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Molecular cloning of the monodehydroascorbate reductase gene from *Brassica campestris* and analysis of its mRNA level in response to oxidative stress

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Abstract

In a majority of living organisms, a fundamental protection mechanism from reactive oxygen species is by the ascorbate–glutathione cycle in which an important antioxidant, ascorbate (vitamin C), is utilized to convert harmful H_2O_2 to H_2O. Monodehydroascorbate reductase (MDHAR) maintains reduced pools of ascorbate by recycling the oxidized form of ascorbate. By screening a *Brassica campestris* cDNA library, we identified a *B. campestris* MDHAR cDNA (*BcMdhar*) which encodes a polypeptide of 434 amino acids possessing domains characteristic of FAD- and NAD(P)H-binding proteins. The predicted amino acid sequence of the open reading frame (ORF) shows a high level of identity to the cytosolic MDHAR of rice, pea and tomato, and does not possess N-terminal leader sequence suggesting that it encodes a cytosolic form of MDHAR. Genomic Southern blot analysis indicated that a single nuclear gene encodes this enzyme. Northern hybridization analysis detected *BcMdhar* transcripts in all plant tissues examined. The level of *BcMdhar* mRNA increased in response to oxidative stress invoked by hydrogen peroxide, salicylic acid, paraquat, and ozone.

Keywords: Antioxidant; Ascorbate–glutathione cycle; Monodehydroascorbate reductase; Oxidative stress; *Brassica campestris*

Harmful reactive oxygen species (ROS) are inevitably generated during cellular energy transfer reactions in all organisms [1]. In plants, photosynthesis and electron transport chain produce superoxide radicals (O_2^-) in plastids and mitochondria. O_2^- is converted into hydrogen peroxide (H_2O_2) spontaneously or by superoxide dismutase (SOD; EC 1.15.1.1) [2]. H_2O_2 is also produced through β-oxidation in glyoxysomes and photorespiration in leaf-type peroxisomes [3]. H_2O_2 is further reduced to H_2O by catalase (EC 1.11.1.6) or ascorbate peroxidase (APX; EC 1.11.1.11).

Various stress conditions invoke the increased production of ROS. If the generation rates of free radicals exceed those of degradation under stress conditions, cells suffer oxidative stress which can result in premature senescence, necrosis, or apoptosis [4]. Plants contain several antioxidant mechanisms that detoxify ROS. The primary components of this system include low molecular weight antioxidants (ascorbate, glutathione, carotenoids, flavonoids, and tocopherols), enzymes (SOD, catalase, glutathione peroxidase, and other peroxidases), and the enzymes involved in the ascorbate–glutathione cycle [monodehydroascorbate reductase (MDHAR; EC 1.6.5.4. also called ascorbate free radical reductase), dehydroascorbate reductase (DHAR; EC 1.8.5.1), glutathione reductase (GR; EC 1.6.4.2), and ascorbate peroxidase (APX)] [5]. Ascorbate is one of the essential water-soluble antioxidants in organisms of all kingdoms. APX catalyses the reduction of H_2O_2 with the simultaneous oxidation of ascorbate and generates the monodehydroascorbate (MDHA) radical as the primary product. MDHAR is critical in maintaining the proper concentration of ascorbate in cells by reducing the MDHA radical directly to ascorbate at the expense of an NAD(P)H [5]. Both NADH and NADPH serve as electron donors, with NADH being the preferred donor. A previous kinetic study

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suggests that NAD(P)H reduces the enzyme's FAD, and the reduced FAD donates electrons to MDHA radicals through two successive transfers of electrons [6]. MDHAR is ubiquitous in all the phylogenetic groups and the activity has been detected in microsomes and mitochondria in mammals [7]. Within plant tissues, isoforms of MDHAR exist in chloroplasts, plyoxysomes, mitochondria and the cytosol [8]. The enzyme has been purified to homogeneity from cucumber and shown to be a soluble monomeric enzyme that contains one molecule of FAD per enzyme molecule [6]. Plant cDNAs of MDHAR have been cloned from cucumber [9], pea [10], tomato [11], *Arabidopsis* (accession no. D84417), rice (accession no. D85764), and leaf mustard (accession no. AF109695). However, among the antioxidant enzymes much of the research effort has been focused on GR and APX until recently, and only limited information is available concerning the *Mdhar* gene and its expression upon oxidative stress. Grantz et al. [11] reported that mRNA levels of Tomato *Mdhar* were increased by wounding, and Nishikawa et al. [12] showed that cytosolic gene expression of broccoli *Mdhar* (*Brassica oleracea*) was stimulated actively after harvest. We isolated a cDNA for MDHAR from *Brassica campestris* var. *Pekinensis* (Chinese cabbage; one of the major commercially cultivated vegetable in Asia and many other countries) and demonstrated *Mdhar* transcript level changes in response to stress conditions known to cause oxidative damages.

