

A class I cytosolic HSP20 of rice enhances heat and salt tolerance in different organisms

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Running title: Chaperone activity of OsHSP20

Supplementary materials

Table S1 Primers used in plasmid construction

Primers	Sequences (5'-3')	Restriction sites ¹
YOs20-NF	GGAATTCCATATGTCGCTGATCCGCCGC	<i>NdeI</i>
YOs20-NR	CGCGGATCCCTACTTGAACACGTGCGCCTC	<i>BamHI</i>
Y-Os20-ACDF	GGAATTCCATATGTCCTCCGAGACCGCGGCC	<i>NdeI</i>
Y-Os20-ACDR	CGCGGATCCCTAGACGTCGGGCTTCTTGGC	<i>BamHI</i>
YOs20-CF	GGAATTCCATATGCAGGAGGAGAAGACGGAC	<i>NdeI</i>
Y-Os20-CR	CGCGGATCCCTAGCCGGAGATCTGGATGGA	<i>BamHI</i>
PE-Os20-NF	CGAGCTCATGTCGCTGATCCGCCGC	<i>SacI</i>
PEOs20-NR	AAGGAAAAAAGCGGCCGCCTACTTGAACACGTGCGCCTC	<i>NotI</i>
PEOs20-ACDF	CGAGCTCATGTCCTCCGAGACCGCGGCC	<i>SacI</i>
PEOs20-ACDR	AAGGAAAAAAGCGGCCGCCTAGACGTCGGGCTTCTTGGC	<i>NotI</i>
PEOs20-CF	CGAGCTCATGCAGGAGGAGAAGACGGAC	<i>SacI</i>
PEOs20-CR	AAGGAAAAAAGCGGCCGCCTAGCCGGAGATCTGGATGGA	<i>NotI</i>
Trs20-F	CGGAGCTCATGTCGCTGATCCGCCGCAG	<i>SacI</i>
Trs20-R	CGCGGATCCCTAGCCGGAGATCTGGATGGAC	<i>BamHI</i>

¹, underlined

Figure S1 Characterization of recombinant OsHSP20 in *E. coli*. (A) Heterologous expression of the intact OsHSP20 and its truncated proteins in *E. coli* assessed by SDS-PAGE analysis and Coomassie brilliant blue staining. Line M, protein molecular weight marker; Lane 1: whole cell lysate of non-induced cells containing the empty vector pET32a(+); Lane 2: whole cell lysate of cells containing the empty vector induced with 1 mM IPTG; Lane 3: whole cell lysate of cells containing pET32a-OsHSP20^N induced with 1 mM IPTG; Lane 4: whole cell lysate of cells containing pET32a-OsHSP20^{ACD} induced with 1 mM IPTG; Lane 5: whole cell lysate of cells containing pET32a-OsHSP20^C induced with 1 mM IPTG; Lane 6: whole cell lysate of cells containing pET32a-OsHSP20^{N+ACD} induced with 1 mM IPTG. Lane 7: whole cell lysate of cells containing pET32a-OsHSP20^{ACD+C} induced with 1 mM IPTG. Lane 8: whole cell lysate of cells containing pET32a-OsHSP20^{Full} induced with 1 mM IPTG. The induced proteins are indicated by black arrows. (B) Western blot analysis of purified recombinant OsHSP20 proteins using anti-His-tag monoclonal antibody. Antibodies were used in dilutions of 1:5000. Lane M, protein molecular weight marker; Lane 1, purified recombinant His-OsHSP20^N protein; Lane 2, purified recombinant His-OsHSP20^{ACD} protein; Lane 3, purified recombinant His-OsHSP20^C protein; Lane 4, purified recombinant His-OsHSP20^{N+ACD} protein; Lane 5, purified recombinant His-OsHSP20^{ACD+C} protein; Lane 6, purified recombinant His-OsHSP20^{Full} protein. The specific mass (in kDa) of the molecular weight markers is indicated on the left.

Figure S2 Cell viability of *E. coli* BL21 containing the empty vector pET32a(+) and transformants grown on LB medium after treatment with heat (50 °C) (A) or salt (800 mM NaCl) (B) stresses for different times.

