Expression characteristics of heat shock protein genes in two comparable inbred lines of Chinese cabbage, Chiifu and Kenshin

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Abstract
Heat-shock proteins (HSPs) and heat-shock transcription factors (HSFs) are central components of the heat-shock regulatory network and are involved in cellular responses to various forms of stresses. To examine the differences in heat shock responses (HSRs) of two comparable inbred lines of Chinese cabbage (Brassica rapa), 51 genes were selected from 130,000 Brassica rapa ESTs that belong to an HSF and six HSP families and examined their expression using RT-PCR. Two Chinese cabbage inbred lines, Chiifu and Kenshin, have different geographic origins, in that Chiifu is from temperate regions, while Kenshin is from subtropical and tropical regions. Among the 51 genes, six genes were induced, eleven were stimulated, and three were reduced in both inbred lines in response to heat shock (HS) treatment. However, eleven genes were differentially expressed between the two inbred lines. Among these genes, several appear to be involved in normal growth and chloroplast development. These data suggest that the two Chinese cabbage inbred lines have similar HSRs, but the unique HSRs allow Kenshin to develop at higher temperatures.

Keywords Brassica rapa; Hsf; Hsp90; Hsp70; Hsp60; sHsp; DnaJ.

Introduction
Heat stress is a major abiotic stress that limits plant growth and productivity, especially during the summer months in warm and temperate climates (Wardlaw and Willenbrink, 1994; Mittler, 2006; Wahid et al., 2007; Huang and Xu, 2008). To reduce stress-induced damage, plants use heat shock responses (HSRs) that are quite similar to animal HSRs. Heat shock proteins (HSPs), acting as molecular chaperones for protein quality control, accumulate in cells and lead to a HSR upon exposure to elevated temperatures. This HSR results in thermotolerance by preventing and repairing damage to heat-labile proteins and membranes (Schöffl et al., 1998; Larkindale and Vierling, 2008). Heat shock transcription factors (HSFs) also play a central role in heat stress responses and in acquired thermotolerance (Vierling, 1991; Rizhsky et al., 2004; Busch et al., 2005; Kotak et al., 2007). Recently, the functions of HSPs and HSFs have been comprehensively reviewed (Wang et al., 2004; Baniwal et al., 2004; Miller and Mittler, 2006; Nakamoto and Vigh, 2007; Kotak et al., 2007).

In plants and other organisms, six major families of HSPs are induced upon heat stress: HSP70 (DnaK/Ssa), J-protein/HSP40 (DnaJ/Ydj1), HSP60 (GroEL), HSP90, HSP100 (Clp), and small HSP (sHSP). Of these, the HSP70 and J-protein families are the most abundant and well-characterized HSPs in both prokaryotic and eukaryotic systems. One HSP70 family member interacts with multiple J-proteins to perform a variety of cellular processes and to form a complex network of chaperones (Babu et al., 2009). Some HSPs, termed chaperones, recognize misfolded proteins and prevent the formation of stable, potentially toxic, protein aggregates during heat stress. Through ATP metabolism, other chaperones can act on formed aggregates to unfold them into natively-refoldable or protease-degradable species (Hinault et al., 2006). The molecular functions of the Hsp100 and sHSP families are relatively well characterized in...
plants, while the other HSP families remain uncharacterized (Kotak et al., 2007).

The Casein lytic proteinase/heat shock protein 100 (Clp3Hsp100) proteins are chaperones belonging to the larger AAA+ (ATPases associated with various cellular activities) family that act to remodel and/or disassemble protein complexes and to resolubilize protein aggregates through ATP metabolism (Sauer et al., 2004; Burton and Baker, 2005; Bösler et al., 2006). The Hsp100 family includes cytosolic and organelle forms. A cytosolic member of the Hsp100 family that can interact with sHSPs (Lee et al., 2005) is essential for tolerance to high temperatures in plants, but is not essential for normal growth (Hong and Vierling, 2001). The role of chloroplast- and mitochondrion-localized Hsp100 proteins, namely ClpB-p and ClpB-m, has not been confirmed with respect to thermotolerance (Lee et al., 2007). All sHsps share the conserved α-crystallin domain of approximately 100 residues (Fu et al., 2006; Nakamoto and Vigh, 2007; Waters et al., 2008) and are responsible for both plant-acquired thermotolerance and normal development (Vierling, 1991; Waters et al., 1996; Wang et al., 2004; Sun and MacRae, 2005; Waters et al., 2008). Most sHsps are highly upregulated during heat stress, conferring thermotolerance by protecting other proteins from irreversible denaturation (Waters et al., 2008). Some sHsps are required for normal plant development, including embryogenesis, seed germination, pollen development, and fruit maturation (Waters et al., 1996; Wang et al., 2004; Sun and MacRae, 2005; Waters et al., 2008).

