Plant Molecular Biology **17**: 255–258, 1991.

Update section

Sequence

Nucleotide sequence of a *Triticum aestivum* cDNA clone which is homologous to the 26 kDa chloroplast-localized heat shock protein gene of maize

Jian Weng, Zi-Fen Wang and Henry T. Nguyen*
*Plant Genetics Laboratory, Mail Stop 2122, Texas Tech University, Lubbock, TX 79409, USA (*author for correspondence)*

Received and accepted 29 March 1991

Key words: Tahsp26.6, chloroplast-localized HSP, *Triticum aestivum* L., wheat

Plants synthesize more multiple low-molecular-weight (LMW) heat shock proteins (HSPs) during heat stress than microbes, insects, and animals. The response of plants to heat stress is similar to that of other organisms; however, a unique feature of the heat shock response in plants is that they also synthesize nuclear-encoded, LMW chloroplast-localized HSPs [2, 4, 6]. Higher-plant major LMW chloroplast HSPs are synthesized as precursor polypeptides with typical chloroplast transit peptides that are removed from the amino termini during import of the proteins into chloroplasts [5], while in the green alga *Chlamydomonas* these proteins synthesized without transit peptides [1]. The major LMW chloroplast HSPs range in size from 21 to 28 kDa in different plant species [7]. We have isolated a cDNA clone encoding one of these HSPs. A cDNA library was constructed in lambda ZAP II vector (Stratagene) using poly(A)$^+$ RNA from leaves of *Triticum aestivum* L. cv. Mustang which were heat-shocked for 120 min at 37 °C. The library was screened using a maize cDNA clone, HSP26 [3], as a probe. A cDNA clone, Tahsp26.6, was sequenced (Fig. 1) and its predicted amino acid sequence is homologous to the 26 kDa chloroplast-localized HSP of maize [3]. Wheat Tahsp26.6 encodes a 238 amino acid, 26.6 kDa protein which is 81.7%, 50.0%, 60.8%, and 38.2% identical to the chloroplast-localized HSPs of maize Zmhsp26.3 [3], pea Pshsp26.1 [5], soybean Gmhsp20.5 [5], and *Chlamydomonas* Crhsp16.8 [1], respectively as aligned in Fig. 2. As previously reported [3], monocots and dicots have more conserved sequences at the carboxyl terminal and more divergent sequences at the amino terminal of major LMW chloroplast HSPs. This is the first complete sequence reported for this major LMW chloroplast HSP in small grain cereal crops.

Acknowledgments

We wish to thank Dr Tuan-Hua David Ho for providing us with the maize cDNA clone, HSP26 and Dr Kerry L. Hendershot for help rendered during preparation of this manuscript. This research was supported in part by grants from the

