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# Cloning and expression analysis of pepper chlorophyll catabolite reductase gene *CaRCCR*

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**ABSTRACT.** Opening the porphyrin macrocycle of pheophorbide *a* and forming the primary fluorescent chlorophyll catabolites are key steps in the chlorophyll catabolism pathway. These steps are catalyzed by pheophorbide *a* oxygenase and red chlorophyll catabolite reductase (RCCR). In this study, a novel RCCR gene, *CaRCCR*, was isolated from the pepper (*Capsicum annuum* L.). The full-length *CaRCCR* complementary DNA is comprised of 1173 bp, contains an open reading frame of 945 bp, and encodes a 314-amino acid protein. This deduced protein belongs to the ferredoxin-dependent bilin reductase family. Amino acid sequence alignment showed that *Ca*RCCR shared a high homology to other higher plant RCCR proteins. *CaRCCR* expression, as determined by quantitative real-time polymerase chain reaction, was higher in the leaves than the roots, stems, flowers, and immature fruits. *CaRCCR* expression was almost constant during

 

all phases of leaf development. It was upregulated by abscisic acid, methyl jasmonate, and salicylic acid. Moreover, *CaRCCR* was induced by high salinity and drought stress treatments; it was also slightly regulated by *Phytophthora capsici*. Taken together, these results suggest that *CaRCCR* is involved in defense responses to various stresses.

**Key words:** *Capsicum annuum* L.; *CaRCCR* gene; Expression analysis; Cloning; Stress response

## INTRODUCTION

Leaf senescence is the final stage of leaf development, ultimately leading to the death of the whole leaf. It is governed by developmental age, but it can also be stimulated by diverse environmental factors. Such environmental factors include plant hormones, drought, salinity, extreme temperature, darkness, nutrient limitation, wounding, and pathogen infection (Lim et al., 2007; Guo and Gan, 2012). Early leaf senescence can affect the yield of plants under these adverse environmental conditions. Therefore, studying leaf senescence will not only strengthen our comprehending of the basic biological process, but it may also lead to the discovery of methods that can delay leaf senescence and improve the agricultural traits of a vegetable crop. The pepper (*Capsicum annuum* L.) is an important vegetable crop; it is extensively cultivated throughout the world. In recent years, prematurity of pepper plants caused by various environmental stresses is a universal phenomenon. It is important to research leaf senescence in the pepper.

Loss of green coloration, induced by the degradation of chlorophyll, is the most obvious sign of leaf senescence and widely used for senescence quantification. In recent years, a pathway of chlorophyll catabolism during senescence has been well established (Hörtensteiner, 1999; Kräutler, 2008; Schelbert et al., 2009; Hörtensteiner and Kräutler, 2011; Sakuraba et al., 2012). The pathway starts with the removal of phytol by chlorophyllase (Willstätter and Stoll, 1913), followed by the removal of $Mg^{2+}$ by Mg-dechelatase (Suzuki et al., 2005) to produce pheophorbide (pheide) *a*. The porphyrin macrocycle of the pheide *a* is oxygenolytically cleaved by pheophorbide *a* oxygenase (PAO) (Hörtensteiner et al., 1998), thus forming a red chlorophyll catabolite (RCC) that is subsequently catalyzed to the primary fluorescent chlorophyll catabolites (pFCCs) by RCC reductase (RCCR) (Mühlecker et al., 1997; Rodoni et al., 1997). These chlorophyll degradation steps occur in chloroplasts; then, the resultant pFCC is transported to the vacuoles (Lu et al., 1998; Tommasini et al., 1998), where pFCC is spontaneously converted into nonfluorescent chlorophyll catabolites in an acidic environment (Oberhuber et al., 2003). The key steps in this catabolism pathway are the ring-opening reaction catalyzed by PAO and RCC-mediated reduction. During these steps, chlorophyll catabolites lose their color and phototoxicity (Pružinská et al., 2005; Sugishima et al., 2009). RCCR strongly participates in chlorophyll degradation and leaf senescence; therefore, it is very important for understanding the molecular mechanism of the *RCCR* gene in higher plants.

