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BRIEF COMMUNICATION

Significance of a β-ketoacyl-CoA synthase gene expression for wheat tolerance to adverse environments

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Abstract

TaCer6 was firstly cloned by rapid amplification of cDNA ends (RACE) and identified as a tissue-specific gene in wheat. To determine if environmental factors such as drought and low temperature induce *TaCer6* transcription, we examined the effects of these factors on *TaCer6* in two wheat cultivars. Our results demonstrated that light was essential for *TaCer6* transcription, salt stress inhibited *TaCer6* expression and application of salicylic acid enhanced *TaCer6* transcripts accumulation. In addition, polyethylene glycol (PEG 6000) and abscisic acid increased the expression of *TaCer6* more in the drought- and cold-tolerant cultivar Jinmai47 than in non-tolerant cultivar Shi4185. Low temperature increased *TaCer6* transcription in Jinmai47 while decreased it in Shi4185.

Additional key words: abscisic acid, cold stress, cuticular wax, drought, salt stress, *Triticum aestivum*, very-long-chain fatty acids.

Plant shoots are covered with wax layers that provide protection against water loss, UV and pathogens (Shepherd and Griffiths 2006, Skórska and Szwarc 2007). The cuticular wax is very compound-rich substance. Results of gas chromatography-mass spectrometry technology (GC-MS) indicated more than 100 compounds, such as very-long-chain fatty acids (VLCFA), fatty aldehydes, primary and secondary alcohols, ketones, esters, cyclic compounds and sterols (Mariani and Wolters-Arts 2000).

Cer6 encoding β-ketoacyl-CoA synthase (KCS) catalyzes biosynthesis of VLCFA, which play an important role in holding water from astomatous dissipating, cell elongation, or fertility (Millar *et al.* 1999, Leide *et al.* 2007, Qin *et al.* 2007a). In *Arabidopsis Cer6* mutant, wax load was reduced to 6 - 7 % of wild-type levels and most of pollens were sterile (Millar *et al.* 1999). In tomato *LeCer6* mutant with leaf and fruit deficient in *n*-alkanes and aldehydes with chain lengths beyond C30, cuticular water loss was about four times larger than in wild tomato (Vogg *et al.* 2004, Leide *et al.* 2007). Application of lignoceric acid (C24:0) in ovule culture medium to cotton lintless mutant *GhCer6* could overcome the inhibition of fiber elongation (Qin *et al.* 2007a,b). In this communication, we report on the isolation and expression of wheat *Cer6*-like β-ketoacyl-CoA synthase cDNA, which was named as *TaCer6* (EU159697).

Wheat (*Triticum aestivum* L. Jinmai 47) seeds were sterilized in 0.001 g dm^{-3} $HgCl_2$ for 15 min and washed four times (each 5 min) in sterile distilled water, then cultivated in Hoagland solution for 3 weeks at temperature of 25 °C, a 16-h photoperiod (irradiance of 400 μmol m^{-2} s^{-1}) and air humidity of 60 - 70 %. For cDNA cloning, total RNA was extracted from leaves of 3-week-old seedlings, stressed for 1 h by 20 % polyethylene glycol (PEG) 6000, and 1 μg was used for cDNA synthesis. A *BLASTn* search of wheat expressed sequences tag (EST) in GenBank database was done using the open reading frame (ORF)

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Abbreviations: ABA - abscisic acid; KCS - β-ketoacyl-CoA synthase; ORF - open reading frame; PEG - polyethylene glycol; RACE - rapid amplification of cDNA ends; RT-PCR - reverse transcription polymerase chain reaction; SA - salicylic acid; VLCFAs - very-long-chain fatty acids.
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nucleotide sequence of *Arabidopsis Cer6* (Q9XF43). According the aligned partial sequence, a 927-bp sequence was cloned by reverse transcription polymerase chain reaction (RT-PCR) from wheat. Rapid amplification of cDNA ends (RACE) was carried out to isolate the complete sequence of *TaCer6*, according to the instruction of *SMART12* RACE cDNA amplification kit user manual (*Clontech*, Mountain View, CA, USA). A 554-bp fragment and a 1 303-bp fragment were isolated by 5'RACE and 3'RACE, respectively. The complete cDNA of *TaCer6* contains 2 113 bp including 92-bp sequence of 5'-untranslated region (5'-UTR) with nucleoside A at the front and a 532-bp sequence of 3'-untranslated region (3'-UTR) plus poly-A tail. There is an in-frame stop codon, TAA, at 27 nucleotide of *TaCer6*, the similar as *Cer6* which has the stop code, TAA, at -15 nucleotide (Millar *et al.* 1999).

