Journal of Biochemistry and Molecular Biology, Vol. 40, No. 6, November 2007, pp. 952-958

JOURNAL OF Biochemistry and Molecular Biology

Characterization of a Stress-Responsive Ankyrin Repeat-Containing Zinc Finger Protein of *Capsicum annuum* (CaKR1)

Eun Soo Seong[1,2,#], Doil Choi[2,3,*], Hye Sun Cho[2,#], Chun Keum Lim[4], Hye Jeong Cho[1] and Myeong-Hyeon Wang[1,*]

[1]School of Biotechnology, Kangwon National University, Chuncheon, Kangwon-do, 200-701, South Korea
[2]Plant Genome Research Center, Korea Research Institute of Bioscience and Biotechnology, Taejeon 305-600, South Korea
[3]Department of Plant Sciences, College of Agricultural and Life Sciences, Seoul National University, Seoul 151-742, South Korea
[4]College of Agriculture and Life Sciences, Kangwon National University, Chuncheon, 200-701, South Korea

Received 30 May 2007, Accepted 16 July 2007

We isolated many genes induced from pepper cDNA microarray data following their infection with the soybean pustule pathogen *Xanthomonas axonopodis* pv. glycines 8ra. A full-length cDNA clone of the *Capsicum annuum* ankyrin-repeat domain C_3H_1 zinc finger protein (CaKR1) was identified in a chili pepper using the expressed sequence tag (EST) database. The deduced amino acid sequence of CaKR1 showed a significant sequence similarity (46%) to the ankyrin-repeat protein in very diverse family of proteins of *Arabidopsis*. The gene was induced in response to various biotic and abiotic stresses in the pepper leaves, as well as by an incompatible pathogen, such as salicylic acid (SA) and ethephon. CaKR1 expression was highest in the root and flower, and its expression was induced by treatment with agents such as NaCl and methyl viologen, as well as by cold stresses. These results showed that CaKR1 fusion with soluble, modified green fluorescent protein (smGFP) was localized to the cytosol in *Arabidopsis* protoplasts, suggesting that CaKR1 might be involved in responses to both biotic and abiotic stresses in pepper plants.

Keywords: Abiotic stress, biotic stress, CaKR1, methyl viologen, pepper plants

[#]These authors are equally contributed to this work.

Abbreviations: AKR, *Arabidopsis* ankyrin repeat; AKRP, *Arabidopsis* ankyrin repeat protein; ANK, Ankyrin; smGFP, green fluorescent protein; SA, salicylic acid; CaKR1, *Capsicum annuum* ankyrin-repeat-domain C_3H_1 zinc finger protein; PR, pathogenesis-related; HR, hypersensitivity response; JA, jasmonic acid.

*To whom correspondence should be addressed.
Tel: 82-33-250-6486; Fax: 82-33-241-6480
E-mail: mhwang@kangwon.ac.kr (Wang, M.H)
Tel: 82-2-880-4568; Fax: 82-2-873-2056
E-mail: doil@snu.ac.kr (Choi, D.)

Introduction

Plants are subject to numerous biotic and abiotic stresses, including attacks by various pathogens and environment through their life cycle. To survive these stresses, plants have developed a diverse and complex set of defense mechanisms (Dixon and Lamb, 1990; Dangl and Jones, 2001). Plants respond by triggering specific signal transduction cascades that result in the induction of genes involved in the defense response and the production of antimicrobial compounds (Yang *et al*., 1997; La Camera *et al*., 2004). Salicylic acid (SA) and ethylene are important secondary signals in the plant defense response against pathogens (Ecker and Davis, 1987; Durner *et al*., 1997). Abscisic acid (ABA) is also involved in the regulation of defense-related signaling in response to biotic stresses (Mauchi-Mani and Mauch, 2005).

An ankyrin repeat-containing gene has been named AKR (*Arabidopsis* anKyrin Repeat), and the protein it encodes has been designated AKRP (*Arabidopsis* anKyrin Repeat Protein) (Zhang *et al*., 1992). The ankyrin (ANK) repeat is a 33-amino acid motif that appears 22 times in tandem in the 89 K domain of the human protein ankyrin (Lux *et al*., 1990). The proteins containing ANK repeats have determined the presence of 105 genes encoding and ANK repeats proteins in the other classes possess additional motifs such as transmembrane domains, kinase signatures, zinc or ring fingers (Becerra *et al*., 2004). The major role of plant ANK repeat proteins is related to signaling in defense (Cao *et al*., 1997) and development mechanisms (Li and Chye, 2004; Hemsley *et al*., 2005). A protein (EMB506) containing ANK repeats organized in tandem, and played a role for embryogenesis and proplastid differentiation (Despres *et al*., 2001). ANK repeats have been found in proteins functions, such as cell cycle regulation, mitochondria, cytoskeleton interactions, and signal transduction (Sedgwick and Smerdon, 1999). The *Arabidopsis* ANK repeat-containing protein may be involved in the regulation of

antioxidation metabolism by both disease resistance and stress responses (Yan *et al*., 2002).