RCCR is a soluble protein and belongs to the ferredoxin-dependent bilin reductase (FDBR) family. RCCR catalyzes the ferredoxin-dependent reduction of the C20/C1 double bond of RCC to produce pFCC in an intriguing stereospecific manner (Hörtensteiner, 2006; Sugishima et al., 2009). Previous studies found that mutants deficient in RCCR accumulated RCC, resulting in the production of excessive reactive oxygen species and, finally, the light-

independent cell death phenotype (Mach et al., 2001; Průzinská et al., 2007). To date, the *RCCR* gene has been cloned from many higher plants such as *Arabidopsis*, rice, tomato, pear, cabbage, and so on (Mach et al., 2001; Tang et al., 2011; Zhang et al., 2011; Cheng et al., 2012). In *Arabidopsis*, *AtRCCR* is a single-copy gene and identical to the *Arabidopsis* accelerated cell death 1 (*ACD2*) gene. *AtRCCR/AtACD2* is constitutively expressed in chloroplasts; however, in young seedlings, it is loosely associated with mitochondria as well (Mach et al., 2001; Yao et al., 2004; Yao and Greenberg, 2006).

Since little is known about the *CaRCCR* gene during leaf senescence in the pepper, this study was conducted to clone this gene from the pepper plant and analyze its molecular characteristics. To learn more about the expression patterns of *CaRCCR*, analyses of tissue-specific expression and various stress-induced expression patterns via quantitative real-time polymerase chain reaction (PCR) were performed. This study will provide the basis for further studies on the function of RCCR in the pepper.

## MATERIAL AND METHODS

### Plant material and growth conditions

In this study, the pepper cultivar B12 was used for cloning and expression analysis of the pepper *CaRCCR* gene. This cultivar, which is an early maturing variety, was selected by the pepper research group in the College of Horticulture, Northwest A&F University, China. The seeds were treated with warm water (55°C) for 20 min and then put in an incubator at 28°C to accelerate germination under the dark condition. The seeds were rinsed twice every day until budding. The germinated seeds were sown in pots containing compost. Seedlings were placed in a growth chamber for a 16-h light/8-h dark cycle at 25°/21°C, respectively.

### *CaRCCR* gene expression pattern analysis

#### *Tissue-specific expression of the CaRCCR gene*

To evaluate the *CaRCCR* gene expression levels in different tissues, samples were collected from the root, stem, leaf, flower, and immature fruit of untreated B12 pepper cultivars. To examine the *CaRCCR* expression pattern during leaf development, leaves were harvested at the young, fully expanded, and senescent stages. All samples were frozen in liquid nitrogen and maintained at -80°C prior to RNA extraction.

#### *Stress treatments*

The signaling molecule, and abiotic and biotic stresses were used to treat the 6-leaf stage pepper plants. For the plant signaling molecule treatments, 3 plant hormones were applied, and the pepper plant leaves were sprayed with 0.57 mM abscisic acid (ABA), 5 mM salicylic acid (SA), and 1 M methyl jasmonate (MeJA), which were dissolved in 0.05% (v/v) ethanol. The control plants were treated with distilled water that contained 0.05% (v/v) ethanol. After 0-, 2-, 4-, 8-, 12-, and 24-h treatments, the pepper leaves were sampled. For salt and drought stress treatments, the pepper plants were uprooted from the soil, and their roots

were soaked in 400 mM sodium chloride (NaCl) and 400 mM mannitol, respectively (Wang et al., 2013b). The control seedlings were treated with sterile water. After the 0-, 2-, 4-, 8-, 12-, and 24-h treatments, the pepper leaves were collected. For the fungal pathogen treatment, pepper plants were inoculated with *Phytophthora capsici* (HX-9 strain) by the root-drenching method; the control plants were treated with sterile distilled water (Wang et al., 2013a). The inoculated and control plants were grown in a growth chamber at 28°C under a 16-h light/8-h dark photoperiod cycle with 60% relative humidity. The samples were collected at 0-, 3-, 6-, 12-, 24-, 48-, 72-, and 96-h intervals.

All plants that were treated with the different chemicals and stress elicitors, and the control plants were sampled at the above mentioned times, immediately frozen in liquid nitrogen, and maintained at -80°C prior to RNA extraction.