Translation of the 1 491-nucleotide-long ORF in the *TaCer6* cDNA sequence induced a putative polypeptide of 496 amino acids with a molecular mass of 55.94 kDa and a pI of 9.54. An amino acid sequence comparison using *BLASTp* (http://www.ncbi.nlm.nih.gov/BLAST) revealed that the predicted protein belonged to the fatty acid-condensing enzyme superfamily involved in the synthesis and degradation of fatty acids. The results of Motif scan (http://myhits.isb-sib.ch/cgi-bin/motif_scan) analysis showed the amino acids of Y81 to V369 had a typical characterization of *FAE1_CUT1/Cer6_RppA* synthase. Cluster homology analysis indicated *Cer6* proteins were highly conserved among different plant species. *TaCer6* protein had 98, 90, 79 and 76 % identity over *HvCer6* (ABG35744), *OsCer6* (NP_001049406), *GhCer6* (ABA01490) and *Cer6* (Q9XF43), respectively. The result of phylogenetic analysis indicated that *TaCer6* was near to other *Cer6* proteins of different plant species when compared with other KCS (KCS1; KCS2) and fatty acid elongation enzymes (FAE1; FDH) (Fig. 1). At N-terminus, there are two transmembrane domains, L21 to A42 and L62 to S84. The signal peptide cleavage site is between A39 and A40 among the first transmembrane domain.

To detect the transcript levels of *TaCer6*, drought-tolerant and cold-tolerant cultivar Jinmai47 and non-tolerant cultivar Shi4185 were cultivated in Hoagland solution. For tissue-specific analysis, total RNA was extracted from leaf, stem and root tissues of 3-week-old seedlings. 3-week-old seedlings were treated with different stresses. Plants were sprayed with 200 μM abscisic acid (ABA), 3 mM salicylic acid (SA), 20 % PEG 6000 or 200 mM NaCl followed by sampling after 0, 3, 6, 12 and 24 h. For low temperature stress, seedlings were transferred to a growth chamber at 4 °C and sampled at 0, 3, 6, 12, and 24 h after treatment. For dark treatment, seedlings were transferred to darkness and sampled at 0, 6, 12, 24 and 48 h. First-strand cDNA was synthesized starting from 1 μg total RNA with *PrimeScriptTM* RTase (*TaKaRa*, Dalian, China). Polymerase chain reaction (PCR) was performed at 94 °C for 2 min; 28 cycles of 94 °C for 45 s, 57 °C for 30 s, 72 °C for 1 min; and finally 72 °C for 10 min. Products (0.008 cm^3) were separated by electrophoresis in a 1.0 % agarose/EtBr gel and three replications were carried out to ensure accurate results. Primers of internal standard (*β-actin*, accession number CD405267) and *TaCer6* were *SQactin-5* (GTTCCA ATCTATGAGGGATACACGC) and *SQactin-3* (GAA CCTCCACTGAGAACAACATTACC), *SQTaCer6-5* (CGCTCAAGTCCAACATCACC) and *SQTaCer6-3* (CGCATCCCACGAATATCC). The size of internal standard and *TaCer6* were 422 bp and 688 bp, respectively. To avoid the influence of the residual genomic DNA, RNA was used as the template for PCR with primers of internal standard before the reversed transcript experiment, RNA without PCR product was used for RT-PCR. To prevent the product from being contaminated by other homogenous gene in wheat, primer *SQTaCer6-3* was designed at the 3'-UTR sequence of *TaCer6*.

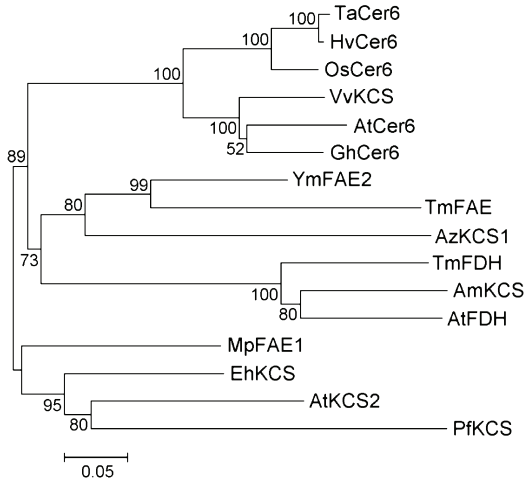

Fig. 1. Phylogenetic analysis of TaCer6 with other predicted condensing enzymes. Neighbor-Joining method was used to build the dendrogram together with the distance-based minimum-evolution method of MEGA 4.0.2. The accession numbers in this study are as follows: TaCer6 EU159697; HvCer6 ABG35744; OsCer6 NP_001049406; GhCer6 ABA0149; AtCer6 Q9XF43; OsCer6 NP_001049406; VvKCS CAN74750; ZmFAE2 NP_001105135; TmFAE AAL99199; AtKCS1 NP_171620; TmFDH AAO47729; AmKCS CAC84082; AtFDH NP_180193; MpFAE1 AAP74371; EhKCS ABS18382; AtKCS2 NP_195177; PfKCS AAU05611. The scale bar denotes the number of substitutions per site.