In the present study, we isolated a *Capsicum annuum* ankyrin-repeat domain C_3H_1 zinc finger protein, CaKR1, from chili pepper inoculated with *Xanthomonas axonopodis* pv. glycines 8ra (*X. ag* 8ra) (Hwang *et al*., 1992). Northern blot analyses revealed that the expression of CaKR1 was rapidly and preferentially induced during the HR of chili pepper to the incompatible interactions with the bacterial pathogens. CaKR1 is targeted to the cytosol of protoplasts from our experiments on cellular localization, providing that the role of CaKR1 is involved in the both biotic and abiotic stress responses in pepper.

Materials and Methods

Plant material and treatment. Chili pepper (*Capsicum annuum*) 'Bukang' seeds were cultured in MS (Murashige and Skoog) medium (MS salts including MS vitamins, 3% sucrose, 0.8% agar, pH 5.8). The germinated plants were transferred to pots and kept in a growth chamber at 24°C for 4 weeks. The bacterial pathogen used for inoculation was *X. axonopodis* pv. glycines 8ra (*X. ag* 8ra), a soy bean pustule pathogen (Hwang *et al*., 1992). Bacterial infiltration was accomplished by syringe infiltration of bacterial suspensions (approximately 4×10^8 cfu/ml). The leaves were detached in a sterilized water solution containing 5 mM SA, 5 mM ethephon and 100 μM MeJA. They were placed in distilled water and kept in a 4°C cold chamber under dim light for 24 h, and they were incubated in 0.25 M NaCl and 50 μM MV (Methyl Viologen) for 24 h. The ABA stock solution was prepared by dissolving ABA in small aliquots of 1 N NaOH. The stock was diluted to 10^{-3} M with distilled water and adjusted to pH 6.0 with 0.1 N HCl. 10^{-4} and 10^{-5} M ABA solutions were concocted by further dilution. The ABA solutions were applied to detached leaves through their petiole.

Multiple amino acid sequence alignment. Multiple alignments of CaKR1 homologs were generated using http://us.expasy.org/tools. The accession numbers are: NP 200670 (*Arabidopsis thaliana*); ABE84364 (*Medicago truncatula*); XP469392 (*Oryza sativa*).

RNA Analyses. The total RNA was isolated from the pepper [subjected to *X. ag* 8ra, SA, MeJA (Methyl Jasmonic acid), ET, NaCl, cold, MV, ABA or various tissues] (Chomczynski and Sacchi, 1987; Yi *et al*., 2004). Plant materials (1-1.5 g) were frozen in liquid nitrogen and homogenized in 10 ml extraction buffer [4 M guanidine isothiocyanate, 25 mM sodium citrate at pH 7.0, 0.55% (w/v) N-laurylsarcosine and 0.1 M 2-mercaptoethanol]. A mixture of 2 M sodium acetate (pH 4.0), water saturated phenol and chloroform-isoamylalcohol (24 : 1) was added to the homogenate. After centrifugation, the pellet was suspended in 2 M LiCl solution and incubated at 4°C for 18 h. The total RNA concentration and purity were determined by spectrophotometer and staining of the ribosomal RNA with ethidium bromide, respectively. Equal quantities of the total RNA (20 μg) were loaded into 1% agarose gel containing 7.4% formaldehyde. The RNA was transferred onto nylon membranes (Hybond N^+, Amersham), then crosslinked under irradiation with UV light. To generate a *CaKR1*-specific probe, each coding sequence was PCR-amplified with two primers: (5'-ATGTGTAGTGGTTCCGGGA-3' and 5'-ATGCACAATCTGCTC CTGCT-3') for *CaKR1*. Hybridization was performed overnight at 65°C in 5% dextran sulfate, 0.25 M disodium phosphate (pH 7.2), 7% (w/v) SDS and 1 mM EDTA. After hybridization, the filter was washed twice with 2 × SSC and 0.1% SDS for 10 min each at room temperature, and twice with 0.1 × SSC and 0.1% SDS for 5 min each at 65°C.

Localization of CaKR1-smGFP fusion protein. *CaKR1* was prepared by amplification using forward primer (5'-ACAGGATCC AAAGAGTAAAGAAGAACAGGATGTGTAGTGGTTCCGG-3') and reverse primer (5'-ATAGGATCCACCGCCTTCTAGCGTTTT CACCAGGGGGAACAAATG-3'). The C-terminus of the amplified *CaKR1* fragment was fused to the N-terminal region of the smGFP (green fluorescent protein) expression vector (David and Vierstra, 1996), and the 35S-smGFP was constructed as a control. For transient expression, plasmid DNA (4 μg each of p35S::CaKR1-sm GFP and p 35S::sm GFP) was introduced into the protoplast of *Arabidopsis*, and incubated for 12 h at 25°C. The fusion construct was introduced into *Arabidopsis* protoplasts prepared from whole seedlings by the polyethylene glycol-mediated transformation procedure (Kang *et al*., 1998) for transient expression of smGFP:CaKR1. Fluorescence images were captured using an UV light fluorescence microscope (ZEISS, Axioskop, Germany) fitted with fluorescein isothiocyanate filters (excitation filter 520 nm and emission filter 488 nm).