## Cloning and sequence analysis of the *CaRCCR* gene

For cloning of the *CaRCCR* gene, total RNA was extracted from green mature leaves using the TRIzol (Invitrogen) method, and first-strand complementary DNA (cDNA) synthesis was performed using the Smart RACE cDNA Amplification Kit (Clontech). The reported nucleotide sequence of *Solanum lycopersicum RCCR* (accession No. XM004234620) was used as the query probe to retrieve homologous expressed sequence tags (ESTs) of the pepper in GenBank using the BLASTN analysis. Three overlapping ESTs of the pepper (accession Nos. GD090771, GD097572, and GD089113) were chosen for assembly in a splicing fragment without a complete open reading frame (ORF). To confirm the authenticity of an assembled sequence in the pepper, a pair of primers, EST-RCCR-F and EST-RCCR-R, was utilized (Table 1). The design of both primers was based on the splicing sequence. The PCR procedure was as follows: 94°C for 5 min; 35 cycles of 94°C for 30 s, 60°C for 30 s, and 72°C for 1.0 min; and, finally, an extension of 72°C for 10 min. The corresponding sequence was obtained, subcloned into the pMD19-T vector (TaKaRa), and sequenced.

To isolate a full-length cDNA of the putative *CaRCCR* gene with complete 5'- and 3'-regions, the RACE method was used. The gene-specific primers (5'-GSP, 5'-NGSP, 3'-GSP, and 3'-NGSP) were designed according to the partial cDNA sequence (Table 1). The primers of 5'-GSP (external primer) and 5'-NGSP (internal primer) were used to isolate 5'-region sequences; the primers of the 3'-GSP (external primer) and 3'-NGSP (internal primer) were used for isolation of 3'-end sequences. The nested PCR was performed via 5'-RACE and 3'-RACE. The amplified PCR products were ligated into the pMD19-T vector and sequenced. Finally, all acquired sequences were assembled into a sequence with a complete ORF using the Contig Expression Software and the BLAST software online.

The cDNA and protein sequences of the putative *Ca*RCCR were analyzed by the DNAMAN software 5.2.2 and the BLAST software online (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Prediction of the putative signal peptide subcellular localization, chloroplast transit peptides, and its cleavage site were carried out using the CBS prediction servers online program (http://www.cbs.dtu.dk/services/). Isoelectric point (pI) and molecular weight (MW) of the putative protein were analyzed by the pI/MW program (http://www.expasy.org/), and its secondary structure was predicted by the scratch protein predictor program online (http://scratch.proteomics.ics.uci.edu). The multiple sequence alignments of *Ca*RCCR and other RCCR proteins were performed by the DNAMAN software. The phylogenetic tree was constructed using the MEGA5.05 program with the neighbor-joining method.

## RNA isolation

Total RNA was extracted from different pepper tissues (without treatment) and leaves (with different treatments, as mentioned above) using the TRIzol method. Contaminated genomic DNA was digested by RNase-free DNase I (Promega). The concentration and purity of total RNA were determined spectrophotometrically using a NanoDrop instrument (Thermo Scientific NanoDrop 2000C Technologies, Wilmington, DE, USA). The first-strand cDNA was synthesized according to the instructions of the PrimeScript™ Kit (TaKaRa).

## Quantitative real-time PCR analysis

In order to identify the expression patterns of *CaRCCR* in the different pepper tissues without treatment and the leaves under various stresses, quantitative real-time PCR was performed using the SYBR® Premix Ex Taq™ II (TaKaRa). The reaction system for real-time reverse transcription (RT) PCR analysis was a 20-μL reaction volume containing 10.0 μL SYBR® Premix Ex *Taq*™ II, 2.0 μL diluted cDNA, and 0.8 μL forward and reverse primers. The amplification was completed with the cycling parameters as follows: 95°C for 1 min, followed by 95°C for 10 s, 57°C for 20 s, and 72°C for 20 s for 40 cycles. The gene of *UBI3* (accession No. AY486137.1) was used as the internal control (reference gene) in this study (Table 1). The relative expression levels of the gene were calculated with the comparative threshold method of Livak and Schmittgen (2001). All treatments were performed in 3 independent biological replicates.

## Primers used in this study

Primers used for cloning and quantitative real-time PCR analysis of the *CaRCCR* gene are listed in Table 1.

