

Supplementary Material

Supplementary Table S1. Details of primer pairs STE1_1F_1R and STE1_2F_2R which were applied for amplification of the first and the second exons of the *HvSTE1* gene with the aim of mutations occurrence confirmation.

Primer Name	Primer Sequence	GC%	T _m (°C)
STE1_1F_1R	F: 5' GGCATTTGTGTCACGCTCT 3'	52,6	64,0
	R: 5' CCTCACCAACAAATCGACCT 3'	50,0	63,9
STE1_2F_2R	F: 5' GTTCCCCCTTTCTGTTC 3'	50,0	63,4
	R: 5' CATCCCAGAGGAACAAAGCTA 3'	47,6	63,0

Supplementary Table S2. PCR profiles for the STE1_1F_1R and STE1_2F_2R primers.

	Temperature [°C]		Duration	No. of cycles
	STE1_1F_1R	STE1_2F_2R		
Initial denaturation	95°C	95°C	5 mins	1
Denaturation	95°C	95°C	45 sec	3
Annealing	66°C	61°C	50 sec	
Extension	72°C	72°C	1 min 30 sec	
Denaturation	95°C	95°C	45 sec	3
Annealing	64°C	59°C	50 sec	
Extension	72°C	72°C	1 min 30 sec	
Denaturation	95°C	95°C	45 sec	36
Annealing	62°C	57°C	50 sec	
Extension	72°C	72°C	1 min 30 sec	
Final extension	72°C	72°C	5 mins	1
Pause	15°C	15°C	Pause	1

Supplementary Table S3. Details of primer pairs STE1_B, EF1, and H2A which were used with the aim of *HvSTE1* gene expression analysis.

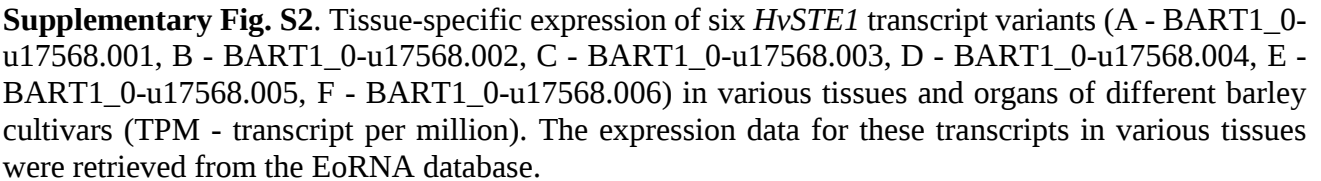
Primer Name	Primer Sequence	GC%	T _m (°C)
STE1_B	F: 5' AGACGGACTGGTACAACGAGA 3'	52	57
	R: 5' AGATGACGAAGCACCAGAGG 3'	55	57
EF1	F: 5' CCCTCCTCTTGGTCGTTTTG 3'	55	58
	R: 5' ATGACACCAACAGCCACAGTTT 3'	45	58
H2A	F: 5' AGCGTTTAGCTGTGCTCCTTCC 3'	54	58
	R: 5' TGA CTCAATCGGTACCAGGAAAC 3'	50	58