B. campestris var. *Pekinensis* was grown in a greenhouse on a 25 °C day/20 °C night cycle. Illumination of 16 h at 350 μE m^{-2} s^{-1} was used for the day period. Plant samples were harvested before and after oxidative treatments, frozen in liquid nitrogen, and stored at − 80 °C for DNA or RNA extraction.

A *B. campestris* cDNA library was kindly provided by Dr. Won-Il Jung at the Korea Advanced Institute of Science and Technology. The leaves of 3-week-old *Brassica* plants were used for mRNA extraction and double-stranded cDNA was synthesized using reverse transcriptase. λZAPII vector (Stratagene) was used to entrap the synthesized cDNA to construct the *Brassica* cDNA library. The titer of the constructed library was 2.6×10^6 plaque forming units/ml indicating that cDNA of the most expressed genes were represented. The library was amplified using *E. coli* strain XL-1 blue to the titer of 8.24×10^9 and used for screening. A ^{32}P-labeled *Mdhar* EST clone of Chinese cabbage, RF1041, was used as a probe. DNA probes were labeled using a random primer kit (Amersham, UK) and purified by Sephadex G-50 Quick Spin Column (Amersham).

Six positive clones containing 1.6-kilobase (kb) inserts were identified from two rounds of screening the cDNA library. After in vivo excision of the cDNA clones into pBluescript II SK(−) plasmids following the manufacturer's instruction (Stratagene), double-stranded DNA sequencing of the inserts was done by the dideoxy chain termination method using an ALFexpress Auto Cycle Sequencing Kit and an automated ALFexpress DNA sequencer (Pharmacia, Uppsala). Sequence data were analyzed with Genetyx sequence analysis software (SDC Software Development, Tokyo) and NCBI sequence-similarity search programs. The DNA sequence of the inserts revealed a 1305-base pair (bp) open reading frame (ORF) which can encode 434 amino acids with an expected molecular weight of 46.5 kilodalton. The ORF is flanked by a 35-bp 5′-UTR and a 223-bp 3′-UTR. The nucleotide sequence and its deduced amino acid sequence are available at GenBank (accession no. AY039786). The amino acid sequence deduced from the nucleotide sequence of the ORF was aligned with those of MDHAR from other plant species (Fig. 1). A substantial degree of identity was found to amino acid sequences of cytosolic forms of MDHAR from tomato (*Lycopersicon esculentum* Mill., 79%), rice (*Oryza sativa*, 78%), pea (*Pisium sativum* L., 76%), and cucumber (*Cucumis sativus*, 75%). In contrast, the amino acid sequence of the *B. campestris* ORF showed lower than 50% identity to those of the plastidic MDHAR; *Brassica juncea*, 44% and, *Spinacia oleracea* 43%. The ORF does not appear to contain an N-terminal leader sequence (Fig. 1) suggesting the cytosolic location of its product. We concluded that the ORF encodes most probably a cytosolic isoform of MDHAR and named it *BcMdhar*.

The predicted amino acid sequence of the *BcMdhar* gene possesses three putative regions that could be involved in the binding of FAD or NAD(P)H (Fig. 1). The first two consensus regions (domains I and II in Fig. 1) consist of an 18-amino-acid domain with 11 amino acids in the center being the 'core consensus'. These two domains are thought to bind an ADP moiety of FAD (domain I) or an ADP moiety of NAD(P)H (domain II) [13]. The third consensus region (domain III) consists of 13 amino acids and is known to be involved in the binding of the flavin moiety of FAD [13]. These consensus motifs have been shown to be involved in FAD binding from the crystal structure analysis of some of the flavin enzymes [14–17]. The presence of these motifs in the deduced amino acid sequence implies the involvement of FAD and NAD(P)H in the catalytic activity of the putative protein and also supports the identification of the cloned ORF as *Mdhar*.

