

## 34. Cloning of the Mitochondria-localized Small Heat Shock Protein Gene in Rice (*Oryza sativa* L.)

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### 벼의 미토콘드리아에 있는 small heat shock protein 유전자의 클로닝

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Key words : Mitochondria, Small heat shock protein.

#### <Objectives>

The diversity of small HSPs (small heat shock proteins) is specific to plants, since other eukaryotes have far fewer sHSPs. It has been shown that small HSPs act as molecular chaperone. However, the roles of small HSPs in the molecular mechanism of the heat shock response *in vivo* are mostly unknown. we therefore tried to isolate cDNA for the mitochondria-localized small heat shock protein from rice gene for novel molecular characterization.

#### <Materials and Methods>

1. Materials: Rice (*Oryza sativa* L.) cv. Dongjin
2. Methods: A cDNA for rice mitochondrial small HSP was isolated by RT-PCR. Southern and Northern blot analyses.

#### <Results and Discussion>

A rice cDNA clone, *Osmthsp*, encoding the mitochondria-localized small heat shock protein was isolated. DNA sequence analysis of *Osmthsp* revealed that the cDNA has only one open reading frame (ORF) of 663 bp which encodes a polypeptide of 220 amino acid residues with a predicted molecular mass of 27 KDa (Fig. 1). The amino acid sequence deduced from the nucleotide sequence of the *Osmthsp* has a structure characteristic of the mitochondria-localized small HSP (Fig. 2). Southern blot analysis revealed that *Osmthsp* is encoded by a single gene. Expression of the *Osmthsp* gene under heat-stress and other oxidative stress conditions will be investigated by Northern blot analysis.

ATTCTCAACGCAAAATCCAAAAGATAAGCACAGTTACCGCAGCGAGAGCGAGAGAGAGTGG 60  
 GAGAGCCATGGCTTCCATCGTGGCATCGAAGAGGATCCCACTGTTCCGCTCGTGGAGCA 120  
 M A S I V A S K R I P L F R L V E Q  
 GCTCCTCGCCGCTCGCCGCCCCAAGGCGCCGCTCCGCTCTCAGGCGGTTGGCCGCTCGC 180  
 L L A A S P A Q G A A S A L R P V A V A  
 CGCCGGTCCCCTGCATCAACACCGGCGCGCAGCTCCGCGGCGCAGAGGGACGAGTC 240  
 G G S R A Y N T G A Q L R R H E R D E S  
 GGACGACGACGCGCCGCTGGGTACGACACCGGCGCCCCACACGCGCAGCCACCATGCC 300  
 D D D S G R G Y D T R R P T R D A T M P  
 CGCCTTCTCTCAGATGTTTCCGTTGATCCGTTTCAAGCGGCGCGCAGAGCCCTCGGCGGCT 360  
 A F F S D V F R D P F S A P Q S L G R L  
 GCTGAGCCTCATGGACGACCTGGCGACCGCCGCGGCGCGCGCGCGCGCGACGCTCGC 420  
 L S L M D D L A T P A G R A G A A T L R  
 GCGCGGTGGACCGGAGGAGGAGCGAGGAGGCGCTGCACCTGAGGDTGGACATGCGGG 480  
 R G W N A K E S E E A L H L R V D M P G  
 CTTGGGAGGAGGACGCTGAAAGGTGTGGCGGACGAGACAGCCTCGTGTCAAGGGCGA 540  
 L G K E H V K V W A E Q N S L V I K G E  
 GGGGAGAGGAGGCGCGCGGAGGACGAGGCGCGCCCGCGCGGAGGTACAGCGCCCGCAT 600  
 G E K E A G E D E G A A P A R Y S G R I  
 CGAGCTCGCGCGGAGGTGTACAGGATGGACCATCAAGGCGGAGATGAAGAACGGCGT 660  
 E L A P E V V R M D Q I K A E M K N G V  
 GCTCAAGGTGCTCGTCCCAAGGTGAAGGAGGACGAGCGCAGGAGCCTCTTCAAGTCAA 720  
 L K V V V P K V K E E Q R R D V F Q V N  
 GTCGAGTGGAGCTCAATCCGCGCGCGCTTCCGCGTGGAGGTTGAGGATGCGGATGGA 780  
 V E  
 TCGTCTCCTTTTATCCATAAGAAATGCGGCGTCTGTAGCTTTGTTTTTTTTCGAAGT 840  
 TATGGAGATAAAGTTTTTGGTTACTTATGATAAAAAAATTTTACTTATGCGATTGATCT 900  
 CGTATCGGAGATCTCTCGCGCTCGTGGTCAAATTCAGGCGGACTCCGCGCGAAAACGGAG 960  
 CGAGCGCGCACGTTGAGCGGAGAACCGTGCAGGACAAATTAAGCCAGGCGCTCCTTC 1020  
 ATTTTCTCTACATATATCCATGAAAAATCRAAACTTGGCGAGGAGATCATTTTCTGT 1080  
 GCATGATAAAATTCAAAATTTGGTTAGAGG 1110

Fig. 1. Nucleotide and deduced amino acid sequences of cDNA for the mitochondria-localized small heat shock protein of rice.