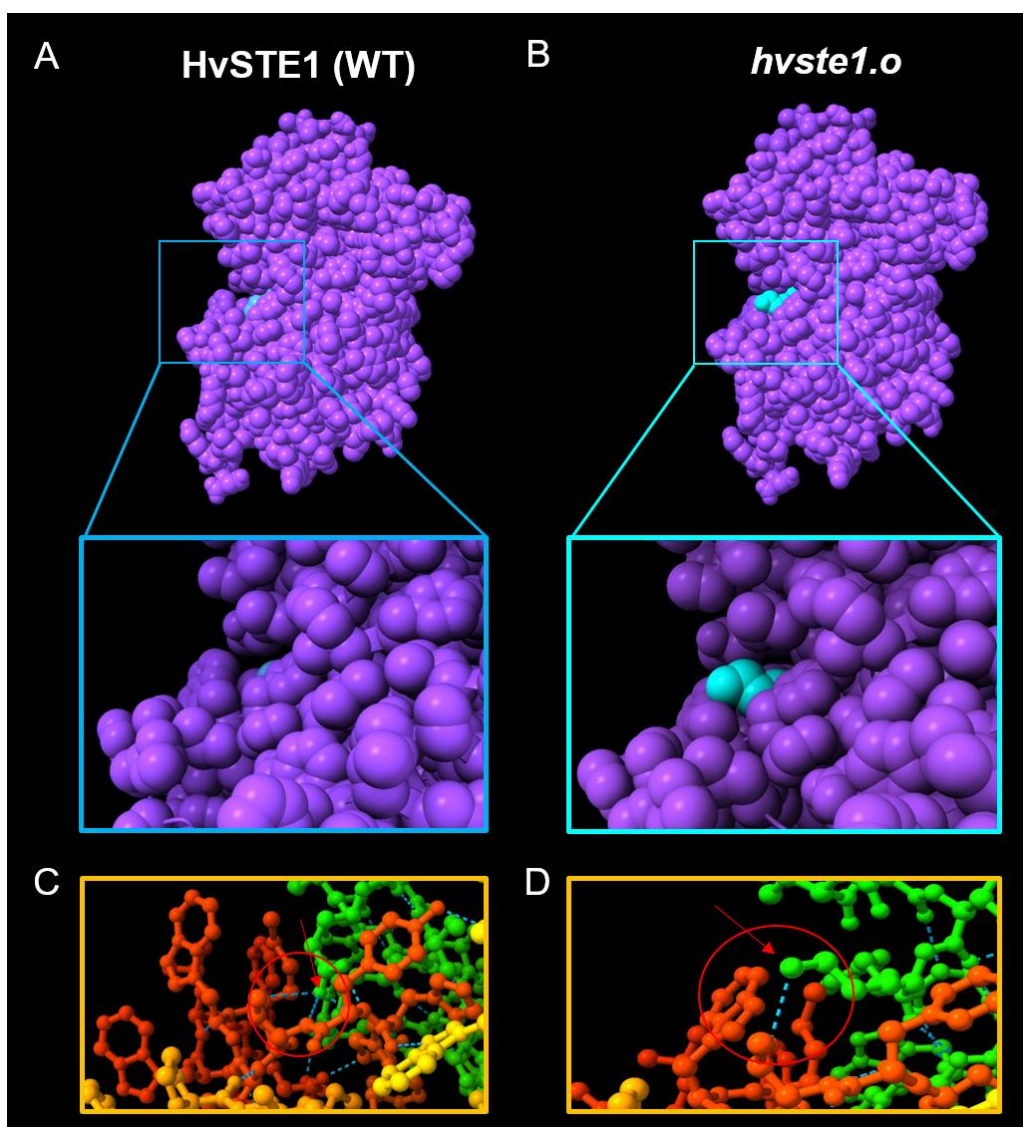
Supplementary Table S4. RT-PCR profile for the STE1_B, EF1, and H2A primer pair.

	Temperature [°C]	Duration	No. of cycles
Initial denaturation	95°C	3 mins	1
Denaturation	95°C	45 sec	4
Annealing	63°C	45 sec	
Extension	72°C	1 min	
Denaturation	95°C	45 sec	4
Annealing	61°C	45 sec	
Extension	72°C	1 min	
Denaturation	95°C	45 sec	36
Annealing	57/58°C	45 sec	
Extension	72°C	1 min	
Final extension	72°C	5 mins	1
Pause	8°C	Pause	1