**Table 1.** Primer sequences used in this study.

| Primer | Sequence (5'-3') |
|---|---|
| Cloning of *CaRCCR* gene | |
| EST-RCCR-F | TGGTGGAACTTGTATCTACTCTG |
| EST-RCCR-R | TATTTATTCGATCCCTTTTAGC |
| 5'-RACE-GSP | CTCTGCTTGTCTAACTGTGTGTCTTC |
| 5'-RACE-NGSP | CAAGTATGAAATCAACCTGAGAAGC |
| 3'-RACE-GSP | GATTTCATACTTGGTAGTTGGGTTC |
| 3'-RACE-NGSP | GAAGACACACAGTTAGACAAGCAGAG |
| Quantitative real-time PCR | |
| RT-RCCR-F | TGGTGGAACTTGTATCTACTCTGG |
| RT-RCCR-R | CTACCAAGTATGAAATCAACCTGAG |
| CaUBI3F | TGTCCATCTGCTCTCTGTTG |
| CaUBI3R | CACCCCAAGCACAATAAGAC |

# RESULTS

## Cloning and sequence analysis of *CaRCCR*

The full-length cDNA, designated *CaRCCR*, was obtained using the bioinformatic

analysis and RACE techniques. The transcript consisted of 1173 nucleotides, including a 9-bp 5'-untranslated region (UTR), 945-bp ORF, and 219-bp 3'-UTR (GenBank accession No. KC176711; Figure 1). *CaRCCR* was predicted to encode 314 amino acids, the theoretical molecular mass of the encoded protein was 35.2 kDa, and the calculated pI was 5.45. Structural analysis revealed that *Ca*RCCR belonged to the FDBR family and contained 2 conserved acidic residue sites (Glu151 and Asp288), which are involved in catalysis and/or substrate binding. Subcellular localization analysis demonstrated that *Ca*RCCR was located in the chloroplast. Furthermore, sequence analysis indicated that the deduced *Ca*RCCR protein contains a chloroplast transit peptide with 37-amino acid residues; its cleavage site was located between $P_{37}$ and $M_{38}$. The deduced *Ca*RCCR did not have a signal peptide region or transmembrane helix.

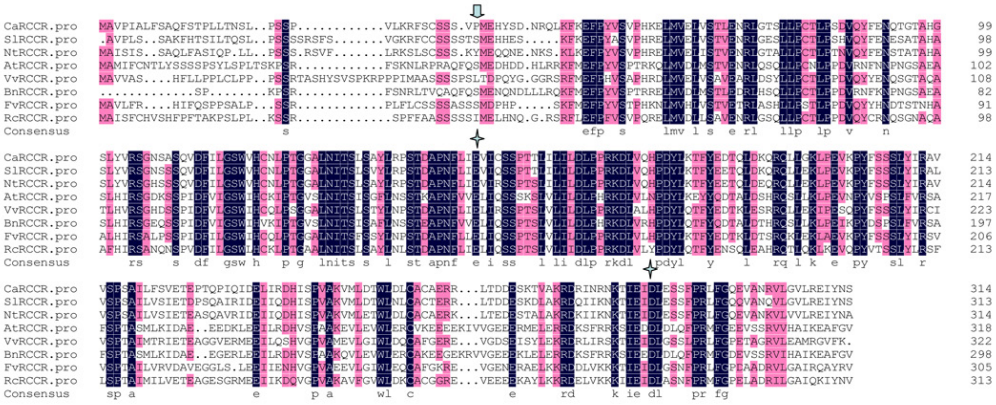


**Figure 1.** Multiple-sequence alignment of the *Ca*RCCR protein and RCCR proteins from other plants using the DNAMAN software. Blue and gray shaded regions reflect 100 and 80% sequence conservation, respectively. The arrow indicates the cleavage site of the chloroplast peptide, and the asterisks indicate 2 conserved acidic residue sites (Glu151 and Asp288). In addition to *Ca*RCCR, other amino acid sequences included in this alignment were *Solanum lycopersicum* RCCR (CAJ80766.1), *Nicotiana tabacum* RCCR (ABY19386.1), *Vitis vinifera* RCCR (XP_002277744.1), *Ricinus communis* RCCR (XP_002523576.1), *Fragaria vesca* subsp *vesca* RCCR (XP_004307021.1), *Brassica napus* RCCR (CAJ80767.1), and *Arabidopsis thaliana* RCCR (NP_195417.1).