Figure S3 Western blot analysis with an anti-HSP20 polyclonal antibody demonstrating the presence of the recombinant protein in the *P. pastoris* strain SMD1168 cells. Proteins were extracted from *P. pastoris* cell cultures that were cultivated in buffered methanol complex medium until the optical density at 600 nm (OD₆₀₀) reached 1.4. Antibodies were used in dilutions of 1:5000. M, protein molecular weight marker - the specific mass (in kDa) of the molecular weight marker is indicated on the left; pPIC3.5K, SMD1168 cells carrying empty vector; N, SMD1168 cells carrying vector pPIC3.5K-OsHSP20^N; ACD, SMD1168 cells carrying vector pPIC3.5K-OsHSP20^{ACD}; C, SMD1168 cells carrying vector pPIC3.5K-OsHSP20^C; N+ACD, SMD1168 cells carrying vector pPIC3.5K-OsHSP20^{N+ACD}; ACD+C, SMD1168 cells carrying vector pPIC3.5K-OsHSP20^{ACD+C}; Full, SMD1168 cells carrying vector pPIC3.5K-OsHSP20^{Full}.

Figure S4 qRT-PCR and western blot analysis of transgenic OsHSP20 rice lines. (A) qRT-PCR analysis. Total RNA was extracted from leaves of 4-week-old WT and T2 generation transgenic OsHSP20 rice. The *OsUBCE2* (LOC_Os02g42314) gene was used as an internal control. (B) Western blot analysis using an anti-HSP20 polyclonal antibody. Proteins were extracted from leaves of 4-week-old WT and T2 generation transgenic OsHSP20 rice. Western blot analysis using anti-Actin monoclonal antibody was used as an internal control. Antibodies were used in dilutions of 1:5000. WT, wild-type; Ubi:OsHSP20-L5, -L16 and -L20, three transgenic rice lines.

Figure S5 Integral blots of Figure 1E for detection of OsHSP20 in rice seedlings following heat stress (45 °C), in which the exposure time is 20 s for OsHSP20 protein (A) and 30 s for Actin (B).

Figure S6 Integral blots of Figure 1F for detection of OsHSP20 in rice seedlings following salinity stress (50 mM NaCl), in which the exposure time is 150 s for OsHSP20 protein (A) and 30 s for Actin (B).

Figure S7 Integral blots of Figure S4B for detection of OsHSP20 in transgenic rice plants, in which the exposure time is 30 s for both OsHSP20 protein (A) and Actin (B).