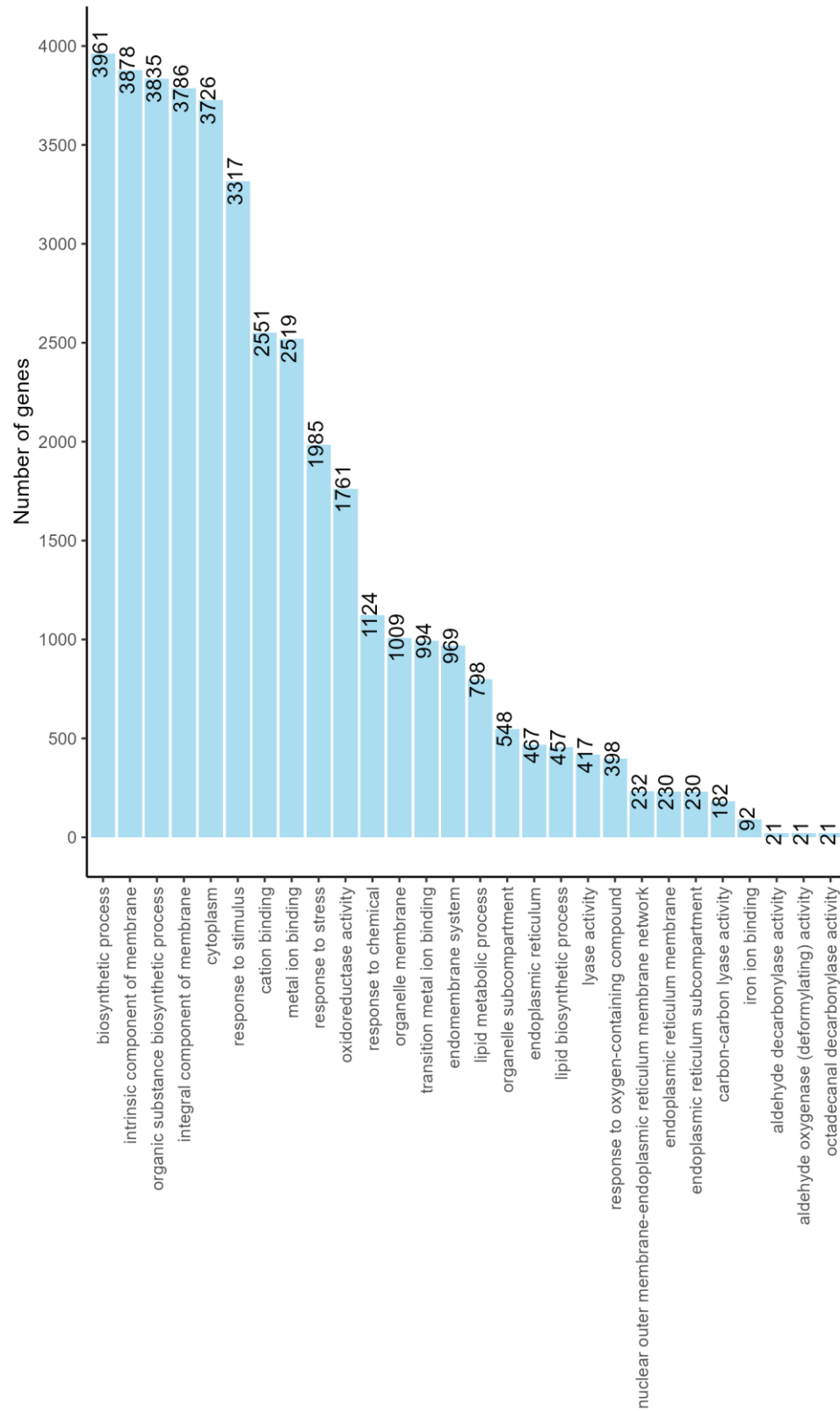
AratSTE	-----MAADNAYLMQFVDETSFYNRIVLSHLLPA--NLWEPLPHFLQTWLRNYLAGTL	51
SorbSTE	---MAAAAHGGDYLRRFVAETEWYNAVVL SAVAPG--DWRGLPHPVQSWMRNCVGGYL	54
ZeamSTE	-----MAVHGGDYLRRFVAETEWYNEVVL SAVAPG--DWRGLPHPVQSWMRNCVGGYL	52
HorvSTE	---MAAAAAAGEDYWGLFREETDWEYNEIFLSAVVPGGGGWRRALPHPLRSWLRNCIGGYL	57
TriaSTE	---MAAAAAAGQDYWGLFREETDWEYNEIFLSAVVPGGGGWRRALPHPLRSWLRNCIGGYL	57
AegtSTE	---MAAAAAAGQDYWGLFREETDWEYNEIFLSAVVPGGGGWRRALPHPLRSWLRNCIGGYL	57
BradSTE	MAAAAHGGGGGAEYSVLFREETGWYNEIFLSAVVPG--DWRALPHPLQSWLRNGVGAYL	58
OrysSTE	-----MAGGGGEYLRQFVEETAWEYNEIFLSHVVPG--DWRALPHPLQSWLRNGLGGYL	52
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AratSTE	LYFISGFLWC FYIYYL KINVYLPKDAIPTIKAMRLQMFVAMKAMPWYTL LPTVSESMIER	111
SorbSTE	LYFISGFLWC FVIYYWKRHAYIPKDAIPTNEAMKKQIVVASKAMPFYCALPTLSEYMIES	114
ZeamSTE	LYFISGFLWC FVIYYWKRHAYIPKDAIPTNEAMKKQIIVASKAMPFYCALPTLSEYMIES	112
HorvSTE	LYFATGFLWC FVIYYWKRNAYIPKDAVPTVEAMKKQIIVASKAMPFYCALPSVSEHMIES	117
TriaSTE	LYFATGFLWC FVIYYWKRNAYIPKDAVPTVEAMKKQIIVASKAMPFYCALPSVSEHMIES	117
AegtSTE	LYFATGFLWC FVIYYWKRNAYIPKDAVPTVEAMKKQIIVASKAMPFYCALPSVSEHMIES	117
BradSTE	IYFLTGFLWC FVIYYWKRHAYIPKDSIPTLEAMKKQIIVASKAMPFYCALPTISEYMIES	118
OrysSTE	IYFACGFLWC FVIYYWKRHAYIPKDSIPTLEAMKKQIIVASKAMPFYCALPTLSEYMVEN	112
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AratSTE	GWTKCFASIDFGWILYFVYIAIYLVFVFGIYWMHRELHDIKPLYKYLHATHHIYNKQN	171
SorbSTE	GWTQCYFNISEVGFSMYLCYMYLIFVFGIYWMHRELHDIKPLYKYLHATHHIYNKEN	174
ZeamSTE	GWTRCYFNISEMGFSAYLCYMYLIFVFGIYWMHRELHDIKPLYKYLHATHHIYNKEN	172
HorvSTE	GWTRCFHFHISEVGWPMYFVYVALYLT FVFGIYWMHRELHDIKPLYKYLHATHHIYNKEN	177
TriaSTE	GWTRCFHFHISEVGWPMYIIVSLYLIFVFGIYWMHRELHDIKPLYKYLHATHHIYNKEN	177
AegtSTE	GWTRCFHFHISEVGWPMYIIVSLYLIFVFGIYWMHRELHDIKPLYKYLHATHHIYNKEN	177
BradSTE	GWTRCFHFHISEVGWPMYLVYVALYLT FVFGIYWMHRELHDIKPLYKYLHATHHIYNKEN	178
OrysSTE	GWTQCYFNISEVGWPMYLVYVALYLT FVFGIYWMHRELHDIKPLYKYLHTYHHIYNKEN	172
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AratSTE	TLSPFAGLAFHPVDGILQAVPHVIALFIVPIHFTTHIGLLFMEAIWTANIHDCHIHNKIWP	231
SorbSTE	TLSPFAGLAFHPLDGILQAIPHVFALFLPPTHFRTHIALLFLEAVWTTNIHDCHIHKIWP	234
ZeamSTE	TLSPFAGLAFHPLDGILQAIPHVALFLLPPTHFRTHIALVFLGVTNTNIHDCHIHKVWP	232