The deduced *Ca*RCCR amino acid sequence showed high homology to other plant RCCR sequences via multiple alignment using the DNAMAN software (Figure 1). The percent identities of *Ca*RCCR relative to *Solanum lycopersicum* RCCR (CAJ80766.1), *Nicotiana tabacum* RCCR (ABY19386.1), *Vitis vinifera* RCCR (XP_002277744.1), *Ricinus communis* RCCR (XP_002523576.1), *Fragaria vesca* subsp *vesca* RCCR (XP_004307021.1), *Brassica napus* RCCR (CAJ80767.1), and *Arabidopsis thaliana* RCCR (NP_195417.1) were 82, 79, 60, 59, 59, 53, and 50%, respectively.

A phylogenetic tree constructed using the MEGA5.05 software was used to investigate the evolutionary relationships of the *Ca*RCCR amino acid sequence in comparison to the RCCR proteins of other plants. Two groups were formed using the 13 RCCR protein sequences from *C. annuum* L., *S. lycopersicum*, *N. tabacum*, *R. communis*, *V. vinifera*, *B. napus*, *A. thaliana*, *F. vesca* subsp *vesca*, *Medicago truncatula*, *Theobroma cacao*, *Glycine max*, *Citrullus lanatus* subsp *vulgaris*, and *Cucumis sativus* (Figure 2). *Ca*RCCR clustered in the first group, which included *Sl*RCCR, *Nt*RCCR, *Cs*RCCR, *Rc*RCCR, and so on. Phylogenetic

analysis showed that *Ca*RCCR was more closely related to *Sl*RCCR and *Nt*RCCR than the RCCR proteins of other plants.

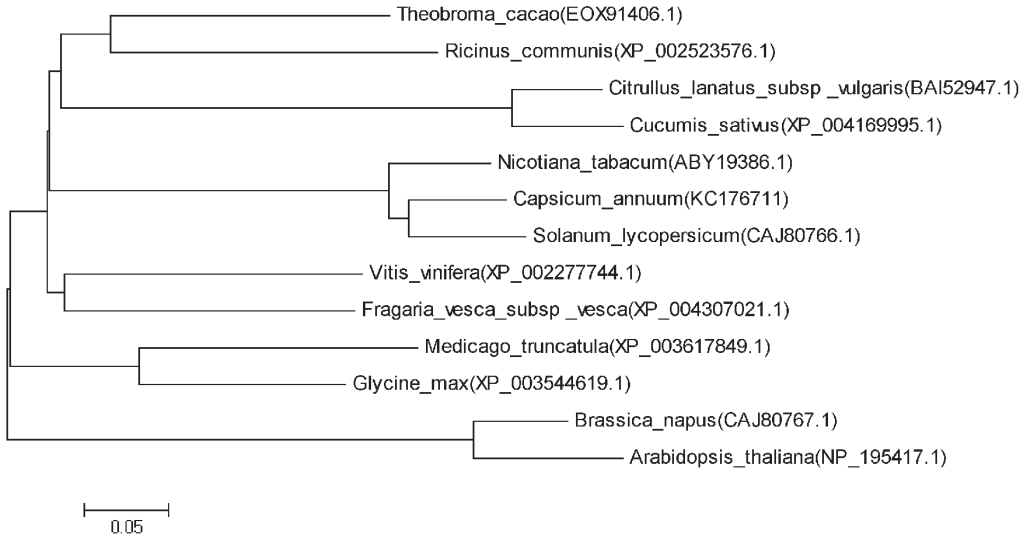


**Figure 2.** Phylogenetic analysis of the *Ca*RCCR protein and RCCR proteins of other plant species. The phylogenetic tree was constructed by the neighbor-joining method using MEGA 5.05. Branches were labeled with the names and GenBank accession Nos. of the different plant species.

## Tissue-specific expression of *CaRCCR*

In order to investigate the expression levels of the *CaRCCR* gene in different tissues of B12 pepper cultivar plants, total RNA was extracted from the roots, stems, leaves, flowers, and immature fruits, and quantitative real-time PCR was performed (Figure 3a). *CaRCCR* transcripts were detected in all of these tissues and found to be higher in the leaves than in the other tissues. During the 3 developmental stages, the transcript level of *CaRCCR* was almost constant in the young leaves, fully expanded leaves, and senescent leaves (Figure 3b).