TaCer6 is a tissue-specific gene. In both wheat cultivars, it was mainly expressed in leaf and stem tissues but not in root tissue. In addition, *TaCer6* transcripts were more abundant in stem tissue than in leaf tissue (Fig. 2*A*). The expression of *TaCer6* in different wheat tissues was similar to *Cer6* expression in different *Arabidopsis* tissues (Hooker *et al.* 2002).

In *Arabidopsis*, application of ABA increased *Cer6* transcripts accumulation (Hooker *et al.* 2002). In wheat, application of ABA also increased *TaCer6* transcripts accumulation, while transcript profiles of *TaCer6* in two cultivars to ABA treatment were different (Fig. 2*B*). In

drought- and cold-tolerant cv. Jinmai 47, *TaCer6* transcripts increased when seedling was treated for 3 h but decreased gradually with the elongation of treatment time. In non-tolerant cv. Shi4185, *TaCe6* transcripts increased gradually. ABA plays an important role in mediating the expression of a large number of genes that respond to drought and salt stresses (Kim *et al.* 2004). These stresses trigger ABA accumulation, which in turn induces the expression of some genes which are also induced by exogenous application of ABA (Aroca *et al.* 2008).

To further examine how *TaCer6* responds to drought and salt stress, PEG and NaCl were applied because they could simulate drought and salt stress, respectively (Miki *et al.* 2001, Lei *et al*. 2007, Cancado *et al.* 2008). Under PEG treatment, the transcripts of *TaCer6* increased, while transcript profiles were different in two wheat cultivars (Fig. 2*B*). In Jinmai47, the expression of *TaCer6* was extremely sensitive to PEG treatment, and the amount of *TaCer6* transcripts had been already very high within 3 h. However, in Shi4185, the expression of *TaCer6* was insensitive to PEG treatment, the amount of *TaCer6* transcripts did not increase until treated for 24 h. Similarly, application of PEG also increased *Cer6* transcript accumulation in *Arabidopsis* (Hooker *et al.* 2002). However, response of *TaCer6* to salt stress was different to that of *Cer6*. Application of NaCl inhibited the expression of *TaCer6* in wheat (Fig. 2*B*), while in *Arabidopsis*, application of NaCl increased *Cer6* expression (Hooker *et al.* 2002). Because Jinmai47, as a drought-tolerant cultivar, responds to drought rapidly, the responses of *TaCer6* to ABA and PEG treatments may be different from those in Shi4185.

In Jinmai 47, response of *TaCer6* transcripts to 4 °C treatment was similar to that to ABA treatment (Fig. 2*B*). *TaCer6* transcripts increased during 3 h of treatment but decreased gradually with the treatment duration. However, in cv. Shi4185, *TaCer6* transcripts decreased under 4 °C. The results indicate that the amount of *TaCer6* transcript is related to wheat cold-tolerance. Light-regulated genes are considered to be related to cold-tolerance in wheat because temperature and photoperiod participate in vernalization (Kosová *et al.* 2008). Thus we further studied the effect of light on *TaCer6* expression and found that light was essential. Transcripts of *TaCer6* decreased gradually in response to darkness in both cultivars (Fig. 2*C*). Under darkness, *TaCer6* expression in wheat was similar to *Cer6*