Results and Discussion

Sequence analysis of *CaKR1*. A pepper cDNA microarray was probed with RNA extracted from hot pepper leaves infected by *X. ag* 8ra to isolate pepper genes induced during the non-host bacterial pathogen HR. *X. ag* 8ra is not a pathogen of pepper, but induce the expression of a number of PR genes, as well as does occur an HR in pepper leaves (Lee *et al*., 2004), suggesting that 40 (out of 350 total ESTs) gene sequences were up-regulated more than 2-fold following *X. ag* infiltration. One of the upregulated genes showed high sequence similarity to *Arabidopsis* ankyrin repeats protein that designated as a *CaKR1*. Sequence analysis of the 2.5 kb EST clone of *CaKR1* revealed that the cDNA was cloned into T vector and then sequenced. The complete sequence of *CaKR1* contained an open reading frame encoding a putative 597 amino acid protein. (Fig. 1). Nucleotide and protein database searches showed that CaKR1 has a 98 amino acid ANK domain that includes a 26 amino acid C_3H_1 zinc finger domain (Fig. 1). CaKR1 has a 46% and a 40% identity with the ANK-domain proteins of *Arabidopsis* (accession no. NP200670) and of rice (accession no. XP469392), respectively. By evaluating the sequence conservation, it became obvious that the terminal repeats of the arrays deviated from the general consensus and the same tendency has been observed in animal proteins (Bork, 1993). The conservation of the hydrophobic positions in *Arabidopsis* suggests that ANK repeats might