The HSP90 protein directs protein folding, but also plays a key role in signal transduction, cell cycle control, protein degradation, and protein trafficking (Wang et al., 2004). HSP90 proteins act as part of a multi-chaperone machine together with HSP70 proteins (Krishna and Gloor, 2001). HSP70 proteins participate in preventing aggregation and assist in refolding of non-native proteins under normal and stress conditions (Hartl, 1996; Frydman, 2001). Protein import, translocation processes, and facilitating the proteolytic degradation of unstable proteins by targeting the proteins to lysosomes or proteasomes (Hartl, 1996). Plant Hsp70s are composed of four major subgroups: cytosol, ER, plastids, and mitochondrial Hsp70 (Sung et al., 2001). cpHsp70-1 enhances heat tolerance (Sung et al., 2003) and is essential for plant development (Su and Li, 2004), while Hsc70-1 contributes to plant growth and development and abiotic stress tolerance (Sung and Guy, 2003; Cazalé et al., 2009). HSP60 proteins play a crucial role in assisting newly synthesized and newly translocated proteins achieve their native forms (Bukau and Horwich, 1998; Frydman, 2001). Additionally, pTCPn60β1 is essential for plastid division by folding proteins required for plastid division (Suzuki et al., 2009). J-proteins (Hsp40s/DnaJ) are co-chaperones of the Hsp70 machine, which plays a critical role in HSRs by stimulating the Hsp70 ATPase activity, thereby stabilizing its interaction with client proteins. J-proteins are required accessory factors for Hsp70 and regulate Hsp70 function in vivo (Qiu et al., 2006; Babu et al., 2009). Plants, specifically Arabidopsis and tomato, have over 20 HSFs that can be subsequently subdivided into the HsfA1, HsfA2, and HsfB1 families (Baniwal et al., 2004). Some HSFs function in thermotolerance.

Chinese cabbage (Brassica rapa ssp. pekinensis) includes two distinct inbred lines, Chifu and Kenshin, which have different geographic origins. It has been proposed that these two lines respond to temperature and vernalization differently. In this study, the expression of HSP and HSF genes was examined under short-term heat shock stress conditions. The expression of 11 genes was different between the two inbred lines, suggesting possible involvement in HSRs reflecting their different geographic origins. The data presented here provide the foundation for future studies of HSRs in Chinese cabbage for development of heat-tolerant cultivars.

Materials and Methods

Plant materials and heat shock treatments

Two distinct Chinese cabbage (Brassica rapa ssp. pekinensis) inbred lines, Chifu and Kenshin, which are different in heading type and geographic origin, were used in this study. Chifu was previously established in the Shan Dong Province of North-East China, and does not overlap after head formation. Kenshin was previously established in the Fu Jian Province of South-East China and in Taiwan, both relatively warm regions, and has an overlapping phenotype. Leaf discs (1 cm in diameter) were prepared from plants grown in a greenhouse (15-22 °C) for 30 days and exposed either to 100 μmolm⁻²s⁻¹ at 40 °C or the indicated temperatures. After treatment, leaf discs were blotted out, frozen immediately in liquid nitrogen, and stored at -70 °C.

Selection of HSP and HSF genes from Chinese cabbage

Of the 130,000 ESTs generated by 30 cDNA libraries of Brassica rapa ssp. pekinensis, the inbred Chifu line (enrolled in NCBI) contained 87 redundant HSP and HSF genes. Among them, a total of 51 HSP and HSF genes were selected by the best match to Arabidopsis counterparts in Table 2. Primers for reverse-transcription (RT)-PCR with melting temperatures of approximately 59 °C were designed using the Primer3 program (Table 1).