Figure S1

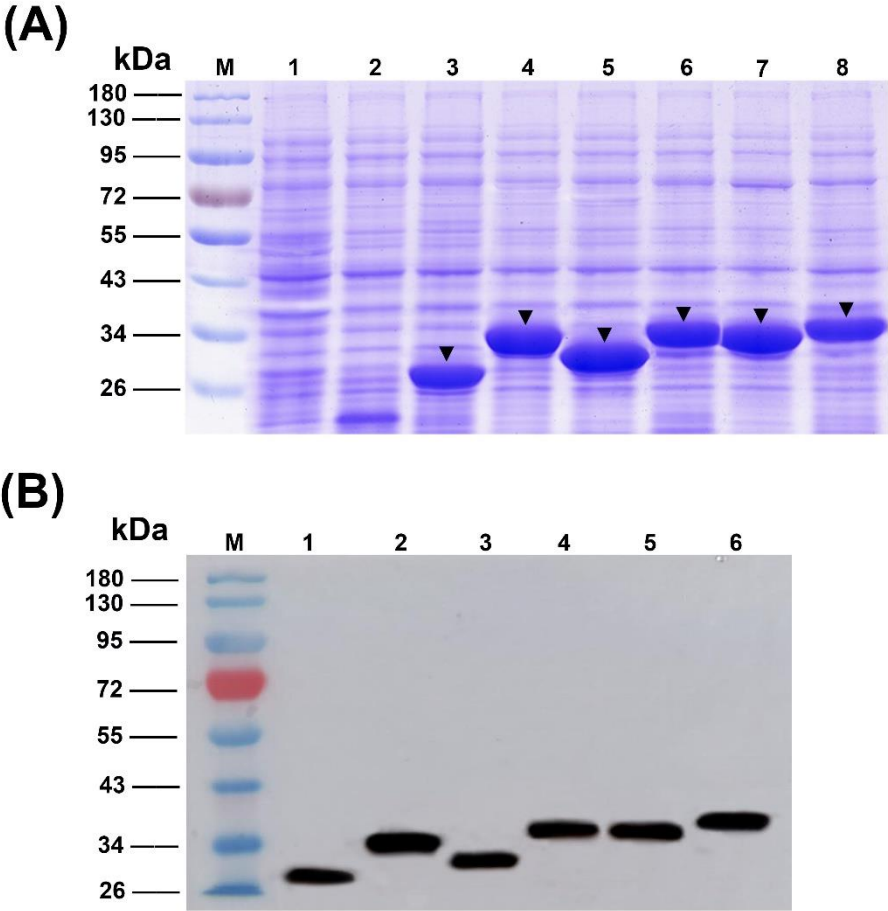


Figure S2

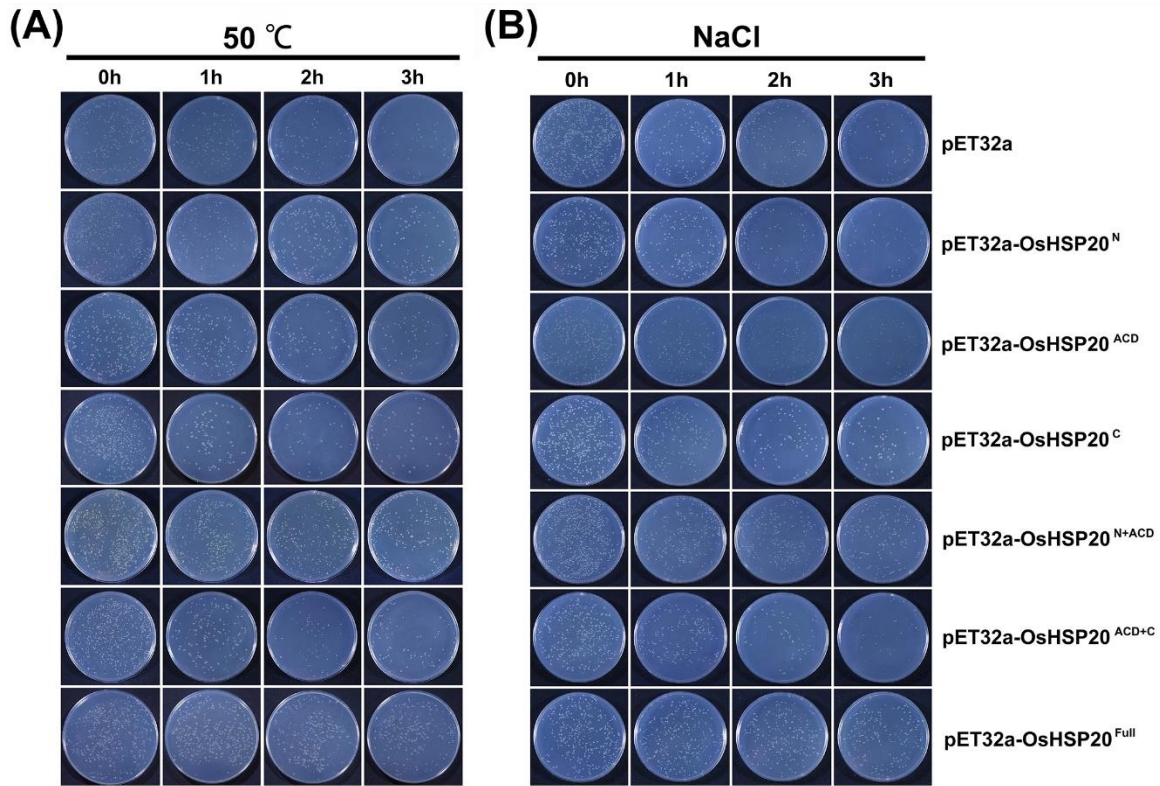


