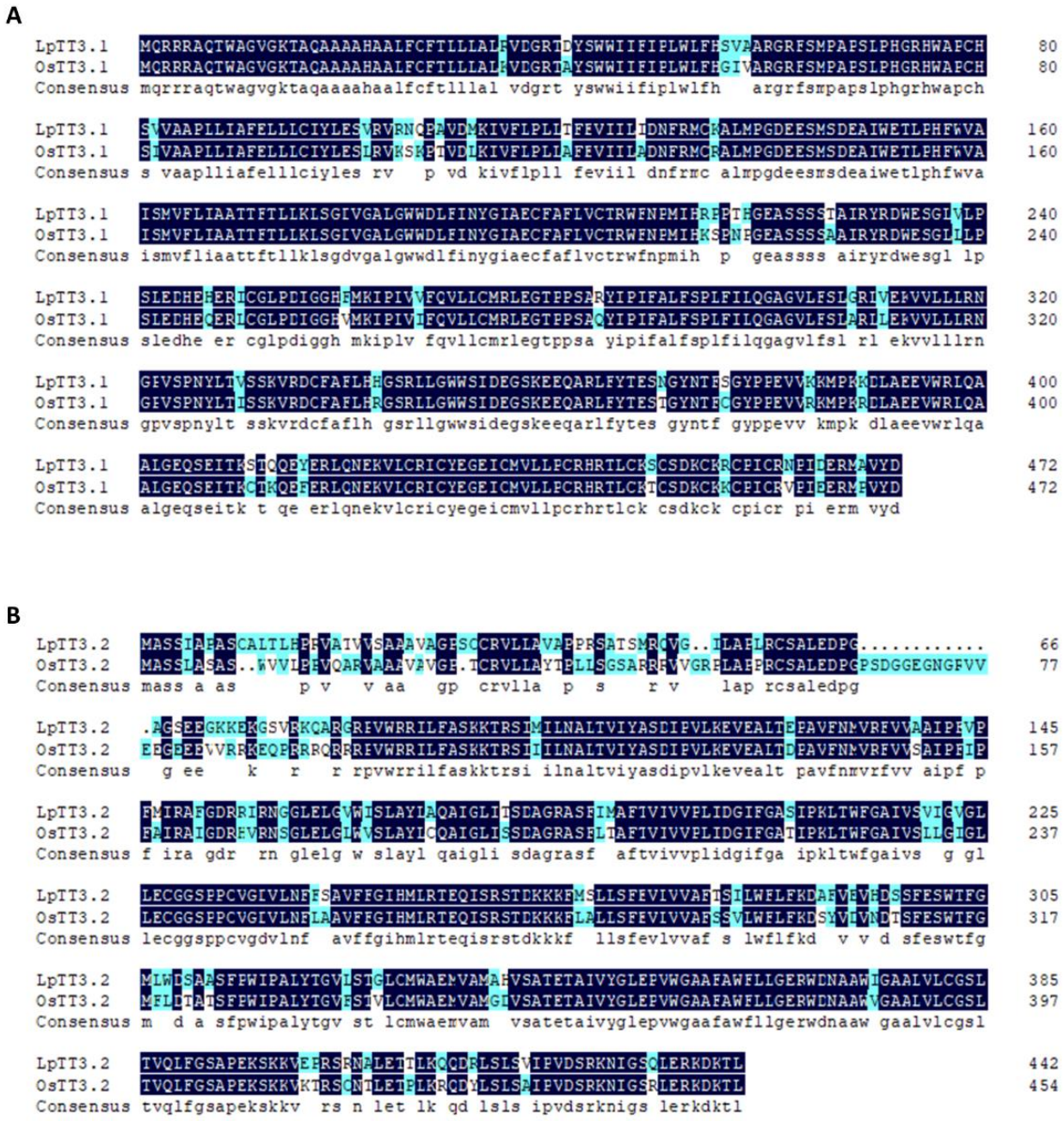
Supplemental Table 1 Ka/Ks analysis of *OsTT3.1*/*LpTT3.1* and *OsTT3.2*/*LpTT3.2*