The *BcMdhar* clones were fused under the cauliflower mosaic virus 35S promoter and introduced into tobacco plants. Leaf extract of the transgenic tobacco plants showed a significantly higher level of MDHAR activity than that of wild-type tobacco plants. MDHAR enzyme activity was determined by monitoring the decrease in A_{340} as a result of NADH oxidation (Lee, B.-H., personal communication).

Southern blot analysis indicated that the *BcMdhar* gene exists as a single copy in the *B. campestris* genome. *B. campestris* genomic DNA (10 μg) was completely digested with either *Bam*HI, *Eco*RI, or *Xba*I, and separated on an agarose gel. After transferring digested DNA onto a Nytran-Plus nylon membrane (Schleicher & Schuell, Germany) by the alkaline transfer method, *BcMdhar* cDNA probe labeled with ^{32}P was used for Southern hybridization as described

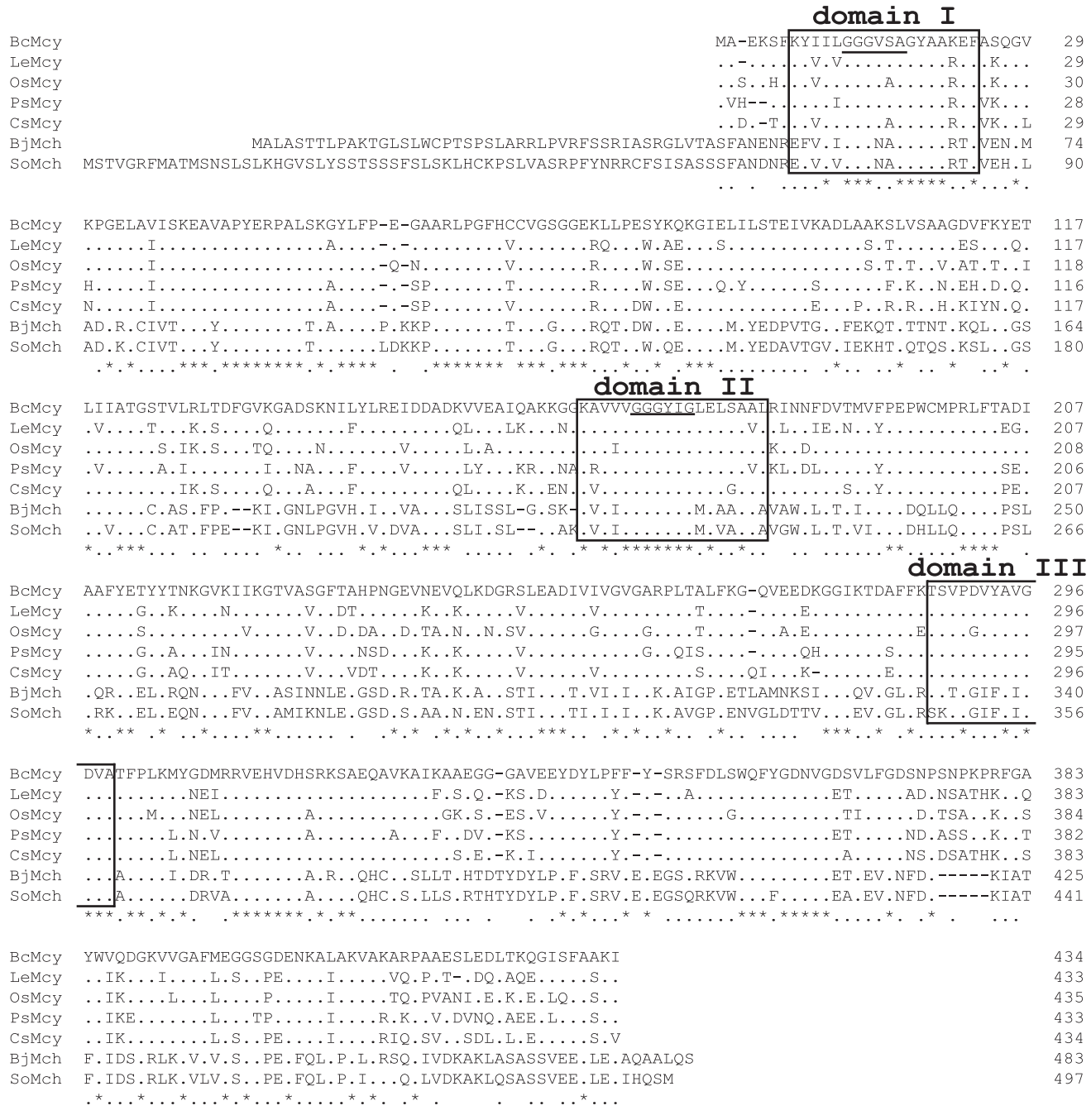

Fig. 1. Multiple alignment of the deduced amino acid sequences of *BcMdhar* with cytosolic *Mdhar* genes from *L. esculentum* Mill. (accession no. T06407), *O. sativa* (accession no. D85764), *P. sativum* (accession no. A55333), and *C. sativus* (accession no. JU0182), and chloroplastic *Mdhar* from *B. juncea* (accession no. AAD28178) and *S. oleracea* (accession no. BAB63925). Asterisks represent amino acid residues identical to those of *BcMdhar*. Dots indicate amino acid residues similar in their chemical properties. Gaps in sequences, which were introduced to improve the alignment, are indicated by dashes. Amino acid domains involved in the binding of FAD and NAD(P)H are boxed. The 6-amino-acid core consensus of domains I and II was underlined in the amino acid sequence of *BcMdhar*.

previously [18]. The probe hybridized strongly to single bands of approximately 20 and 6 kb in *Bam*HI- and *Xba*I-digested DNA, respectively (Fig. 2). The two bands of approximately 12 and 2.8 kb in *Eco*RI-digested DNA are due to the internal *Eco*RI site within the *BcMdhar* gene.