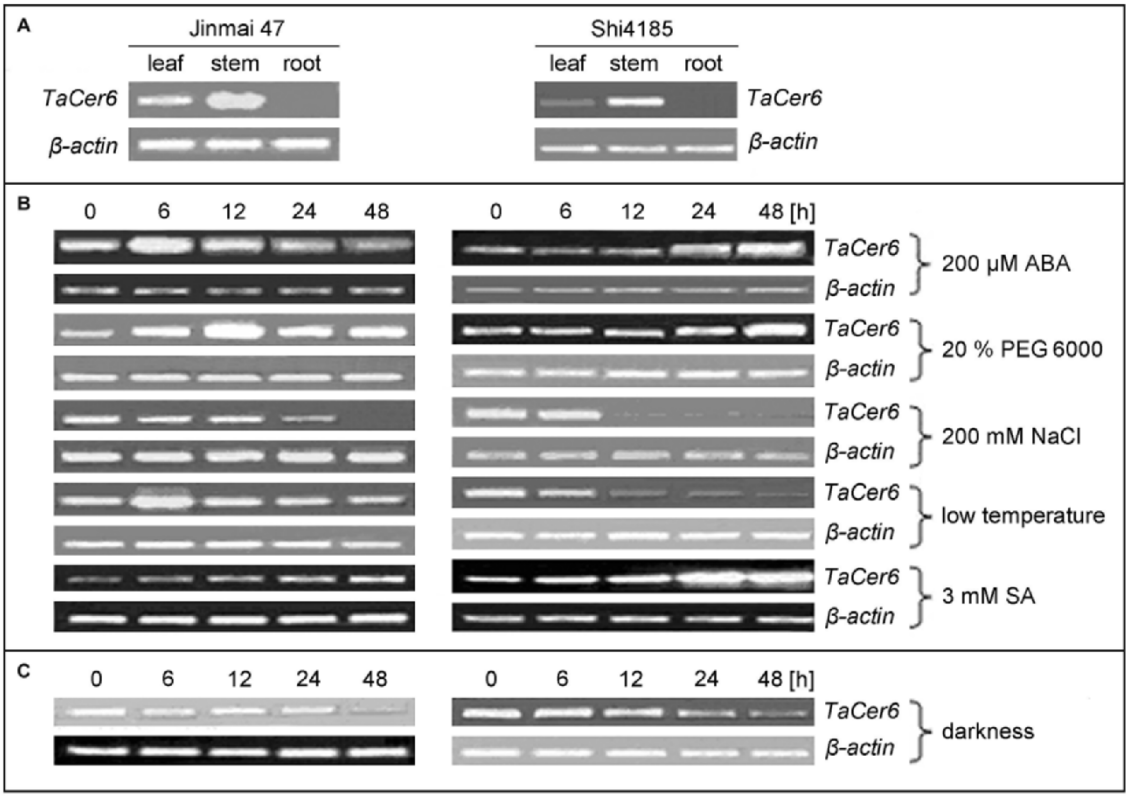

Fig. 2. Expression analysis of *TaCer6* transcripts in different tissues of wheat (*A*) and in leaf tissue under different treatments (*B*, *C*). The *left panel* is for drought-tolerant and cold-tolerant cultivar Jinmai47 and the *right panel* is for non-tolerant Shi4185. For tissue-specific analysis, DNA-free total RNA from different tissues (roots, stems, leaves) of 3-week-old wheat seedlings under normal hydroponics condition were analyzed by semi-quantitative RT-PCR. For stress-response analysis, DNA-free total RNA was extracted from leaves of 3-week-old wheat seedlings under different stress treatments. The *β-actin* (accession number CD405267) transcript was used as an internal control. A 668-bp fragment of *TaCer6* and a 422-bp fragment of *β-actin* were amplified by 28 cycles. Experiments were repeated at least three times with similar results.

expression in *Arabidopsis* (Hooker *et al.* 2002). Light is vital to plant cuticular wax biosynthesis and transport. Light deficiency leads to decrease of wax loads and relative gene expression (Hooker *et al.* 2002, Pighin *et al.* 2004, Luo *et al.* 2007).

SA is an important signal molecule in regulating plant defense in response to a wide variety of pathogens (Horváth *et al.* 2007, Loake *et al.* 2007). Therefore, we examined *TaCer6* transcripts accumulation under SA treatment. In both wheat cultivars, application of SA increased *TaCer6* transcripts accumulation (Fig. 2*B*). The results indicate that the amount of *TaCer6* transcript might have relation to wheat diseases tolerance.

In summary, this is the first time cloning of tissue-specific *TaCer6* in wheat. Through the analysis of *TaCer6* expression in two wheat cultivars with different drought- and cold-tolerance under different stresses, we deduce that the amount of *TaCer6* is related to wheat tolerance to drought, cold and diseases. Moreover, light is essential to *TaCer6* expression and salt stress inhibits *TaCer6* expression. Because *Cer6* protein was reported as β-ketoacyl-CoA synthase, which catalyzes synthesis of VLCFA from LCFA (Millar *et al.* 1999), we suggest that VLCFA, an important components in wax layer, take part in drought and cold tolerance and in response to biotic stresses.

References

Aroca, R., Alguacil, M.M., Vernieri, P., Ruiz-Lozano, J.M.: Plant responses to drought stress and exogenous ABA application are modulated differently by mycorrhization in tomato and an ABA-deficient mutant (*Sitien*). - Microbiol. Ecol. **56**: 704-719, 2008.