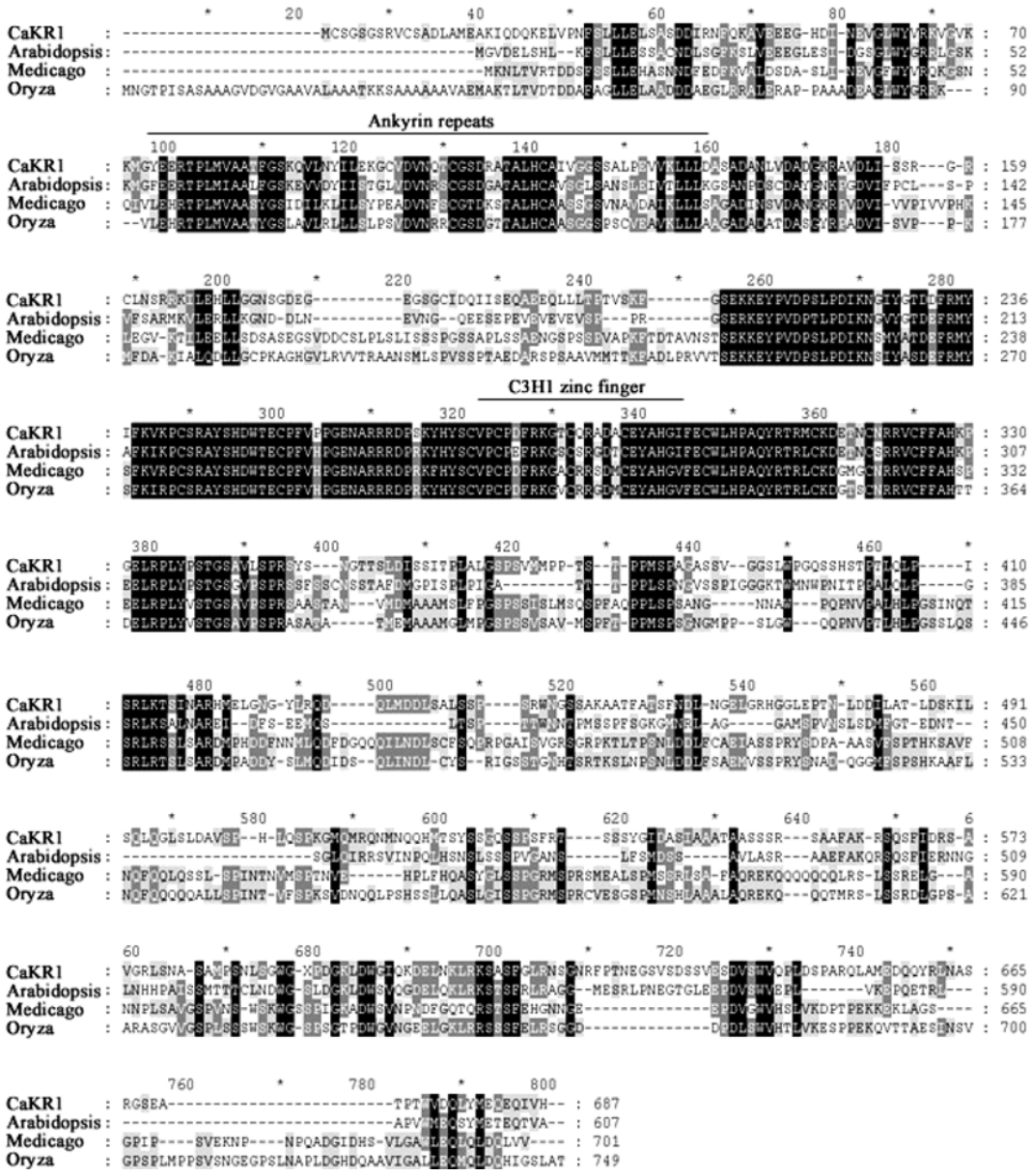

Fig. 1. Comparison of the deduced amino acid sequences of ankyrin-repeat-containing proteins having high sequence similarity to CaKR1. The black bar above the sequences represents the ankyrin repeats and the C_3H_1 zinc finger domain. Dashes indicate gaps used to optimize the alignment. The GenBank, DDBJ, EMBL, and NCBI accession numbers of the nucleotide sequences are as follows: DQ862464 (pepper cDNA [CaKR1]); NP200670 (*Arabidopsis* cDNA); ABE84364 (Medicargo cDNA), and XP469392 (*Oryza sativa* cDNA).

have similar functions in plants and animals. All the ANK repeats in *Arabidopsis* proteins were identified a total of 509 ANK repeats coded by 105 genes. Six genes of these proteins encode amino acids with ANK repeats and zinc finger domains. They are divided into two families (E1 and E2) (Becerra *et al*., 2004). Family E1 contains five genes coding proteins with two or three ANK repeats in the N-terminal region and one or two zinc-fingers in the central part of the protein (Becerra *et al*. 2004). Similar proteins are present in *Oryza sativa* and *Medicago* from our alignments (Fig. 1). The E2 family has an array of six ANK repeats. The functions of none of these proteins have been determined, but expression to *Botrytis* infection had analyzed. ZFAR1 encodes a protein harboring ankyrin repeat domains (AbuQamar *et al*. 2006).

Organ-specific expression of *CaKR1*. Northern blot analysis with RNA isolated from various tissues of pepper was performed with a *CaKR1* probe (Fig. 2). There was a signal in the root and flower tissues of non-stressed plants, but no signal in non-stressed leaves and stems. Some of the Ankyrin repeats genes seem to have more specific patterns of expression (Becerra *et al*. 2004). For example, EN14 and

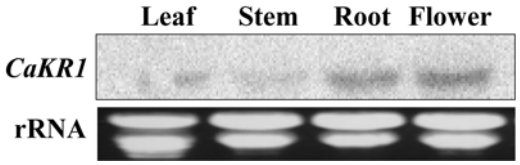

Fig. 2. Tissue RNA expression of the *CaKR1* gene. *CaKR1* RNA (20 μg) levels were monitored in chili pepper leaves, stem, roots and flowers. The size of *CaKR1* is 2.1 kb.

EN17 genes amplified only in roots and EN11 gene detected only in leaves. The pattern of expression of EN11 is consistent with ACD6 gene reported by Lu *et al*. (2005).

***CaKR1* is induced by an incompatible pathogen.** To determine if CaKR1 induction results from bacterial infection, the time course of gene transcript accumulation was analyzed by RNA blotting (Fig. 3A). The level of *CaKR1* transcripts increased following pathogen infection (Fig. 3A), indicating that CaKR1 is induced by incompatible pathogen interactions in leaf tissue of pepper. The *Capsicum annuum* pathogen-related gene (*CaPR1*) is also induced by various stresses in chili pepper (Kim *et al*., 2005). Interactions between incompatible pathogens and plants lead to rapid cell death that prevents the spread of harmful pathogens to neighboring cells (Heath, 2000). The HR response affects the levels of signal molecules, such as SA, jasmonic acid (JA), and ethylene (Reymond and Farmer, 1998). CaKR1 expression was strongly induced by treatment with SA and ethephon (an ethylene generator), and weakly induced by MeJA (Fig. 3B, C, and D). This result indicated that CaKR1 is expressed and functions earlier than that of CaPR-1 in response to SA exposure. *Capsicum annuum Pin* II (*CaPIN* II), the positive control for the MeJA treatment, significantly increased the expression level after 3 h of MeJA treatment. *CaAccoxidase*, the positive control for the ethephon treatment (Chung *et al*., 2003), was induced after 6 h of ethephon treatment, indicating that *CaKR1* transcription is regulated by incompatible pathogens as well as plant stress-related chemicals. The ACD6 protein, which contains an ANK-repeat domain, is an essential positive regulator of the defense response in *Arabidopsis*. The best-characterized ANK protein in plants, PR1/noninducible immunity 1 (NPR1/NIM1), is involved in SA-dependent disease resistance and in SA-independent resistance responses elicited by certain root-associated bacteria (Pieterse *et al*., 1998).