Figure S3

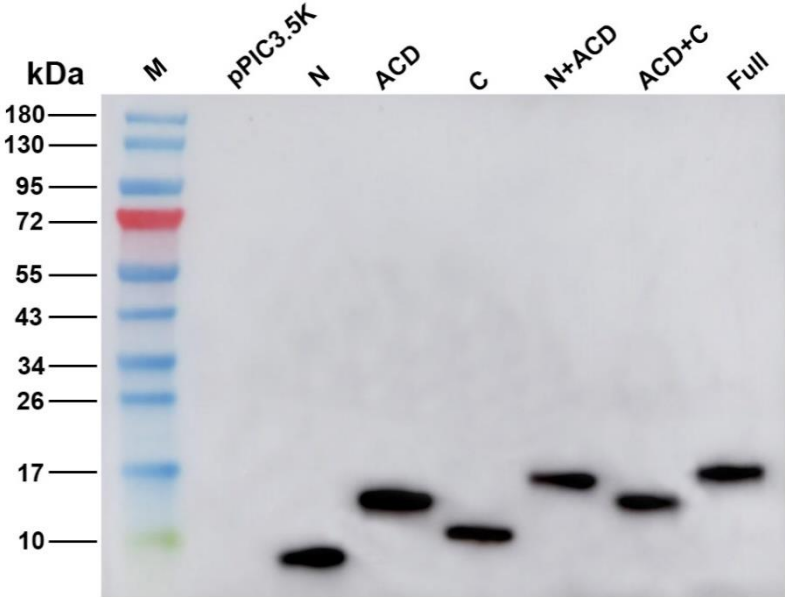
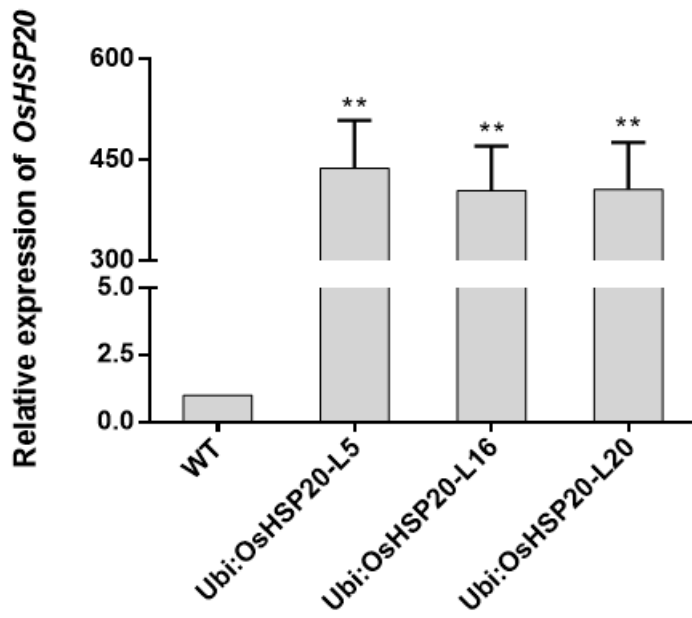


Figure S4

(A)



(B)