## Expression analysis of the *CaRCCR* gene in pepper plants treated with signaling molecules

To examine whether signal molecules induce *CaRCCR* expression, 3 phytohormones were used in the pepper leaves. As shown in Figure 4, the expression levels of *CaRCCR* in the leaves sprayed with ABA, MeJA, and SA were elevated to different levels. ABA treatment could quickly induce expression of the *CaRCCR* gene within the first 4 h after treatment. In comparison to the control, the highest transcript levels (4.67-fold) of *CaRCCR* were detected at 8 h. Then, the expression of *CaRCCR* decreased gradually after 8 h. Spraying pepper plants with MeJA caused rapid upregulation of *CaRCCR* at 2 h to the highest observed expression level (2.85-fold) and then quickly declined at 4 h. However, *CaRCCR* transcripts increased gradually from 8 to 12 h. In contrast, the treatment of SA induced *CaRCCR* transcript abun-

dance at 2 h; *CaRCCR* expression peaked by 4 h, which was 4.05-fold greater than that of the control, and was then gradually downregulated. Interestingly, there was a slight upregulation at 12 h. These results indicate that the *CaRCCR* gene could be induced and upregulated by all 3 signaling molecules tested (i.e., ABA, MeJA, and SA).

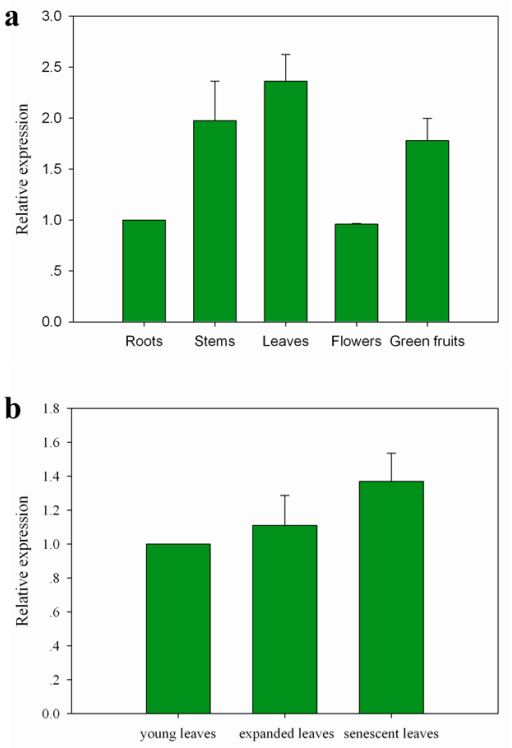


**Figure 3. a.** Tissue-specific expression of *CaRCCR* in pepper roots, leaves, stems, flowers, and immature fruits. Relative expression levels of the *CaRCCR* transcript were determined in different tissues in comparison to that in roots. **b.** Expression profiles of *CaRCCR* during the leaf developmental stage. RNA was extracted from the young leaf, fully expanded leaf, and senescent leaf. Error bars represent SD for 3 independent replicates.

## Expression patterns of *CaRCCR* induced by abiotic stresses

To analyze the expression pattern of the *CaRCCR* gene in response to abiotic stresses, pepper plants were exposed to 400 mM NaCl or 400 mM mannitol. The abundance of *CaRCCR* transcripts was analyzed via quantitative RT-PCR. As shown in Figure 5a, the *CaRCCR* expression level in plants treated with 400 mM NaCl exhibited little change within the first 4 h after treatment; it then began to increase gradually at 8 h and peaked (2.51-fold) at 12 h when compared to the control (0 h). For the drought treatment, the transcript level of *CaRCCR* was induced in the pepper plants (Figure 5b). The increased expression was shown at 2 h (1.75-fold), upregulated strongly at 12 h (3.95-fold), and maintained a relatively steady level from 12 to 24 h (4.03-fold). However, there was a slight increase in downregulation at 8 h. The above-mentioned results indicate that the *CaRCCR* gene could be induced and upregulated under abiotic stresses, including high salinity and drought stress.