To characterize the tissue specific expression of *BcMdhar*, we analyzed the total RNAs isolated from different vegetative tissues of *B. campestris* and from embryogenic calli by Northern blot analysis using full-length *BcMdhar* cDNA as a probe (Fig. 3).

Total *B. campestris* RNA was isolated by the guanidine thiocyanate method [19] from stems, roots, young leaves (2-week-old growing leaves with less than 1 cm in width), mature leaves (5- to 6-week-old healthy leaves with 6–7-cm

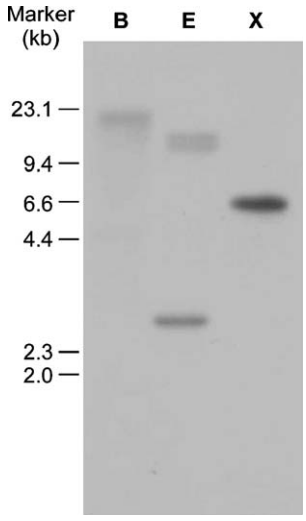

Fig. 2. Southern blot analysis of *B. campestris* genomic DNA digested with either *Bam*HI, *Eco*RI, or *Xba*I (lanes are labeled with B, E, X, respectively) and probed with a ^{32}P-labeled *BcMdhar* cDNA. Numbers at the left side of the blot are the sizes of a DNA marker in kb.

width), and actively dividing calli. RNA samples (15 μg) were denatured with formaldehyde, separated by electrophoresis on a 1.2% formaldehyde-agarose gel, and transferred to Hybond-N nylon membrane (Amersham) with 10 × SSC. The blot was then hybridized as described previously [18]. The level of *BcMdhar* transcripts was moderate in all tissues examined (Fig. 3). Leaf tissues of *B. campestris* had a higher level of *BcMdhar* transcript than roots or stems. Mature leaves seemed to accumulate a slightly higher level of transcripts than young leaves. This result is dissimilar to the expression pattern of another antioxidant enzyme of *B. campestris*, glutathione reductase [18], indicating the independent regulation of the two genes in the ascorbate–glutathione cycle.

It has been shown by many research groups that the enzyme activity of the ascorbate–glutathione cycle increases in response to oxidative stresses (reviewed in Ref. [20]). We investigated the changes in the steady-state mRNA levels of *BcMdhar* under oxidative stress inducing conditions. *B. campestris* plants were grown in a growth chamber for 3 weeks, misted with an aqueous solution containing 10 mM H_2O_2, 20 mM salicylic acid, or 2.38 mM paraquat. Added as a surface active agent was 0.01% Tween 20. Plants were then maintained in the growth chamber until the sampling times for RNA extraction.

