

Isolation and characterization of the third gene encoding a 16.9 kDa class I low-molecular-mass heat shock protein, *Oshsp 16.9C*, in rice

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Abstract. Using rice cDNA *pTS1* encoding a 16.9 kDa heat shock protein (HSP) as a probe for Southern hybridization analysis, we observed five prominent bands of 9.5, 5.9, 3.4, 2.5, and 1.7 kb in the *Eco* RI digests of rice genomic DNA and found them to contain six individual genes. The 5.9 kb DNA fragment was further digested with *Hind* III to generate three fragments of 3.5, 1.7 and 0.7 kb, and we found, using *pTS1* cDNA as a probe, the 3.5 kb fragment contained a putative low-molecular-mass (LMM) HSP gene. The DNA sequencing of 3.5 kb fragment revealed encoding of a presumptive 16.9 kDa HSP (149 amino acid residues) with predicted pI value of 6.42. The nucleotide sequence of this gene was highly homologous to the coding regions of two rice class I LMM HSP genes, *Oshsp 16.9A* and *Oshsp16.9B*, sharing 93.1% and 94.3% sequence identity, respectively, as published previously in our laboratory. The deduced amino acid sequence of this gene is similar to those of the *Oshsp16.9A* and *Oshsp16.9B* genes with a difference of only 11 and 10 amino acids, respectively. It was hence designated as *Oshsp16.9C* (accession no. U81385). We used 3' *UTRs* (untranslated regions) for analysis of *Oshsp16.9C* gene expression since the 3' *UTRs* of these three genes showed very low sequence homology.

Keywords: Heat shock protein; Heat shock gene; Low-molecular-mass heat shock protein; *Oryza sativa* L.

Abbreviations: HS, heat shock; HSP, heat shock protein; LMM, low-molecular-mass; *UTR*, untranslated region.

Introduction

Heat shock proteins (HSPs) have been induced during thermal stress in all organisms ever examined, ranging from bacteria to human beings (Schlesinger et al., 1982), and they appear to be involved in thermoprotection (Lin et al., 1984; Chou et al., 1989; Krishnan et al., 1989; Vierling, 1991; Jinn et al., 1997). The HSPs are usually divided into high-molecular-mass (HMM) proteins of more than 30 kDa and low-molecular-mass (LMM) proteins of about 17 to 28 kDa (Lindquist and Craig, 1988; Vierling, 1991). In contrast to animal systems, plants synthesize more abundant LMM HSPs than HMM HSPs. The LMM HSPs superfamily is unusually complex, consisting of at least five gene families (LaFayette et al., 1996; Waters et al., 1996). The role

of LMM HSP in heat stress is not completely clear yet. However, the sequence conservation of genomic and cDNA clones of plant LMM HSP genes isolated and characterized from a number of species suggests that they may play an important role in plants coping with HS.

We have been studying the physiological function of class I LMM HSPs in soybean and rice (Lin et al., 1984; Chou et al., 1989; Jinn et al., 1989; Jinn et al., 1993; Jinn et al., 1995; Yeh et al., 1995; Jinn et al., 1997; Yeh et al., 1997). We have isolated and characterized three cDNA clones: *pTS1* (encoding a 16.9 kDa HSP, Tseng et al., 1992), *pTS3* (encoding a 17.3 kDa HSP, Tseng et al., 1992), and *pYL* (encoding a 18.0 kDa HSP, Lee et al., 1995), and also five genomic clones, *Oshsp16.9A*, *Oshsp16.9B*, *Oshsp18.0*, *Oshsp17.3*, and *Oshsp17.7* of rice class I LMM HSPs (Tseng et al., 1992; 1993; Guan et al., 1998, respectively). The *Oshsp16.9A* contains the sequence of *pTS1* cDNA. The three cDNA clones are highly homologous in their sequences except for the 3' untranslated regions (*UTRs*), which show a low degree of homology. Because of the abundance and complexity of these proteins, we have tried to isolate and characterize additional genes for rice class I LMM HSPs for the purpose of studying their differences in gene expression under heat stress. So far, we have isolated and characterized all the genes for rice class I LMM

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