Cancado, G.M.A., Nogueira, F.T.S., Camargo, S.R., Drummond, R.D., Jorge, R.A.: Gene expression profiling in maize roots under aluminum stress. - Biol. Plant. **52**: 475-485, 2008.

Hooker, T.S., Millar, A.A., Kunst., L.: Significance of the expression of the *CER6* condensing enzyme for cuticular wax production in *Arabidopsis*. - Plant Physiol. **129**: 1568-1580, 2002.

Horváth, E., Pál, M., Szalai, G., Páldi, E., Janda, T.: Exogenous 4-hydroxybenzoic acid and salicylic acid modulate the effect of short-term drought and freezing stress on wheat plants. - Biol. Plant. **51**: 480-487, 2007.

Kim, J.B., Kang, J.Y., Kim, S.Y.: Over-expression of a transcription factor regulating ABA-responsive gene expression confers multiple stress tolerance. - Plant Biotechnol. J. **2**: 459-466, 2004.

Kosová, K., Prášil, I.T., Vítámvás, P.: The relationship between vernalization- and photoperiodically-regulated genes and the development of frost tolerance in wheat and barley. - Biol. Plant. **52**: 601-615, 2008.

Lei, Y., Yin, C., Ren, J., Li, C.: Effect of osmotic stress and sodium nitroprusside pretreatment on proline metabolism of wheat seedlings. - Biol. Plant. **51**: 386-390, 2007.

Leide, J., Hildebrandt, U., Reussing, K., Riederer, M., Vogg, G.: The developmental pattern of tomato fruit wax accumulation and its impact on cuticular transpiration barrier properties: effects of a deficiency in a β-ketoacyl-coenzyme A synthase (*LeCER6*). - Plant Physiol. **144**: 1667-1679, 2007.

Loake, G., Grant, M.: Salicylic acid in plant defence - the players and protagonists. - Curr. Opin. Plant. Biol. **10**: 466-472, 2007.

Luo, B., Xue, X.Y., Hu, W.L., Wang, L.J., Chen, X.Y.: An ABC transporter gene of *Arabidopsis thaliana, AtWBC11*, is involved in cuticle development and prevention of organ fusion. - Plant Cell Physiol. **48**: 1790-1802, 2007.

Mariani, C., Wolters-Arts, M.: Complex waxes. - Plant Cell **12**: 1795-1800, 2000.

Miki, Y., Hashiba, M., Hisajima, S.: Establishment of salt stress tolerant rice plants through step up NaCl treatment *in vitro*. - Biol. Plant. **44**: 391-395, 2001.

Millar, A.A., Clemens, S., Zachgo, S., Giblin, E.M., Taylor, D.C., Kunst, L.: *CUT1*, an *Arabidopsis* gene required for cuticular wax biosynthesis and pollen fertility, encodes a very-long-chain fatty acid condensing enzyme. - Plant Cell **11**: 825-838, 1999.

Qin, Y.M., Hu, C.Y., Pang, Y., Kastaniotis, A.J., Hiltunen, J.K., Zhu, Y.X.: Saturated very-long-chain fatty acids promote cotton fiber and *Arabidopsis* cell elongation by activating ethylene biosynthesis. - Plant Cell **19**: 3692-3704, 2007a.

Qin, Y.M., Pujol, F.M., Hu, C.Y., Feng, J.X., Kastaniotis, A.J., Hiltunen, J.K., Zhu, Y.X.: Genetic and biochemical studies in yeast reveal that the cotton fibre-specic *GhCER6* gene functions in fatty acid elongation. - J. exp. Bot. **58**: 473-481, 2007b.

Pighin, J.A., Zheng, H.Q., Balakshin, L.J., Goodman, I.P., Western, T.L., Jetter, R., Kunst, L.A., Samuels, L.: Plant cuticular lipid export requires an ABC transporter. - Science **306**: 702-706, 2004.

Shepherd, T., Griffiths, D.W.: The effects of stress on plant cuticular waxes. - New Phytol. **171**: 469-499, 2006.

Skórska, E., Szwarc, W.: Influence of UV-B radiation on young triticale plants with different wax cover. - Biol. Plant. **51**: 189-192, 2007.

Vogg, G., Fischer, S., Leide, J., Emmanuel, E., Jetter, R., Levy, A.A., Riederer, M.: Tomato fruit cuticular waxes and their effects on transpiration barrier properties: functional characterization of a mutant deficient in a very-long-chain fatty acid β-ketoacyl-CoA synthase. - J. exp. Bot. **55**: 1401-1410, 2004.