Note

Isolation and Expression Analysis of *Brassica rapa* WRKY 7

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The cDNA clone of *Brassica rapa* WRKY7 (*BrWRKY7*) was obtained from EST collection in *Brassica* genomics team and its DNA sequence was determined. The cDNA clone is 1,037 bp long in nucleotides and encodes an open reading frame of 307 amino acids. Based on a phylogenetic tree, *BrWRKY7* belongs to group II_d. *BrWRKY7* was induced by wound and SA. It was also induced by pathogen attack such as *Xanthomonas campestris* pv. *campestris* (*Xcc*), suggesting that this *BrWRKY* may play an essential role in defense response of chinese cabbages.

Keywords : *Brassica rapa*, WRKY transcription factor

Plants have a variety of active defense mechanisms to protect themselves from pathogen infection. Plant defense responses result from the transcriptional activation of a large number of genes upon pathogen infection or treatment with pathogen elicitors (Ruston and Somssich, 1998; Schreiber and Desveaux, 2008; Yang et al., 1997). Most WRKY genes are rapidly induced by pathogens, pathogen elicitors, or salicylic acid (SA) treatment in mostly *Arabidopsis* and rice (Chen and Chen, 2000; Dong et al., 2003; Eulgem et al., 1999; Ryu et al., 2006). Therefore, it has been implicated that WRKY proteins are major transcriptional regulators in defense signaling (Chen and Chen, 2000, 2002; Chen et al., 2002; Cormack et al., 2002; Deslandes et al., 2002; Du and Chen, 2000; Eulgem et al., 1999; Kim et al., 2000; Liu et al., 2005, 2006; Maleck et al., 2000; Robatzek and Somssich, 2001, 2002; Yoda et al., 2002; Yu et al., 2001). In this study, based on global expression profiling analysis of *Brassica rapa* genes on 25K cDNA oligomer chip, *BrWRKY7* was isolated and characterized.

Chinese cabbage (*Brassica rapa* ssp. *pekinensis* cv. *chiibu*) was grown in a greenhouse for 3 weeks. Three-week old chinese cabbage seedlings were treated with salicylic acid (SA) at 1 mM or buffer, respectively. Chinese cabbage

leaves were harvested at the times indicated in the figures. For bacterial inoculations, a compatible strain of *Xcc* to *Brassica rapa* ssp. *pekinensis* cv. *chiibu* was grown in PSA

Fig. 1. Nucleotide sequence and deduced amino acids of *BrWRKY7*. Typical DNA binding domain in the most WRKY proteins was boxed.

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medium (10 g peptone, 10 g sucrose, 1 g sodium-glutamate, and 15 g agar per liter) for 2 days and then resuspended in 1 mM MgCl₂ to a final OD₆₀₀ of 0.5. Inoculum of *Xcc* was sprayed on 3-week-old Chinese cabbage seedlings. After inoculations, plants were incubated in a humidity chamber for 24 hr. Samples were taken at 24 hr. Leaf samples were ground to powder in liquid nitrogen and total RNA was extracted using Trizol reagent according to the manufacturer's instructions (Invitrogen, Carlsbad, CA). RT-PCR analysis using M-MLV RTase (Promega, Madison, WI) was performed with 1 µg of total RNA according to manufacturer's instructions and method described before (Lee, J.-H. et al., 2008). Subsequent PCR was performed for 25–35 cycles using specific primers 5'-TTCATAACCCATCATCCCAAATC-3' and 5'-AAACAAGAGTAGGGTCACTTCCAAC-3' for *BrWRKY7*. PCR product with primers 5'-TGTGAACGATTCCTGCACCT-3' and 5'-GGAAGGATCTGTACGGTAAAC-3' for *BrActin* (In-Ah Lee, MS thesis, 2005) was used as equal loading controls.

The cDNA clone of *BrWRKY7* was obtained from the collection of expressed sequence tags (ESTs) in our institute. Its cDNA clone is 1,037 bp long in nucleotides and encodes a protein of 307 amino acids. Its nucleotide sequences and deduced amino acid sequences are shown in Fig. 1. The WRKY domain of *BrWRKY7* is underlined. To investigate the function of *BrWRKY7*, a phylogenetic tree was constructed based on WRKY domain sequences (60 amino acids) of WRKY proteins as shown in Fig. 2. *BrWRKY7* belongs to group IId. Seven members of *AtWRKY* proteins (*AtWRKY7*, *AtWRKY11*, *AtWRKY15*, *AtWRKY17*, *AtWRKY21*, *AtWRKY39*, and *AtWRKY74*) belong to group IId (Fig. 2). According to the phylogenetic tree shown in Fig. 2, *BrWRKY7* is a *Brassica rapa* homolog of *AtWRKY7*. To investigate the percentage of identity of two