***CaKR1* is induced by abiotic stresses.** Detached leaves of chili pepper plants were subjected to abiotic salt stress (NaCl, 0.4 M). *CaKR1* transcript levels increased in response to a high-salt concentration (Fig. 4A) and were strongly induced by 24 h storage in a cold chamber (Fig. 4B). The *Capsicum annuum* dehydrin gene (*Cadhn*) is a marker gene that is regulated by abiotic stresses in chili pepper plants. *Cadhn* is responsive to ABA treatment, and its transcripts are abundant in green pepper fruits (Chung *et al*., 2003). There was a high level of expression up to 6 h after exposure to methyl viologen. Exposure of plants to abiotic stress factors, such as water deficit and a high concentration of salt, results in elevated ABA biosynthesis (Cohen and Bray, 1990), and increased ABA induces a number of genes (Bray, 1993). Expression of CaKR1 was also induced following treatment of chili pepper leaves with 100 μM ABA (Fig. 4D). However, induction by ABA was lower than in the samples exposed to NaCl, methyl viologen or cold (Fig. 4). The role of ABA was reported that it can positively or negatively regulate plant defense signaling pathways (Mauchi-Mani and Mauch, 2005), but the importance of ABA in plant resistance responses is still unknown.

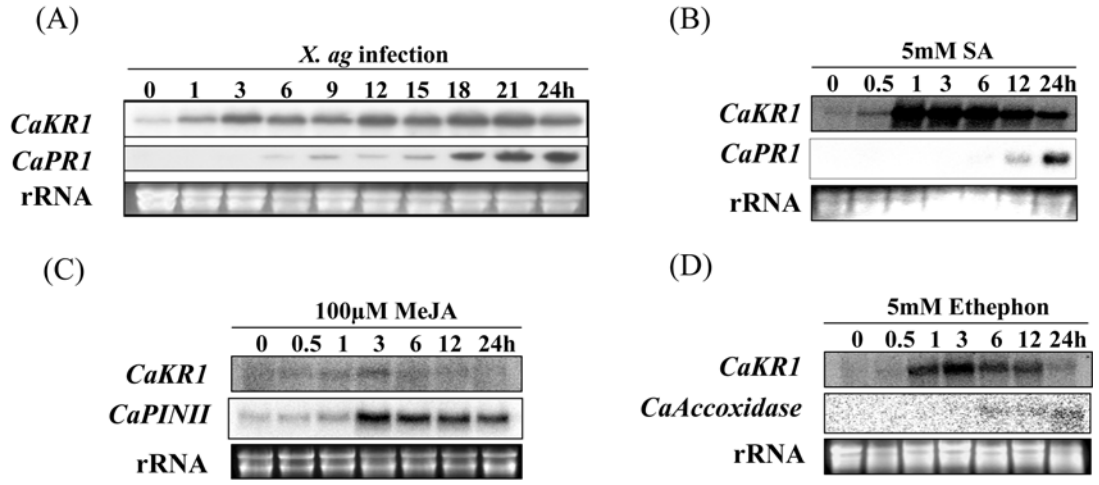

Fig. 3. Expression of *CaKR1* mRNA in response to bacterial pathogens and chemicals. (A) Pepper leaves were infiltrated with *X. axonopodis* pv glycines 8ra (non-host pathogen). The *CaPR1* (*Capsicum annuum P*athogenesis related protein *1*) gene was used as a positive control. (B-D) Expression of CaKR1 mRNA (2.1 kb) following treatment with SA (salicylic acid), MJ (methyl jasmonic acid) or ethephon. *CaPR1*, *CaPINII* and *CaAccoxidase* used as positive control markers in chemical treatments of pepper. These results were assayed three times.

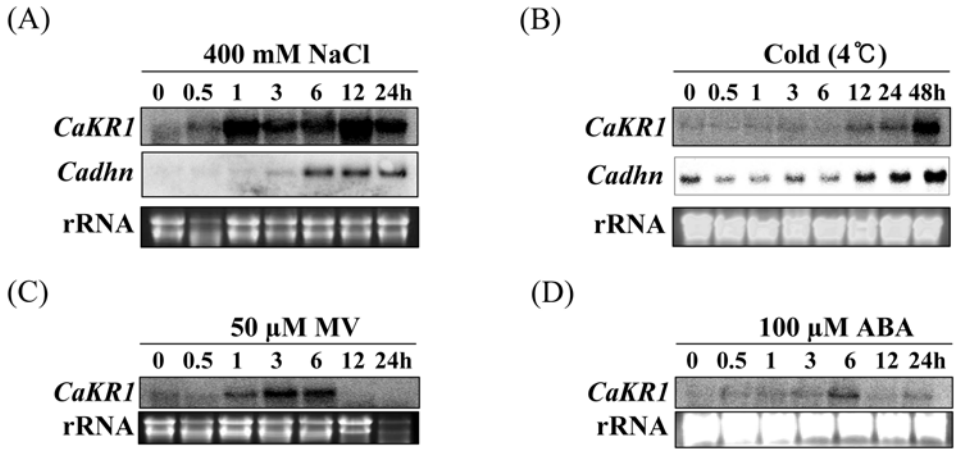

Fig. 4. Expression of *CaKR1* mRNA in response to abiotic stress. The total RNA(A-D) was prepared from 2-month-old pepper plants transferred to a cold chamber and subjected to 0.4 M NaCl, 50 μM methyl viologen, and 100 μM ABA. *Cadehydrin (Cadhn)* served as positive control marker. The total RNA was extracted from the leaf tissue at the times indicated. The size of *CaKR1* is 2.1 kb.

