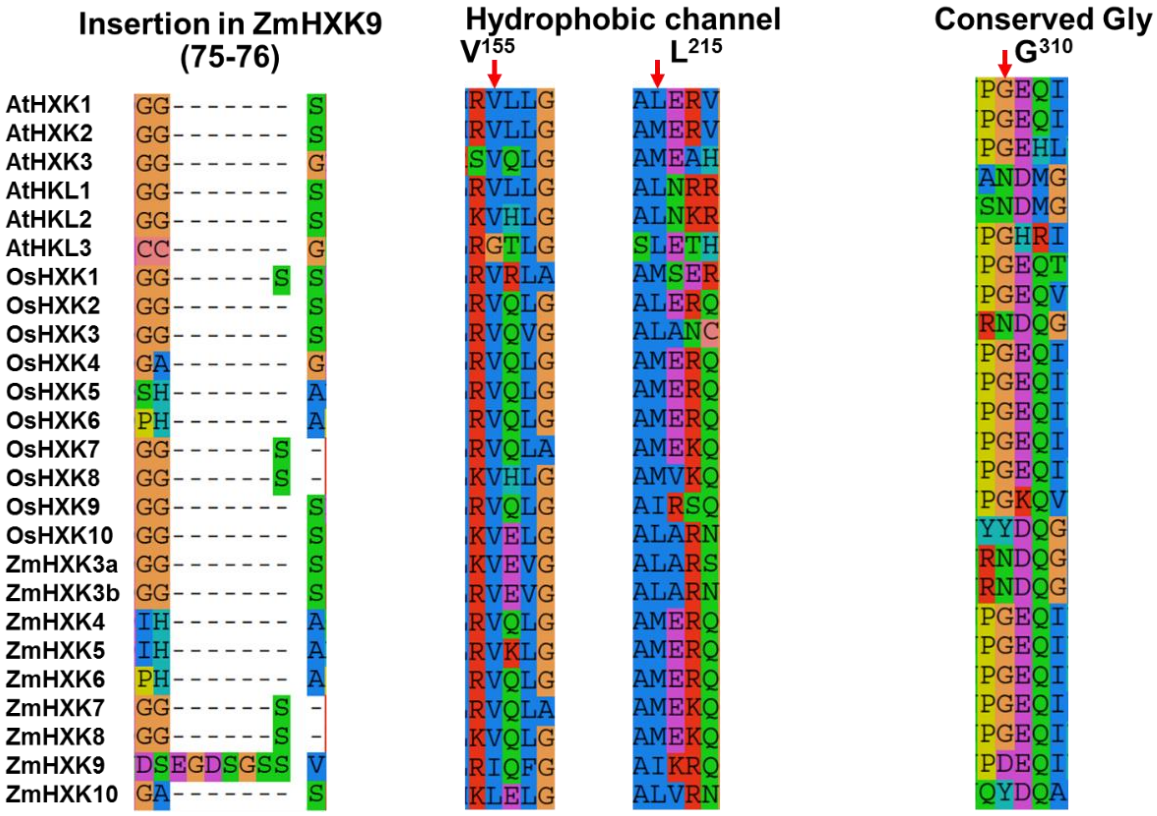

Fig S7. Changes in the amino acids of ZmHXK9 that could explain its low activity. The sequences were aligned using SeaView 4 [32].

Table S4. List of primers used for qPCR analysis, subcloning each maize HXK and PCR analysis in the yeast mutant.