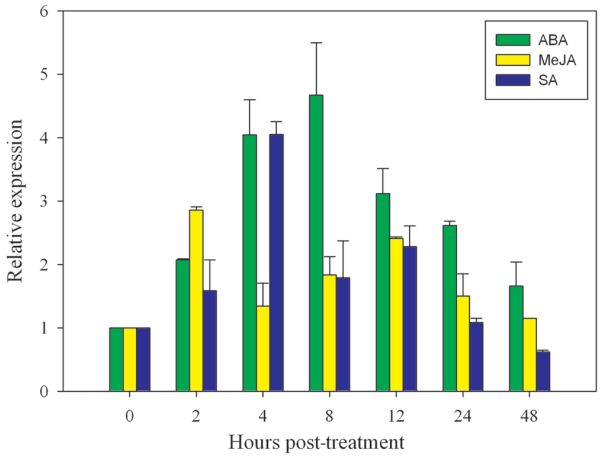


**Figure 4.** *CaRCCR* expression patterns in pepper leaves treated with various phytohormones, including abscisic acid (ABA), methyl jasmonate (MeJA), and salicylic acid (SA). The relative transcriptional expression of *CaRCCR* was calculated in phytohormone-treated leaves in comparison to that in the mock controls. Error bars represent SD for 3 independent replicates.

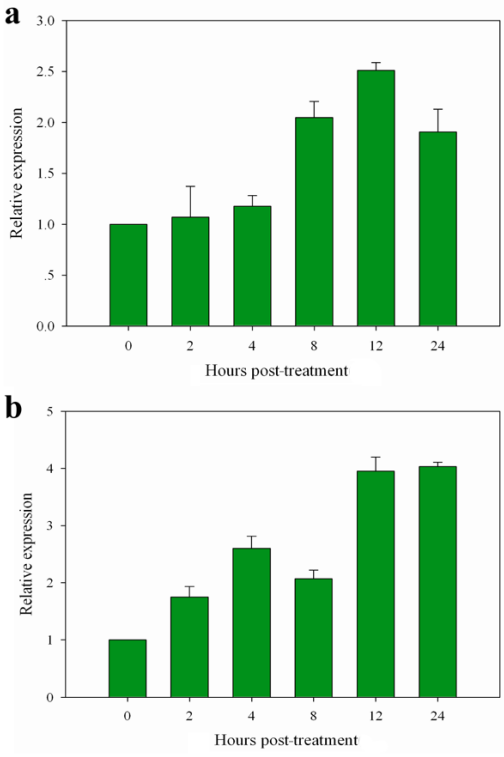


**Figure 5.** Expression patterns of *CaRCCR* in pepper leaves treated with abiotic stresses. (**a**) Salt stress. (**b**) Drought stress. The relative expression of *CaRCCR* was calculated in various treated leaves in comparison to that in the mock controls. Error bars represent SD for 3 independent replicates.

## Expression analysis of the *CaRCCR* gene in response to biotic stresses

In this study, the expression of *CaRCCR* slightly increased when the pepper plants were infected with *Phytophthora capsici* (Figure 6). The transcript level of *CaRCCR* maintained nearly the same degree at 3 h and then gradually decreased from 6 to 24 h when compared to the mock control. The transcript expression level increased rapidly and reached its peak (1.75-fold) at 48 h. Subsequently, the expression level began to decrease at 72 h (0.84-fold) but slightly increased to 1.28-fold at 96 h, which was the lowest actual time point in comparison to the control. These results suggest that there are changes in gene expression patterns under *P. capsici* infection.

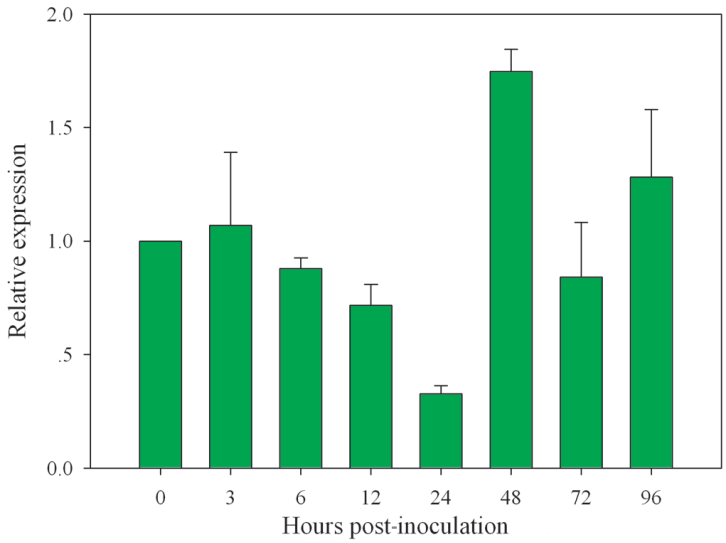


**Figure 6.** Expression analysis of *CaRCCR* in pepper leaves after infection with *Phytophthora capsici*. *CaRCCR* relative expression was calculated in *P. capsici*-infected leaves in comparison to that in the mock controls. Error bars represent SD for 3 independent replicates.