The stress response in plants is evoked by the production of ROS (reactive oxygen species), which include O_2^-, H_2O_2, and OH radical (Salin, 1988), and AKR2 can affect ROS production (Yan *et al*., 2002). The OH radical can be oxidized many cellular components and cause cellular damage (Cheeseman and Slater, 1993). Therefore, it is essential for plants to scavenge H_2O_2 from cells, but H_2O_2 serves as a messenger to trigger defense gene expression in host plant cells during pathogen infection (Levine *et al*., 1994). Accurate regulation in H_2O_2 production and destruction is critically important to the survival of plants, and it is governed by mechanisms that can involve antioxidant proteins and novel signal transduction pathways. The functions of ZFAR1, zinc finger protein harboring ankyrin repeats domain, was determined to increased salinity, ABA, and oxidative stress generated by paraquat (AbuQamar *et al*., 2006). Treatment with MV results in the formation of chloroplast-associated ROS species and is used to study general plant stress responses (Fujibe *et al*., 2003). Responses to various stresses, such as wounding, pathogens, cold, drought, salt, and high-light stress, were identified to the transcriptional level in a broad variety of stress response pathways. A model that integrates the multiple pathways involved in salt, drought, and cold responses have been published (Cheong *et al*., 2003).

CaKR1-smGFP fusion proteins are targeted to the cytosol of *Arabidopsis* protoplasts. To investigate the cellular location of CaKR1, we performed an *in vivo* targeting experiment employing CaKR1 fused to a modified form of smGFP (David and Vierstra, 1996). Protoplasts of *Arabidopsis* were transformed with the 35S-CaKR1-smGFP construct, or with 35S-smGFP alone (Fig. 5A). GFP fluorescence in the epidermal cells containing the 35S-CaKR1-smGFP construct was detected exclusively in the cytosol (Fig. 5B). NPR1 localizes to the nucleus and functions as a transcriptional coactivator to regulate the defense response (Fan and Dong, 2002).

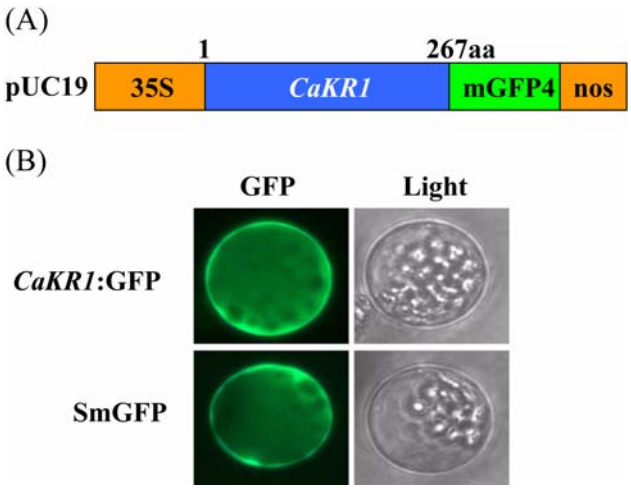

Fig. 5. The fusion construct CaKR1-smGFP and its subcellular localization. (A) *CaKR1* cDNA as a template with forward primer and reverse primer. The *CaKR1* coding region was fused in-frame to the N-terminus of the smGFP protein. The fusion protein is driven by the 35S CaMV promoter. (B) smGFP (lower panels) and CaKR1-smGFP (upper panels) were introduced into *Arabidopsis* protoplasts. Expression of the introduced genes was examined after 24 h using a fluorescence microscope. The results were assayed three times.

The LlANK protein containing five tandem ankyrin repeats and a RING zinc-finger domain possesses ubiquitin ligase activity *in vitro* (Huang *et al*., 2006). The primary function of ubiquitin ligase was the rapid degradation of proteins with abnormal conformations and of many regulatory proteins to facilitate in the regulation of their activities (Weissman, 2001). We expect that CaKR1 is possible role of E3 ligase activity, but none of it has been characterized in this study. In conclusions, plant ANK domain proteins are involved in signaling pathways (Lu *et al*., 2005), stress responses

(Chinchilla *et al*., 2003), and plant defense (Kuhlmann, 2003). We identified a 98 amino acid region containing ANK repeats in CaKR1 isolated from chili pepper. The ANK repeat protein of *Arabidopsis* is transiently down-regulated transcriptionally by pathogen attack (Kuhlmann, 2003). Ankyrin1 homologous proteins function in pathogen defense (Kuhlmann, 2003). We found that the *CaKR1* transcript levels in pepper leaves increased in response to infection with an incompatible pathogen, *X. ag 8ra*, as well as to abiotic stresses. In this report, we show that CaKR1 is regulated by both abiotic and biotic stresses in pepper plants, suggesting that that it is involved in the signaling pathway. Understanding of how CaKR1 functions in plant defense and environmental stress responses should provide insights into plant disease resistance and abiotic stress tolerance.