qPCR primers	
ZmHXK4-RT-Fw	GTGATCGAGGAGGTCGAGAG
ZmHXK4-RT-Rv	AACAGCCCATGTTCATCTCC
ZmHXK5-RT-Fw	GTGTGCTGCGAGTCCAACTA
ZmHXK5-RT-Rv	GGAAGGAAAACGTGAAACCA
ZmHXK6-RT-Fw	CATTGCTGCTGAGTTGGAAA
ZmHXK6-RT-Rv	CTTTCCATGGCCCTACTCAA
ZmHXK7-RT-Fw	CTGGTTTCGGGCATGTATCT
ZmHXK7-RT-Rv	GACCACCATCTTCCTCGTGT
ZmHXK8-RT-Fw	TGCTCCTCTCCTACGTCGAT
ZmHXK8-RT-Rv	GCCGACTTCTCTGGAGTCAC
ZmHXK9-RT-Fw	TTCAGTTGCATCTGGCACTC
ZmHXK9-RT-Rv	CATATCTCCCAGCAGCCAAT
Subcloning primers and adapters	
(Bases necessary for recombination and not present in original template are underlined)	
AtHXK1-GW-Fw	AAAAAGCAGGCTTCGAAGGAGATAGAACCATGGGTAAAGTAGCTGTTGGAG
AtHXK1-GW-Rv	AGAAAGCTGGGTGAGAGTCTTCAAGGTAGAGAGAGTG
ZmHXK4-GW-Fw	AAAAAGCAGGCTTCGAAGGAGATAGAACCATGGTGAAGGCCGTGGTGGT
ZmHXK4-GW-Rv	AGAAAGCTGGGTGGTCACTCGCGCCATACTGATACTG
ZmHXK5-GW-Fw	AAAAAGCAGGCTTCGAAGGAGATAGAACCATGGGGAAGTCCGTGGTGGT
ZmHXK5-GW-Rv	AGAAAGCTGGGTGGTCACTCTCGCCATACTGGGAGT
ZmHXK6-GW-Fw	AAAAAGCAGGCTTCGAAGGAGATAGAACCATGGCGAAGGGCGGTGCGGT
ZmHXK6-GW-Rv	AGAAAGCTGGGTGTGCAGCTTCAGCATACTGGGAGTGC
ZmHXK7-GW-Fw	AAAAAGCAGGCTTCGAAGGAGATAGAACCATGGTGGCGGCGGCGGAC
ZmHXK7-GW-Rv	AGAAAGCTGGGTGGTACTGCGAGTGGGCAGCTGCAA

ZmHXK8-GW-Fw	AAAAAGCAGGCTTCGAAGGAGATAGAACCATGGCGGCAGCTGCGCTGG
ZmHXK8-GW-Rv	AGAAAGCTGGGTGCTGAGATTGCGAGGCAGCAATCAGGG
ZmHXK9-GW-Fw	AAAAAGCAGGCTTCGAAGGAGATAGAACCATGCGGAAGCCGGCGGCACT
ZmHXK9-GW-Rv	AGAAAGCTGGGTGATCATCCACGGCCTGGAGACGTTG
ZmHXK4Δ30-GW-Fw	AAAAAGCAGGCTTCGAAGGAGATAGAACCATGGACGCCGACCTCCTGGGG
ZmHXK5Δ30-GW-Fw	AAAAAGCAGGCTTCGAAGGAGATAGAACCATGGACGCCGCGCTCCTGGG
ZmHXK6Δ30-GW-Fw	AAAAAGCAGGCTTCGAAGGAGATAGAACCATGAGGAGGAGTCGGCGGCGG
ZmHXK9Δ30-GW-Fw	AAAAAGCAGGCTTCGAAGGAGATAGAACCATGGCGAGGCGATGGGCACGC

Primers for the PCR analysis in the yeast mutant

		Size (bp)
ZmHXK4-Fw	CACGGTTGGTGAAGATGTTG	480
ZmHXK4-Rv	TGACATATCTGGCGTCCTCA	
ZmHXK5-Fw	CACGGTTGGTGAAGATGTTG	480
ZmHXK5-Rv	TGACATATCTGGCGTCCTCA	
ZmHXK6-RT-Fw	GGGACTCTAATTAAGTGGACCAAAG	147
ZmHXK6-RT-Rv	AGCCAATGTGCCTACAGTATC	
ZmHXK7-RT-Fw	GCTGTTGGTGAGGATGTCG	130
ZmHXK7-RT-Rv	CGTCCTCATCGTTGTACCG	
ZmHXK8-RT-Fw	TCTCCTACGTCGATAAGCTCC	180
ZmHXK8-RT-Rv	GAATGCCGACTTCTCTGGAG	
ZmHXK9-RT-Fw	GAAATTGTCCGAAGGGTCTTG	137
ZmHXK9-RT-Rv	TCAGGCGATGTGTCTTGATG	