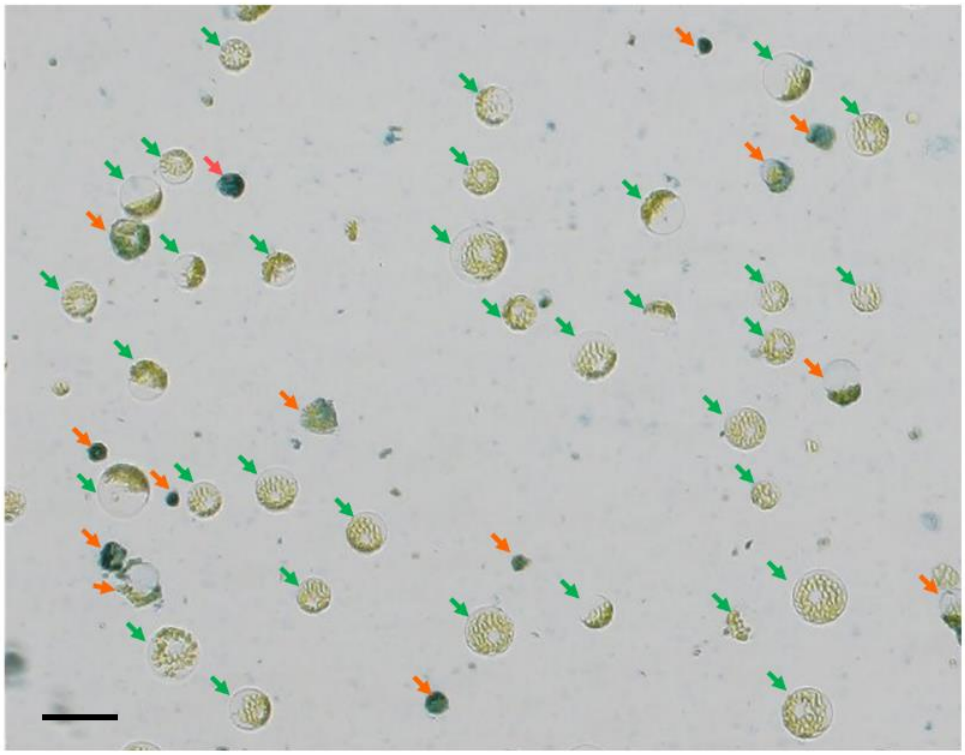
Gene1	Gene2	Ka	Ks	Ka/Ks	Evolutionary selection
LpTT3.1	*OsTT3.1*	0.046431568	0.50551727	0.091849618	Purifying
LpTT3.2	*OsTT3.2*	0.119958113	0.785088532	0.152795651	Purifying

Supplemental Table 2 Primers used in PCR amplification of *OsTT3.1*/*LpTT3.1* and *OsTT3.2*/*LpTT3.2*

Primer name	Sequence (5′–3′)
LpTT3.1 forward	ATGCAGCGGCGGCGGG
LpTT3.1 reverse	AACATCATATACGGGCATGCGTTC
LpTT3.2 forward	ATGGCTTCCTCCATCGC
LpTT3.2 reverse	CAATGTTTTATCCTTCCTCTCTA



Supplemental Figure 1 Sequence alignments of *OsTT3.1*/*LpTT3.1* (A) and *OsTT3.2*/*LpTT3.2* (B)



Supplemental Figure 2 A representative photo taken for protoplasts' viability observation after heat stress. The protoplasts were stained with Evans blue for 1 min and then observed under a light microscope. The 'green' arrows mark the viable cells with intact round shapes, while those in 'red' mark the dead or broken protoplasts with dark blue staining. The black bar in the photo represents 36 μm.

Supplemental data 1 Enzymatic hydrolysate (20 mL)

Solution name	Dosage	Method
0.6M D-Mannitol	2.186 g	metage
10mM MES·H2O	take 1 mL of 0.2M MES·H2O	0.2M MES·H2O mother solution
Cellulase	0.3 g	metage
Macerozyme	0.15 g	metage

Supplemental data 2 MES·H_2O (100 mL)

Solution name	Dosage	Method
0.2M MES·H_2O	4.265 g	weigh 4.265 g MES·H_2O，deionized water fixed volume 100mL，with KOH to adjust pH to 5.7

Supplemental data 3 0.1% BSA and 3.4 mM $CaCl_2$ (100 mL)

Solution name	Dosage	Method
0.1% BSA	0.02 g	metage
3.4mM $CaCl_2$	take 34 μL of 2 M $CaCl_2$	2M $CaCl_2$：weigh 29.345 g $CaCl_2·2H_2O$, deionized water volume to 100mL

Supplemental data 4 W5 (200 mL)

Solution name	Dosage	Method
154mM NaCl Solution	take 15.4mL of 2M NaCl	2M NaCl：weigh 11.7gNaCl，deionized water volume to 100mL
2mM MES·H_2O Solution	take 1mL of 0.2M MES·H_2O	0.2M MES·H_2O：weigh 4.265 g MES·H_2O，deionized water fixed volume 100mL，with KOH to adjust pH to 5.7
125mM $CaCl_2$ Solution	take 12.5mL of 2M $CaCl_2$	2M $CaCl_2$：weigh 29.345 g $CaCl_2·2H_2O$，deionized water volume to 100mL
5mM KCl Solution	take 1mL of 1M KCl	1M KCl：weigh 0.7455g KCl，deionized water volume to 100mL
Deionized water volume to 200mL and filter sterilized.		

Supplemental data 5 MMG (20mL)

Solution name	Dosage	Method
0.6M D-Mannitol	2.186 g	metage
4mM MES·H_2O	take 400μL of 0.2M MES·H_2O	0.2M MES·H_2O solution
15 mM $MgCl_2$ Solution	take 300μL of 1M $MgCl_2$	1M $MgCl_2$：weigh 2.033g $MgCl_2$，deionized water volume to 100mL
Deionized water volume to 20 mL and filter sterilized.		

Supplemental data 6 PEG-4000 Solution (20mL)

Solution name	Dosage	Method
0.6 M D-Mannitol	2.186 g	metage
PEG 4000	8g	metage
Deionized water volume to 20mL**.**Cool to room temperature after bathing at 42°C for 30min.		