---

The nucleotide sequence data reported will appear in the EMBL, GenBank and DDBJ Nucleotide Sequence Databases under the accession number X58280.

```
                  -90                                          -51
                   AATTC  GCGGC  CGCTC  GGCTT  CCAAG  TTCCA  ACGAT  CTCGT

-50                                                                 -1
AGACA  CTCTC  TCGTT  TCAAT  CTCGA  GCGAC  TTTTA  TTATT  TCAAA  GTGCA

1                                                                   45
ATG  GCT  GCA  GCG  AAC  GCT  CCC  TTC  GCT  CTC  GTC  AGC  CGC  CTC  TCC
 M    A    A    A    N    A    P    F    A    L    V    S    R    L    S

46                                                                  90
CCG  GCC  GCG  CGC  CTG  CCC  ATC  CGT  GCC  TGG  AGA  GCC  GCG  AGG  CCG
 P    A    A    R    L    P    I    R    A    W    R    A    A    R    P

91                                                                 135
GCG  CCG  CTC  TCG  ACC  GGC  GGG  AGA  ACC  CGC  CCG  CTC  TCC  GTG  GCC
 A    P    L    S    T    G    G    R    T    R    P    L    S    V    A

136                                                                180
TCC  GCG  GCG  CAG  GAG  AAT  AGG  GAC  AAC  TCC  GTC  GAC  GTC  CAA  GTC
 S    A    A    Q    E    N    R    D    N    S    V    D    V    Q    V

181                                                                225
AGC  CAG  GCC  CAG  AAC  GCC  GGC  AAC  CAG  CAG  GGC  AAT  GCA  GTC  CAG
 S    Q    A    Q    N    A    G    N    Q    Q    G    N    A    V    Q

226                                                                270
CGC  CGC  CCT  CGT  CGC  GCT  GGA  TTT  GAC  ATC  TCC  CCG  TTC  GGG  CTA
 R    R    P    R    R    A    G    F    D    I    S    P    F    G    L

271                                                                315
GTG  GAC  CCG  ATG  TCG  CCG  ATG  AGG  ACG  ATG  CGG  CAG  ATG  CTG  GAC
 V    D    P    M    S    P    M    R    T    M    R    Q    M    L    D

271                                                                360
ACG  ATG  GAC  CGG  CTG  TTC  GAC  GAC  GCC  GTG  GGG  TTC  CCC  ACG  CGT
 T    M    D    R    L    F    D    D    A    V    G    F    P    T    R


361                                                                405
CGC  TCG  CCA  GCG  GCG  CGA  GCG  AGA  CGC  CGG  ATG  CCG  TGG  GAC  ATC
 R    S    P    A    A    R    A    R    R    R    M    P    W    D    I

406                                                                450
ATG  GAG  GAC  GAG  AAG  GAG  GTG  AAG  ATG  CGG  TTT  GAC  ATG  CCT  GGG
 M    E    D    E    K    E    V    K    M    R    F    D    M    P    G

451                                                                495
CTG  TCG  CGG  GAG  GAG  GTG  AGG  GTG  ATG  GTG  GAG  GAC  GAC  GCG  CTG
 L    S    R    E    E    V    R    V    M    V    E    D    D    A    L

496                                                                540
GTC  ATC  CGC  GGC  GAG  CAC  AAG  AAG  GAG  GCC  GGC  GAA  GGG  CAG  GGC
 V    I    R    G    E    H    K    K    E    A    G    E    G    Q    G

541                                                                585
GAA  GGC  GGC  GAC  GGG  TGG  TGG  AAG  GAG  CGC  AGC  GTG  AGC  TCC  TAC
 E    G    G    D    G    W    W    K    E    R    S    V    S    S    Y

586                                                                630
GAC  ATG  CGC  CTT  GCT  CTG  CCG  GAC  GAG  TGC  GAC  AAG  AGC  CAG  GTG
 D    M    R    L    A    L    P    D    E    C    D    K    S    Q    V

631                                                                675
CGG  GCC  GAG  CTC  AAG  AAC  GGC  GTG  CTG  CTC  GTG  TCC  GTG  CCC  AAG
 R    A    E    L    K    N    G    V    L    L    V    S    V    P    K

676                                                                720
AGG  GAG  ACC  GAG  CGC  AAG  GTC  ATC  GAC  GTG  CAG  GTC  CAG  TGA  TGA
 R    E    T    E    R    K    V    I    D    V    Q    V    Q    *    *

721                                                                770
GTTCG  TGTGA  GACTG  TACCC  TGCAT  CTGAG  GCTTT  AAGAT  TTCAG  CTGTC

771                                                                820
CGAGG  TGTGC  TCTTT  GTAGA  GTGTG  CCGTT  TTTCT  CCAAT  CCTCT  TTGCG

821                                     850
CACTC  GTTGT  GCGAA  AGCGG  CCGCG  AATTC
```