Acknowledgment This research was supported by the grant from Research Institute of Agricultural Sciences at Kangwon National University.

References

AbuQamar, S., Chen, X., Dhawan, R., Bluhm, B., Salmeron, J., Lam, S., Dietrich, R. A. and Mengiste, T. (2006) Expression profiling and mutant analysis reveals complex regulatory networks involved in *Arabidopsis* response to *Botrytis* infection. *Plant J.* **48**, 28-44.

Becerra, C., Jahrmann, T., Puigdomenech, P. and Vicient, C. M. (2004) Ankyrin repeat-containing proteins in *Arabidopsis*: characterization of a novel and abundant group of genes coding ankyrin-transmembrane proteins. *Gene* **340**, 111-121.

Bork, P. (1993) Hundreds of ankyrin-like repeats in functionally diverse proteins: mobile modules that cross phyla horizontally? *Proteins* **17**, 363-374.

Bray, E. A. (1993) Molecular responses to water deficit. *Plant Physiol.* **103**, 1035-1040.

Cao, H., Glazebrook, J., Clarke, J. D., Volko, S. and Dong, X. (1997) The *Arabidopsis NPR1*gene that controls systemic acquired resistance encodes a novel protein containing ankyrin repeats. *Cell* **88**, 57-63.

Cheeseman, K. H. and Slater, T. F. (1993) An introduction to free radical biochemistry. *Br. Med. Bull.* **49**, 481-493.

Cheong, Y. H., Chang, H. S., Gupta, R., Wang, X., Zhu, X. and Luan, S. (2003) Transcriptional profiling reveals novel interactions between wounding, pathogen, abiotic stress, and hormonal responses in *Arabidopsis*. *Plant Physiol.* **129**, 661-677.

Chinchilla, D., Merchan, F., Megias, M., Kondorosi, A., Sousa, C. and Crespi, M. (2003) Ankyrin protein kinases: a novel type of plant kinase gene whose expression is induced by osmotic stress in alfalfa. *Plant Mol. Biol.* **51**, 555-566.

Chomczynski, P. and Sacchi, N. (1987) Single step method of RNA isolation by acid guanidium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.* **162**, 156-159.

Chung, E., Kim, S. Y., Yi, S. Y. and Choi, D. (2003) *Capsicum annuum dehydrin*, an osmotic-stress gene in chili pepper plants. *Mol. Cells* **15**, 327-332.

Cohen, A. and Bray, E. A. (1990) Characterization of three mRNAs that accumulate in wilted tomato leaves in response to elevated levels of endogenous abscisic acid. *Planta* **182**, 27-33.

Dangl, J. L. and Jones, J. D. G. (2001) Plant pathogens and integrated defense responses to infection. *Nature* **411**, 826-833.

David, S. J. and Vierstra, R. D. (1996) Soluble derivatives of green fluorescent protein (GFP) for use in *Arabidopsis thaliana*. *Weeds World* **3**, 43-48.

Despres, B., Delseny, M. and Devic, M. (2001) Partial complementation of embryo defective mutations: a general strategy to elucidate gene function. *Plant J.* **27**, 149-159.

Dixon, R. A. and Lamb, C. J. (1990) Molecular communication in interactions between plants and microbial pathogens. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **41**, 339-367.

Durner, J., Shah, J. and Klessig, D. F. (1997) Salicylic acid and disease resistance in plants. *Trends Plant Sci.* **2**, 266-274.

Ecker, J. and Davis, R. W. (1987) Plant defense genes are regulated by ethylene. *Proc. Natl. Acad. Sci. USA* **84**, 5202-5206.

Fan, W. and Dong, X. (2002) In vivo interaction between NPR1 and transcription factor TGA2 leads to salicylic acid-mediated gene activation in *Arabidopsis*. *Plant Cell* **14**, 1377-1389.

Fujibe, T., Saii, H., Arakawa, K., Yabe, N., Takeuchi, Y. and Yamamoto, K. T. (2003) A methyl viologen-resistant mutant of *Arabidopsis*, which is allelic to ozone-sensitive rcd1, is tolerant to supplemental ultraviolet-B irradiation. *Plant Physiol.* **134**, 275-285.

Heath, M. C. (2000) Hypersensitive response-related death. *Plant Mol. Biol.* **44**, 321-334.

Hemsley, P. A., Kemp, A. C. and Grierson, C. S. (2005) The TIP GROWTH DEFECTIVE1S-acyl transferase regulates plant cell growth in *Arabidopsis*. *Plant Cell* **17**, 2554-2563.

Huang, J., Chen, F., Casino, C. D., Autino, A., Shen, M., Yuan, S., Peng J., Shi, H., Wang, C., Cresti, M. and Li, Y. (2006) An ankyrin repeat-containing protein, characterized as a ubiquitin ligase, is closely associated with membrane-enclosed organelles and required for pollen germination and pollen tube growth in lily. *Plant Physiol.* **140**, 1374-1383.