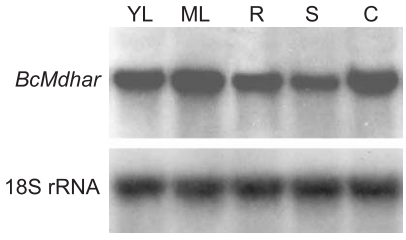

Fig. 3. Northern blot analysis of total RNA from various *B. campestris* tissues. Total RNA (15 μg) from each tissue was blotted onto a membrane and probed with ^{32}P-labeled *BcMdhar* cDNA. Transcript size is approximately 1.5 kb. YL, young leaf tissue; ML, mature leaf tissue; R, roots; S, stems; C, calli. Lower panel is the same blot that was stripped and re-probed with ^{32}P-labeled 18S rDNA to show equal loading.

Upon treatment of H_2O_2, which is the direct substrate of the ascorbate–glutathione cycle, a burst of expression of *BcMdhar* was observed in 30 min (approximately 1.8-fold, Fig. 4A). The transcripts were then slightly reduced at 1 and 2 h after the H_2O_2 treatment but increased again to a high level at 4 and 6 h.

Although the mode of action of salicylic acid during the plants' response against oxidative attack is still under debate [21], a suggestion is that salicylic acid inhibits catalase activity resulting in the accumulation of H_2O_2 in plant tissues [22]. When 20 mM salicylic acid was applied, *BcMdhar* transcripts increased gradually to a higher level (1.5-fold at hour 4) over the experimental period (Fig. 4B).

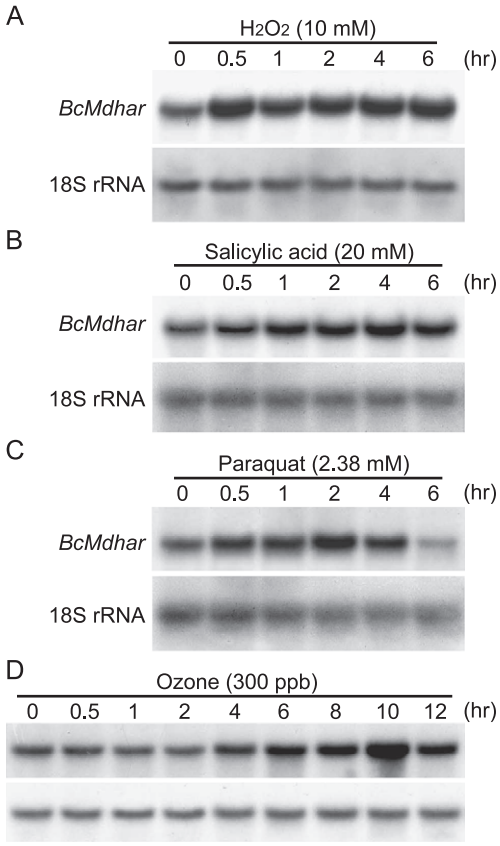

Fig. 4. The effect of H_2O_2, salicylic acid, paraquat, and ozone on the mRNA abundance of *BcMdhar* in *B. campestris*. RNA was prepared from treated plants which were sampled at the indicated times after each stress treatment. Total RNAs (10 μg) were separated by electrophoresis on a 1.2% formamide-containing agarose gel and probed with ^{32}P-labeled *BcMdhar* cDNA. 10 mM H_2O_2 (A), 20 mM salicylic acid (B), or 2.38 mM paraquat was delivered as mist in an aqueous solution. 300 ppb ozone (D) was fumigated in an ozone fumigation open-top chamber. The lower autoradiogram of each panel is 18S rRNA to serve as a loading control. The autoradiograms were scanned and the band intensity was determined by an image processing and analysis program NIH Image (NIH, USA).

The mRNA induction appeared to be delayed and less pronounced than that of the H_2O_2 treatment.

Paraquat (methyl viologen, 1, 19-dimethyl-4, 49-bipyridinium dichloride; Sigma, St. Louis) is a herbicide that induces the generation of superoxide anions and H_2O_2 in the plant cell [23]. The mRNA level of *BcMdhar* increased immediately after treating the *B. campestris* leaves with 2.38 mM paraquat, which is the concentration used to control weeds in the field (Fig. 4C). Increases of 1.3- to 1.6-fold *BcMdhar* transcripts relative to untreated plants were detected until 4 h after the paraquat treatment. Visible damage on the plants appeared within 4 h after the paraquat treatment; withering was apparent at 4 h and leaf necrosis started after 6 h. We assume that the decrease of *BcMdhar* transcripts at hour 6 (Fig. 4C) is due to the overall deterioration of cell functions arising from the oxidative attack of paraquat.