Fig. 1. Nucleotide and deduced amino acid sequences of Tahsp26.6. Negative numbers refer to the 5′-noncoding region. Nucleotide +1 was assigned to the A of the first methionine codon. Amino acids are indicated in the standard one-letter code under the nucleotide sequence. The stop codon is denoted by an asterisk (*).

```
                                                           25
Tahsp26.6   M A A A N A P F A L V S R L S P A A R . . L P I R
Zmhsp26.3   * * * * . . * * * I A G * * * * V * * . . * * V *
Pshsp26.1   * * Q S . . . . . . * * . * * T I * S P I * S Q K
Gmhsp20.5   . . . . . . . . . . . . . . . . . . . . . . . . .
Crhsp16.8   . . . . . . . . . . . . . . . . . . . . . . . . .

                                                           50
Tahsp26.6   A W R A A R P A P L . . . S T G G R T R P L S . .
Zmhsp26.3   * * . . . * * * H G F . A * S . * * A * S * A . .
Pshsp26.1   P G S S V K S T * P C M A * F P L * . * Q * P R L
Gmhsp20.5   . . . . . . . . . . . . . . . . . . . . . . . . .
Crhsp16.8   . . . . . . . . . . . . . . . . . . . . . . . . .

                                                           75
Tahsp26.6   . . . . V . A S A A Q E . . N R D N S V D V Q V S
Zmhsp26.3   . . . . * . * * * * * * . . * * * * * * * * * * *
Pshsp26.1   G L R N * R * Q * G G D G D * K * * * * E * . . .
Gmhsp20.5   . . . . . . . . . . G . G D * K * * * * E * . . .
Crhsp16.8   . . . . . . . . . . . . . . . . . . . . . . . . .

                                                          100
Tahsp26.6   Q A Q . N A G N . Q Q G N A V Q R R P R R A G F .
Zmhsp26.3   * . . . * G * * R * * * * * * * * * * * * * T A L
Pshsp26.1   . H R V * K D D . . * * T * * E * K * * * . S S I
Gmhsp20.5   * H . V S K * D . . * * T * * E K K * * * . T A M
Crhsp16.8   M * L S * Y V F . . . * * S . . . . . . . * A . .

                                                          125
Tahsp26.6   D I . . S P F . G . L V D P M S P M R T M R Q M L
Zmhsp26.3   * * S P * * * . * . * * * * * * * * * * * * * * *
Pshsp26.1   * * . . * * * . * L * . * * W * * * * S * * * * *
Gmhsp20.5   * * . . * * * . * I * . * * W * * * * S * * * I *
Crhsp16.8   * . . . . * * F * E . . . . . . . . . . . . . * D

                                                          150
Tahsp26.6   D T M D R L F D D A V G F . P . . T R R S P A A R
Zmhsp26.3   * * * * * * * * * * * * * . * M G * * * * * * T T
Pshsp26.1   * * * * * I * E * * I T I . * . G . . * H I G G G
Gmhsp20.5   * * * * * V * E * T M T I . * . G . . * H I G G G
Crhsp16.8   R A V N * M I N N * L * V A * . . * . . * . * G K

                                                          175
Tahsp26.6   A R R . R M P W D I M E D E K E V K M R F D M P G
Zmhsp26.3   G D V . * L * * * * V * * * * * * * * * I * * * *
Pshsp26.1   E I . . * V * * E * K D E * H * I R * * * * * * *
Gmhsp20.5   E I . . * A * * * * K D E * H * I R * * * * * * *
Crhsp16.8   * G H T H A * M * * I * S P T A F E L H A * A * *

                                                          200
Tahsp26.6   L S R E E V R V M V . E D D A L . V I R G E H K K
Zmhsp26.3   * A * D * * K * * * . * * * T * . * * * * * * * *
Pshsp26.1   V * K * D * K * S * . * * * V * . * * K S D * R E
Gmhsp20.5   * A K * D * K * S * . * * * M * . * * K G G * * S
Crhsp16.8   M G P D D * K * E L Q * G V . * M * T . * * R * *
                                                    L S H T T↑

                                                          225
Tahsp26.6   E A G . E G Q G E G G D G . . W W K E R . S V S .
Zmhsp26.3   * E * A * * . * S * * * * D G * * * Q * . * * * .
Pshsp26.1   * N * G * . . . . . . * C . . . * S . * K T Y * .
Gmhsp20.5   * Q . . * H . . . * * * . D S . * S S * . T Y * .
Crhsp16.8   * * . . . . . . . * * K V . . * R S * * T A Y * F

                                                          250
Tahsp26.6   S Y D M R . L A L P D E C D K S Q V R A E L K N G
Zmhsp26.3   * * * * * . * * * * * * * * * * K * * * * * * * *
Pshsp26.1   C * * T * . * K * * * N * E * E K * K * * * * D *
Gmhsp20.5   * * * T * . * K * * * N * E * D K * K * * * * * *
Crhsp16.8   * . . . * A * S * * E N A N P D G I T * A M D K *
```