WRKY proteins, we aligned amino acid sequences of their full length proteins by ClustalW in DNASTar software Package (Fig. 3). *BrWRKY7* showed 76.5% identity with *AtWRKY7* at amino acid level. Eulgem et al. (2000) reported that group IId WRKY proteins contain a calmodulin binding domain and HARF domain. Fig. 3 showed that these two domains are completely conserved in *BrWRKY7* and *AtWRKY7*, suggesting that *BrWRKY7* functions in a similar way with *AtWRKY7*. However, we cannot exclude the possibility of a different function with *AtWRKY7* because 28 amino acids stretch is not found in the N-terminus of *BrWRKY7* whereas it is present in *AtWRKY7*.

Among seven members of group IId *AtWRKY* proteins the expression of *AtWRKY7*, *AtWRKY11*, *AtWRKY15*, and *AtWRKY17* is strongly induced, whereas the expression of *AtWRKY21*, *AtWRKY39*, *AtWRKY74* is not induced upon *Pseudomonas syringae* pv. *tomato* (*Pst*) infection (Journot-Catalino et al., 2006). The expression profile of *AtWRKY7*, *AtWRKY11*, and *AtWRKY17* are similar whereas the other group II *AtWRKYs* is dissimilar. *AtWRKY7*, *AtWRKY11*, and *AtWRKY17* are reported to be negative regulators in defense signaling against *Pst* infection (Journot-Catalino et al., 2006; Kim et al., 2006). Kim and his colleagues (2006) also demonstrated that *AtWRKY7* is a transcriptional repressor and enhances susceptibility to *Pst* by analyzing T-DNA and RNA silencing *wrky7* mutants. Fig. 4 showed the expression pattern of *BrWRKY7* upon pathogen infection. The transcript level of *BrWRKY7* was induced at 6 hr post-inoculation (hpi) (Fig. 4) and also induced at 6 hr after treatment with SA (Fig. 4).

In conclusion, we demonstrated that *BrWRKY7* was up-regulated upon pathogen infection as *AtWRKY7* and besides a WRKY domain *BrWRKY7* shared two important conserved domains with *AtWRKY7*. This suggests that

Fig. 2. Phylogenetic tree of *BrWRKY7* with other *AtWRKY* proteins. The phylogenetic tree of *BrWRKY7* with other *AtWRKY* proteins was generated using the neighbor-joining method after alignment of the WRKY domain sequences of *AtWRKY6*, *AtWRKY61*, *AtWRKY18*, *AtWRKY60*, *AtWRKY45C*, *AtWRKY51*, *AtWRKY25N*, *AtWRKY7*, *AtWRKY15*, *AtWRKY11*, *AtWRKY22*, *AtWRKY29*, *AtWRKY64*, *AtWRKY70* with ClustalW. Distance in the tree corresponds to evolutionary distance.

Fig. 3. Comparisons of their amino acid sequences between *AtWRKY7* and *BrWRKY7*. Amino acid sequences of two proteins were aligned by ClustalW in DNASTar software package. Identical amino acids are in boxes.

Fig. 4. Expression patterns of *BrWRKY7* in *SA/Xcc* treated chinese cabbage leaves. Three-week-old chinese cabbage seedlings were infected with *Xcc* and treated with *SA*. Immediately after treatment of chinese cabbage seedlings, leaves were harvested as a sample at 0 hr and the rest of the samples were taken at 6, 12, 24, and 48 hr. Total RNA was isolated from each sample and RT-PCR was performed using *BrWRKY7*-specific primer pairs. Transcript levels of *BrActin* show that equal amounts of RNA were used in the RT-PCR samples.

BrWRKY7 appears to act as a negative regulator in defense signaling in relation to pathogen and *SA* as *AtWRKY7*. To verify this hypothesis on the function of *BrWRKY7*, ectopic expression of *BrWRKY7* should be tried and complementation by over-expression of *BrWRKY7* into T-DNA *wrky7* mutant should also be tried in near future.

Accession Numbers

Locus identifiers of the genes from this article are as follows: At1g62300 (*WRKY6*), At1g18860 (*WRKY61*), At4g31800 (*WRKY18*), At2g25000 (*WRKY60*), At3g01970 (*WRKY45*), At5g64810 (*WRKY51*), At2g30250 (*WRKY25*),

At4g24240 (*WRKY7*), At2g23320 (*WRKY15*), At4g31550 (*WRKY11*), At4g01250 (*WRKY22*), At4g23550 (*WRKY29*), At1g66560 (*WRKY64*), At3g56400 (*WRKY70*).

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