Hwang, I., Lim, S. M. and Shaw, P. D. (1992) Cloning and characterization of pathogenecity genes from *Xanthomonas campestris* pv. Glycines. *J. Bacteriol.* **174**, 1919-1923.

Kang, S. G., Jin, J. B., Piao, H. L., Pih, K. T., Jang, H. J., Lim, J. H. and Hwang, I. (1998) Molecular cloning of an *Arabidopsis* cDNA encoding a dynamin-like protein that is localized to plastids. *Plant Mol. Biol.* **38**, 437-447.

Kim, S. Y., Kim, Y. C., Lee, J. H., Oh, S. K., Chung, E., Lee, S., Lee, Y. H., Choi, D. and Park, J. M. (2005) Identification of a CaRAV1 possessing an AP2/ERF and B3 DNA-binding domain from pepper leaves infected with *Xanthomonas axonopodis* pv. glycines 8ra by differential display. *Biochim. Biophy. Acta* **1729**, 141-146.

Kuhlmann, M., Horvay, K., Strathmann, A., Heinekamp, T., Fischer, U., Bottner, S. and Droge-Laser, W. (2003) The alpha-helical D1 domain of the tobacco bZIP transcription factor BZI-1 interacts with the ankyrin-repeat protein ANK1 and is important for BZI-1 function, both in auxin signaling and pathogen response. *J. Biol. Chem.* **278**, 8786-8794.

La Camera, S., Gouzerh, G., Dhondt, S., Hoffmann, L., Fritig, B., Legrand, M. and Heitz, T. (2004) Metabolic reprogramming in plant innate immunity: the contributions of phenylpropanoid and oxylipin pathways. *Immunol. Rev.* **198**, 267-284.

Lee, S., Kim, S. Y., Chung, E., Joung, Y. H., Pai, H. S., Hur, C. G

and Choi, D. (2004) EST and microarray analyses of pathogen-responsive genes in hot pepper (*Capsicum annuum* L.) non-host resistance against soybean pustule pathogen (*Xanthomonas axonopodis* pv. glycines). *Func. Integr. Genomic* **4**, 196-205.

Levine, A., Tenhaken, R., Dixon, R. and Lamb, C. (1994) H_2O_2 from the oxidative burst orchestrates the plant hypersensitive disease resistance response. *Cell* **79**, 583-593.

Li, H. Y. and Chye, M. L. (2004) *Arabidopsis* acyl-coA-binding protein ACBP2 interacts with an ethylene-responsive element-binding protein, AtEBP, via its ankyrin repeats. *Plant Mol. Biol.* **54**, 233-243.

Lu, H., Liu, Y. and Greenberg, J. T. (2005) Structure-function analysis of the plasma membranelocalized *Arabidopsis* defense component ACD6. *Plant J.* **44**, 798-809.

Lux, S. E., John, K. M. and Bennett, V. (1990) Analysis of cDNA for human erythrocyte ankyrin indicates a repeated structure with homology to tissue-differentiation and cell-cycle control proteins. *Nature* **344**, 36-42.

Mauchi-Mani, B. and Mauch, F. (2005) The role of abscisic acid in plant-pathogen interactions. *Curr. Opin. Plant Biol.* **8**, 409-414.

Pieterse, C. M., van Wees, S. C., van Pelt, J. A., Knoester, M., Laan, R., Gerrits, H., Weisbeek, P. J. and van Loon, L. C. (1998) A novel signaling pathway controlling induced systemic resistance in *Arabidopsis*. *Plant Cell* **10**, 1571-1580.

Reymond, P. and Farmer, E. E. (1998) Jasmonate and salicylate as global signals for defense gene expression. *Curr. Opin. Plant Biol.* **1**, 404-411.

Salin, M. A. (1988) Toxic oxygen species and protective systems of chloroplast. *Physiol. Plant* **72**, 681-689.

Sedgwick, S. G. and Smerdon, S. J. (1999) The ankyrin repeat: a diversity of interactions on a common structural framework. *Trends Biochem. Sci.* **24**, 311-316.

Weissman, A. M. (2001) Themes and variations on ubiquitination. *Nat. Rev. Mol. Cell. Biol.* **2**, 169-178.

Yan, J., Wang, J. and Zhang, H. (2002) An ankyrin repeat-containing protein plays a role in both disease resistance and antioxidation metabolism. *Plant J.* **29**, 193-202.

Yang, Y., Shah, J. and Klessig, D. F. (1997) Signal perception and transduction in plant defense responses. *Genes Dev.* **11**, 1621-1639.

Yi, S. Y., Kim, J. H., Joung, Y. H., Lee, S., Kim, W. T., Yu, S. H. and Choi, D. (2004) The pepper transcription factor CaPF1 confers pathogen and freezing tolerance in *Arabidopsis*. *Plant Physiol.* **136**, 2862-2874.

Zhang, H., Scheirer, D. C., Fowle, W. H. and Goodmanav, H. M. (1992) Expression of antisense or sense RNA of an ankyrin repeat-containing gene blocks chloroplast differentiation in *Arabidopsis*. *Plant Cell* **4**, 1575-1588.