Fig. 2.

```
                                                        271
Tahsp26.6     V L L V S V P K R E T E R K V I D V Q V Q #
Zmhsp26.3     * * * * T * * * T * V * * * * * * * * * * #
Pshsp26.1     * * Y I T I * * T K I * * T * * * * * I * #
Gmhsp20.5     * * Y I T I * * T * V * * * * * * * * * * #
Crhsp16.8     * * V * T * * * * * P P A * P * A * T G A #
                                    E P K R↑
```

Fig. 2. Comparison and alignment of the Tahsp26.6 deduced amino acid sequence with other chloroplast-localized HSPs of maize HSP26 (Zmhsp26.3) [3], pea HSP21 (Pshsp26.1) [5], soybean HSP22 (Gmhsp20.5) [5] and *Chlamydomonas* HSP22 (Crhsp16.8) [1]. All sequences represent the complete coding region except soybean HSP22. Identical matches to the Tahsp26.6 sequence are identified by asterisks (*). Gaps (.) are created for better matchings among the sequences. Two underlined peptides should be inserted in the *Chlamydomonas* HSP22 amino acid sequence at the position as indicated by an arrow (↑).

USDA Competitive Research Grant Program (89-37264-4829), Texas Advanced Technology Research Program (003644-198), and NSF Presidential Young Investigator Award (BSR-8552915).

References

1. Grimm B, Ish-Shalom D, Even D, Glaczinski H, Ottersbach P, Ohad I, Kloppstech K: The nuclear-coded chloroplast 22-kDa heat-shock protein of *Chlamydomonas* – Evidence for translocation into the organelle without a processing step. Eur J Biochem 182: 539–546 (1989).
2. Kloppstech K, Meyer G, Schuster G, Ohad I: Synthesis, transport and localization of a nuclear encoded 22-kd heat-shock protein in the chloroplast membranes of peas and *Chlamydomonas reinhardii*. EMBO J 4: 1902–1909 (1985).
3. Nieto-Sotelo J, Vierling E, Ho THD: Cloning, sequence analysis, and expression of a cDNA encoding a plastid-localized heat shock protein in maize. Plant Physiol 93: 1321–1328 (1990).
4. Vierling E, Mishkind ML, Schmidt GW, Key JL: Specific heat shock proteins are transported into chloroplasts. Proc Natl Acad Sci USA 83: 361–365 (1986).
5. Vierling E, Nagao RT, DeRocher AE, Harris L: A heat shock protein localized to chloroplasts is a member of a eukaryotic superfamily of heat shock proteins. EMBO J 7: 575–581 (1988).
6. Vierling E, Harris L, Chen Q: The major heat shock protein in chloroplasts show antigenic conservation among diverse higher plant species. Mol Cell Biol 9: 461–468 (1989).
7. Vierling E: Heat shock protein function and expression in plants. In: Alscher RG, Cumming JR (eds) Stress Responses in Plants: Adaptation and Acclimation Mechanisms, pp. 357–375. Wiley-Liss, New York (1990).