HorvSTE	TLSPFAGLAFHPLDGILQAISHVIALFILPVHFRTHVALFIEAVWTANIHDCHIHKVWP	237
TriaSTE	TLSPFAGLAFHPLDGILQAISHVIALFLLPMHFRTHIALLFIEAVWTANIHDCHIHKIWP	237
AegtSTE	TLSPFAGLAFHPLDGILQAISHVIALFLLPMHFRTHIALLFIEAVWTANIHDCHIHKIWP	237
BradSTE	TLSPFAGLAFHPLDGILQAIPHVFALFLPPTHFRTHIALLFLEAVWTTNIHDCHIHKIWP	238
OrysSTE	TLSPFAGLAFHPLDGILQAIPHVFALYLIPPTHFRTHIALLFIEAVWTTNIHDCHIHKVWP	232
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AratSTE	VMGAGYHTIHHTTYKHNYGHYTIWMDWMFGSLRDP LLEEDDNKDSFKKAE	281
SorbSTE	VMGAGYHTIHHTTYRHNYGHYTIWMDWMFSTLREPEDILKKD-----	276
ZeamSTE	VMGAGYHTIHHTTYRHNYGHYTIWMDWMFGTLREPDDILKKA-----	274
HorvSTE	VMGAGYHTIHHTTYRHNYGHYTIWMDWLFGT LREPEDLLKKD-----	279
TriaSTE	VMGAGYHTIHHTTYRHNYGHYTIWMDWLFGT LREPEDLLKKD-----	279
AegtSTE	VMGAGYHTIHHTTYRHNYGHYTIWMDWLFGT LREPEDLLKKD-----	279
BradSTE	VMGAGYHTIHHTTYRHNYGHYTIWMDWLFGT LREPEDIFKKD-----	280
OrysSTE	VMGAGYHTIHHTTYRHNYGHYTIWMDWMFGTLREPEDILKKD-----	274
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Supplementary Fig. S1. The multiple sequence alignment (MSA) of the STE1 proteins with the use of Clustal Omega tool. The position of substituted amino acid (E146K, allele *hvste1.o*) as a result of the identified mutation is indicated by the red frame. Arat - *Arabidopsis thaliana*, Sorb - *Sorghum bicolor*, Zeam - *Zea mays*, Horv - *Hordeum vulgare*, Tria - *Triticum aestivum*, Aegt - *Aegilops tauschii*, Brad - *Brachypodium distachyon*, Orys - *Oryza sativa*.





Supplementary Fig. S3. Visualization of the 3D structures and surfaces of HvSTE1 (A) and the mutated version of this protein (B) predicted based on the AlphaFold server and UCSF ChimeraX. The localization of the 146 position in each of the structures is indicated by the blue color. (C-D) The localization of the E146 position (C) and E146K substitution (D) (displayed by the red arrows) with atoms in sphere style and rainbow (N to C-terminus) coloring. The changes in hydrogen-bond (blue dashed lines) pattern is indicated by the red oval.



Supplementary Fig. S4. *HvSTE1* gene ontology annotation. The number of genes that take part in each of the biological process is indicated above the bars.