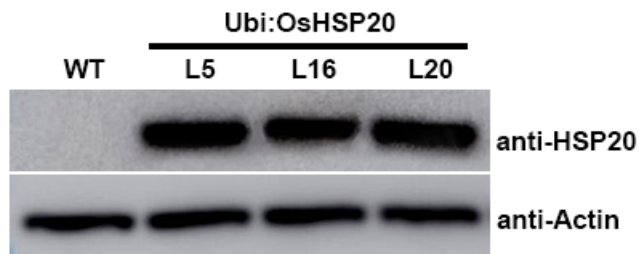


Figure S5

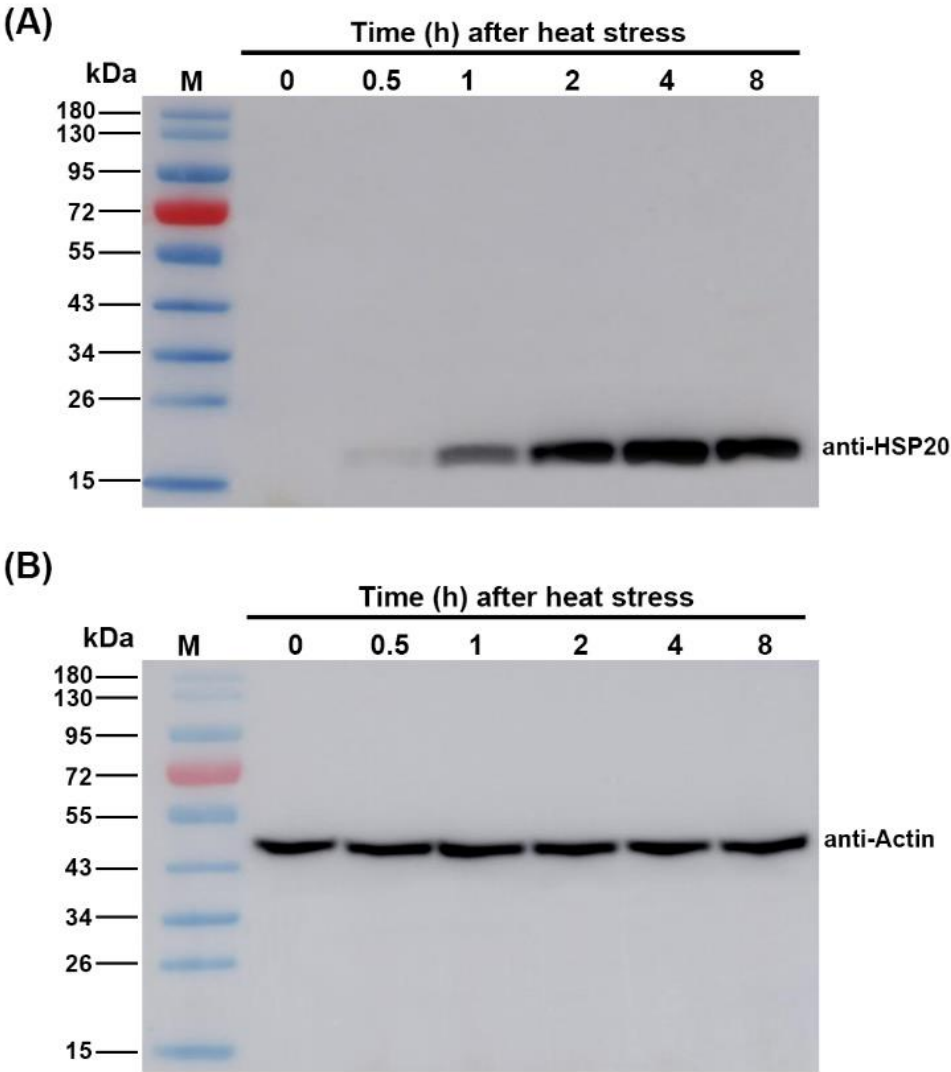


Figure S6

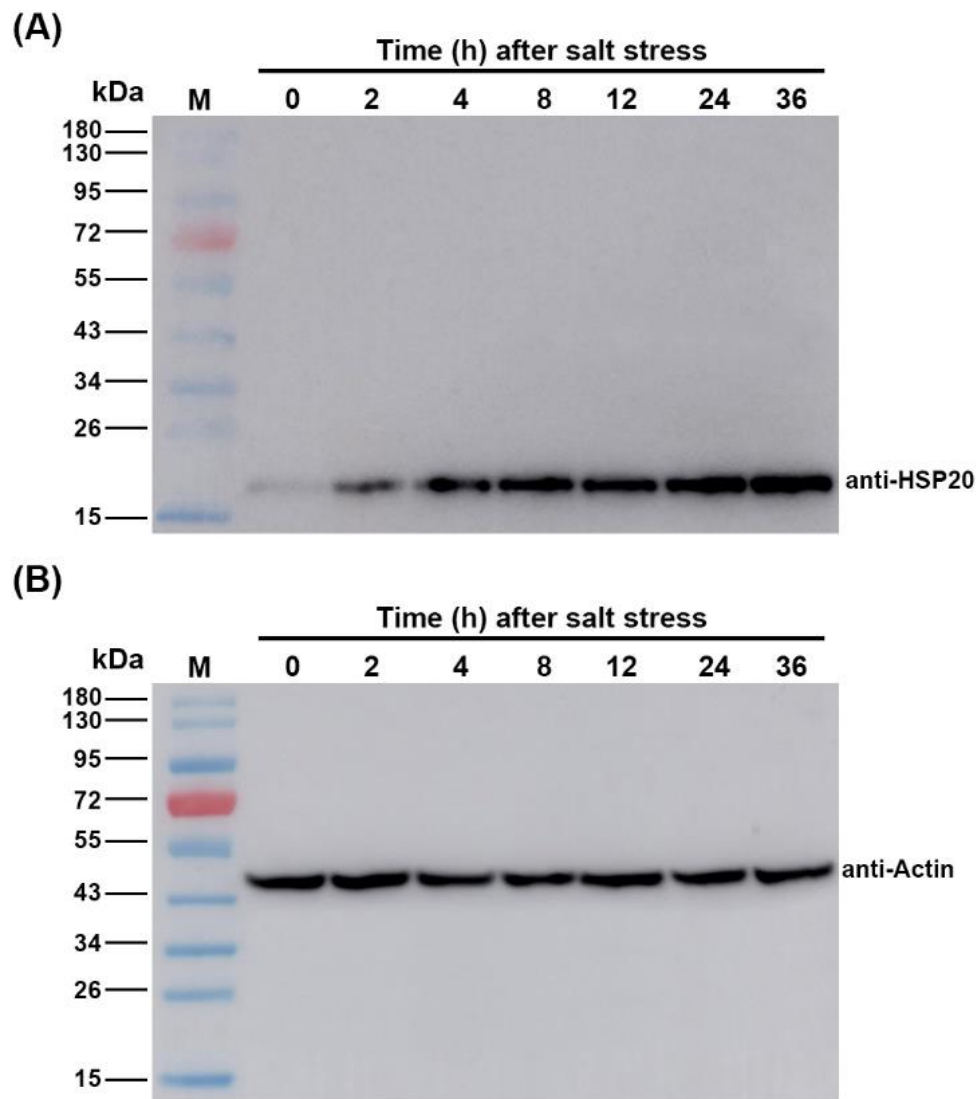


Figure S7