<i>O. sativa</i>	MASTVASKR-IPLFLVEQLLAASPAQGAASALREAVAVAGGSRAYNTGAQLRRHER----	55
<i>Z. mays</i>	MASTVASRRVPLVFALEKLLAASSAPGTGSALREAVAVAGGLRQYNTGAPLRRYEG----	56
<i>T. aestivum</i>	MASRVVFSNRIPLVFAEMENLLAAS----SGSALREAAVAGGVRYNAGAPLRSYDR----	52
<i>P. sativum</i>	---MASS--LALKFLLSSLLSSS-----FLREAVASS-ASRSEFNTN-AMRQYDQHSDD	46
<i>L. esculentum</i>	---MAT---LALRRTASSLFNR-----LVNVRASAFRSEFNTNTQMTAYDQ--DD	44
<i>A. thaliana</i>	---MASSSALALRLLSSSTVAVPR--ALRAVREVSAS--SRLFNTN-AARNYED----	47
<i>G. max</i>	---MASS--LALKFLLSSLLSRS-----LLREASA-SRSEFDTN-AMRQYDNRADD	46
<i>O. sativa</i>	DESDDD SGRGYDTRRPTDATHPAFFSDVFRDPPSAP-QSLGRLLSLMDD----LATPAG	110
<i>Z. mays</i>	AESEDDSVREYDGRHGGRDYAVPFLSDIFRDPLSAP-MSIGRLLNLVDD----LAVAAP	111
<i>T. aestivum</i>	DEAVEDTRR--VARE--FDIGVPSFFSDVFRDPPSAP-QSLGRLLSMLDD----VAAASP	103
<i>P. sativum</i>	RNVVYVRHS---FPRT--RDDL--LLSDVF--DPPS----PPRSLSQVNL---MVDLLTDN	92
<i>L. esculentum</i>	RGVDVDRRSDD---RSVSRDAPFLSDVVF--DPPSPPRSVLSQLLNMDQMDSPFVAAP	100
<i>A. thaliana</i>	-GVDRNHNSN---RHVSRHGCD--FFSDIL--DPPTP--TRLSQMLNFMQVSEIPLVSAT	99
<i>G. max</i>	HSTDIDRHSERSFPSTARDDI--FLRCVG-SIFSDSEFEGSEMDGPGHGQSVPLRVAR	103
<i>O. sativa</i>	R--AGAATLRGWNARESEEDALHLRVDMPGLGKEHWKWAQNSLVITGEEKEAGEDEG	166
<i>Z. mays</i>	----GRAVRRGNAREDEEDALHLRVDMPGLGKEHWKWAQNSLVITGEEKEKEDSEDEA	166
<i>T. aestivum</i>	DGAARAAPMRGNAREDADALHLRVDMPGLGKEHWKWAQNSLVITGEEKE-SEQEG	162
<i>P. sativum</i>	PVLSA--ASRRGWDARETEDALFLRLDMPGLGKEHWKISVEQNTLVITGEEGAKESSEEK	150
<i>L. esculentum</i>	RAMGAVGARCGDVKEDDNLVYIKDMPGLDKEHWKWAVENTLVITGEE-GEKESEN-E	158
<i>A. thaliana</i>	RGMGAS-GVRRGNVREKDDALHLRIDMPGLSREDVIALFQNTLVITGEEGETEEGEDVS	158
<i>G. max</i>	DRSWR--WSGRCWDARETEDALHLRVDMPGLAKEDWKISVEQNTLVITGEE-GAKEGDE-E	159
<i>O. sativa</i>	AAPARYSGRIELAPEVYRMDQIKAEKMGVLRVAVVPRKKEQRDVFQVNVE	220
<i>Z. mays</i>	APPPRYSGRIELAPEVYRMDQIKAEKMGVLRVAVVPRKKEQRKDFVQVNVE	218
<i>T. aestivum</i>	ADAPRYSGRIELAGDVYRMDQIKAEKMGVLRVAVVPRKKEERKDFVEVNV	214
<i>P. sativum</i>	KSGRRFSSRIDLPEKLYKIDQIKAEKMGVLRVAVVPRKKEEERHNVINVKVD	202
<i>L. esculentum</i>	EYRRRYSTLEIPQNIYKLDQIKAEKMGVLRVAVVPRKKEERKDFVVKIE	210
<i>A. thaliana</i>	GDGRFTSRIELPEKLYKIDQIKAEKMGVLRVAVVPRKKEEERHNVIRHINVD	210
<i>G. max</i>	ESARFTSRIELPDKLYKIDQIKAEKMGVLRVAVVPRKKEEERKDFVSVKVE	211

Fig. 2. Alignment of the deduced amino acid sequences of the mitochondria-localized small HSPs from plant species. The boxes indicate the consensus amino acid residues in the sequences.