## DISCUSSION

RCCR catalyzes RCC, the product of the pheide *a* ring opening to the pFCCs. RCCR is an important constituent of the detoxification of photodynamic chlorophylls and their catabolites. Herein, we identified the *CaRCCR* gene from the pepper. The predicted *Ca*RCCR amino acid sequence contained the conserved sites (Glu151 and Asp288 in *Capsicum annuum* L.) that are present in the RCCR protein of other higher plants. Moreover, *Ca*RCCR shared a high degree of identity with *Sl*RCCR and *Nt*RCCR, and they fall into the same group within the phylogenetic tree. Similar to known reported RCCR proteins, *Ca*RCCR existed as a conserved transit peptide at the N terminus. These results demonstrate that *Ca*RCCR should function as an RCCR.

Tissue-specific analysis showed that *CaRCCR* was expressed in all tissues, such as the

roots, stems, leaves, flowers, and fruits, and the expression level of *CaRCCR* in the leaves was higher than that in other tissues. The expression level of *CaRCCR* in the leaves at different developmental stages exhibited little change. The tissue-specific expression of *CaRCCR* was similar to the results in rice. It was reported that expression of the *OsRCCR* gene was detected in all tissues, and expression was the highest in leaves. However, the expression pattern of *CaRCCR* during leaf development was different than that in rice; in rice, the expression of the *OsRCCR1* gene was upregulated in naturally senescent leaves (Tang et al., 2011). These results reveal that *RCCR* is important to the growth and development of all tissues, although its function may be distinct in different species.

Previous studies have shown that chlorophyll degradation is regulated by hormones during the leaf developmental stage and in response to various environmental stresses (Costa et al., 2005; Lim et al., 2007; Bari and Jones, 2009). Chlorophyll degradation of the Chinese cabbage during post-harvest senescence was regulated positively by ethylene and negatively by cytokinin (Zhang et al., 2011). In this study, quantitative real-time PCR analysis indicates that treatment with ABA, MeJA, or SA was found to increase the expression level of *CaRCCR* to different extents. These findings suggest that the *CaRCCR* gene may be involved in ABA-, JA-, and SA-dependent signaling pathways.

Various abiotic and biotic stresses can affect growth and development of pepper plants, causing chlorophyll breakdown, cell death, and, finally, premature senescence. All of these consequences would impact the yield and quality of crop plants. Therefore, it is important to understand the molecular adaptation mechanisms of plants to diverse stresses. Here, using quantitative real-time PCR analysis, we examined the *CaRCCR* expression pattern in pepper leaves treated with high salinity, drought stress, and *P. capsici* infection. The expression of the *CaRCCR* gene was induced under all of the above-mentioned stress conditions. The results showed that *CaRCCR* may be involved in response to abiotic and biotic stresses in the pepper plant, including high salinity, drought stress, and pathogen infection. These results were different from those of a study in *Arabidopsis* reporting that *AtRCCR* expression was rather constant under stress conditions (Průzinská et al., 2007). This different expression pattern of *RCCR* between *Arabidopsis* and the pepper implies that there are differences in the transcriptional regulation of chlorophyll catabolism in different species.

In summary, the *CaRCCR* gene was transcribed at various levels in all tissues. In pepper leaves treated with various stresses, including phytohormones, drought, salt, and *P. capsici* infection, *CaRCCR* was induced, implicating its involvement in abiotic and biotic stresses. Taken together, these data suggest that *CaRCCR* plays a role in whole plant development and involves the response to various environmental stresses of the pepper plant. In the future, transgenic plants exhibiting overexpression or knockdown of the *CaRCCR* gene in the pepper should be used to study the detailed function of *CaRCCR*.

## ACKNOWLEDGMENTS

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