Ozone is the most abundant tropospheric oxidant and an important component of photochemical pollution [24]. Public concerns on the deleterious effects of ozone on human health [25] and vegetation [26] are increasing. Plants respond to high concentration of ozone by triggering various defense mechanisms including the antioxidant defense system [27]. To examine the change of *BcMdhar* expression upon exposure to high levels of ozone, 3-week-old *B. campestris* plants were transferred to an ozone fumigation open-top chamber and fumigated with 300-ppb ozone. The concentration of the ozone was continuously monitored and maintained within 50 ppb of the set limit. Unlike the rapid response to paraquat and H_2O_2 treatments, *BcMdhar* mRNA levels increased approximately 1.4-fold after 6 h with no apparent change at the 1- and 2-h time points (Fig. 4D). The amount of transcript decreased at 12 h after a peak at 10 h (1.9-fold) from the onset of the ozone fumigation. It is conceivable that the gaseous diffusion of ozone may require a longer time to trigger the oxidative stress response in *B. campestris* than the direct contact of the liquid reagents delivered in mist. It is noteworthy that the level of transcript induced at the 10-h time point is comparable to the maximum level of induction seen in H_2O_2 and paraquat treatments.

The cellular levels of ROS must be tightly regulated in plant cells [2,5]. At least part of this regulation is known to be achieved by transcriptional control. mRNAs of several components of the ROS scavenging system including glutathione peroxidases, GR, SOD, and APX are induced in response to many biotic and abiotic stresses [11,12,27–30]. Unexpectedly, only limited reports are available about the regulation of MDHAR, which plays a critical role in the ascorbate–glutathione cycle as a direct reducer of oxidized ascorbate. In this report, we showed the induction of *BcMdhar* transcripts upon H_2O_2, salicylic acid, paraquat, and ozone treatments. As in the case of other components of the ascorbate–glutathione cycle, MDHAR of *B. campestris* appeared to be regulated at the transcriptional level to detoxify increased ROS during stressful conditions. The presence of *BcMdhar* transcripts in all the tissues examined and its rapid response to oxidative stress indicate the importance of this gene in the ROS defense system in plant cells.

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References

[1] B. Halliwell, J.M.C. Gutteridge, Free Radicals in Biology and Medicine, 2nd ed., Oxford Univ. Press, Oxford, UK, 1989.
[2] J.G. Scandalios, Molecular genetics of superoxide dismutase in plants, in: J.G. Scandalios (Ed.), Oxidative Stress and the Molecular Biology of Antioxidant Defenses, Cold Spring Harbor Laboratory Press, New York, USA, 1997, pp. 527–568.
[3] A.H.C. Huang, R.N. Trelease, T.S. Moore, Plant Peroxisomes, Academic Press, New York, USA, 1983.
[4] C.H. Foyer, G. Noctor, Oxygen processing in photosynthesis: regulation and signaling, New Phytol. 146 (2000) 359–388.
[5] K. Asada, Radical production and scavenging in chloroplasts, in: N.R. Baker (Ed.), Photosynthesis and the Environment, Kluwer Academic, Dordrecht, The Netherlands, 1996, pp. 123–150.
[6] M.A. Hossain, K. Asada, Monodehydroascorbate reductase from cucumber is a flavin adenine dinucleotide enzyme, J. Biol. Chem. 260 (1985) 12920–12926.
[7] A. Meister, Glutathione–ascorbic acid antioxidant system in animals, J. Biol. Chem. 269 (1994) 9397–9400.
[8] O. Arrigoni, S. Dipierro, G. Borraccino, Ascorbate free radical reductase, a key enzyme of the ascorbic acid system, FEBS Lett. 125 (1981) 242–244.
[9] S. Sano, K. Asada, cDNA cloning of monodehydroascorbate radical reductase from cucumber: a high degree of homology in terms of amino acid sequence between this enzyme and bacterial flavoenzymes, Plant Cell Physiol. 35 (1994) 425–437.
[10] S.S. Murthy, B.A. Zilinskas, Molecular cloning and characterization of a cDNA encoding pea monodehydroascorbate reductase, J. Biol. Chem. 269 (1994) 31129–31133.
[11] A.A. Grantz, D.A. Brummell, A.B. Bennett, Ascorbate free radical reductase mRNA levels are induced by wounding, Plant Physiol. 108 (1995) 411–418.
[12] F. Nishikawa, M. Kato, H. Hyodo, Y. Ikoma, M. Sugiura, M. Yano, Ascorbate metabolism in harvested broccoli, J. Exp. Bot. 54 (2003) 2439–2448.
[13] G. Eggink, H. Engel, G. Vriend, P. Terpstra, B. Witholt, Rubredoxin reductase of *Pseudomonas oleovorans*. Structural relationship to other flavoprotein oxidoreductases based on one NAD and two FAD fingerprints, J. Mol. Biol. 212 (1990) 135–142.
[14] R.L. Krauth-Siegel, C. Sticherling, I. Jost, C.T. Walsh, E.F. Pai, W. Kabsch, C.B. Lantwin, Crystallization and preliminary crystallographic analysis of trypanothione reductase from *Trypanosoma cruzi*, the causative agent of Chagas' disease, FEBS Lett. 317 (1993) 105–108.
[15] N. Schiering, W. Kabsch, M.J. Moore, M.D. Distefano, C.T. Walsh, E.F. Pai, Structure of the detoxification catalyst mercuric ion reductase from *Bacillus* sp. strain RC607, Nature 352 (1991) 168–172.
[16] A. Mattevi, A.J. Schierbeek, W.G. Hol, Refined crystal structure of lipoamide dehydrogenase from *Azotobacter vinelandii* at 2.2 A reso-

lution. A comparison with the structure of glutathione reductase, J. Mol. Biol. 220 (1991) 975–994.
[17] U. Ermler, G.E. Schulz, The three-dimensional structure of glutathione reductase from *Escherichia coli* at 3.0 A resolution, Proteins 9 (1991) 174–179.
[18] H. Lee, S.H. Won, B.H. Lee, H.D. Park, W.I. Chung, J. Jo, Genomic cloning and characterization of glutathione reductase gene from *Brassica campestris* var. *Pekinensis*, Mol. Cells 13 (2002) 245–251.
[19] R. McGookin, RNA extraction by the guanidine thiocyanate procedure, in: J.M. Walker (Ed.), Methods in Molecular Biology, Humana Press, New Jersey, 1984, pp. 113–116.
[20] C.H. Foyer, M. Lelandais, K.J. Kunert, Photooxidative stress in plants, Physiol. Plant 92 (1994) 696–717.
[21] J.F. Dat, H. Lopez-Delgado, C.H. Foyer, I.M. Scott, Parallel changes in H_2O_2 and catalase during thermotolerance induced by salicylic acid or heat acclimation in mustard seedlings, Plant Physiol. 116 (1998) 1351–1357.
[22] U. Conrath, Z.X. Chen, J.R. Ricigliano, D.F. Klessig, Two inducers of plant defense responses, 2,6-dichloroisionicotinic acid and salicylic acid, inhibit catalase activity in tobacco, Proc. Natl. Acad. Sci. U. S. A. 92 (1995) 7143–7147.
[23] J.L. Donahue, C.M. Okpodu, C.L Cramer, E.A. Grabau, R.G. Alscher, Responses of antioxidants to paraquat in pea leaves (Relationships to resistance), Plant Physiol. 113 (1997) 249–257.
[24] Committee on tropospheric ozone, Rethinking the Ozone Problem in Urban and Regional Air Pollution, National Academy of Sciences, Washington, DC, 1991.
[25] S.Y. Kim, J.T. Lee, Y.C. Hong, K.J. Ahn, H. Kim, Determining the threshold effect of ozone on daily mortality: an analysis of ozone and mortality in Seoul, Korea, 1995–1999, Environ. Res. 94 (2004) 113–119.
[26] A.S. Lefohn, Surface Level Ozone Exposures and Their Effects on Vegetation, Lewis, Chelsea, UK, 1992.
[27] H. Willekens, W. Van Camp, M. Van Montagu, D. Inze, C. Langebartels, D. Inze, H. Sandermann Jr., Ozone, sulfur dioxide, and ultraviolet B have similar effects on mRNA accumulation of antioxidant genes in *Nicotiana plumbaginifolia* L, Plant Physiol. 106 (1994) 1007–1014.
[28] S. Karpinski, C. Escobar, B. Karpinska, G. Creissen, P.M. Mullineaux, Photosynthetic electron transport regulates the expression of cytosolic ascorbate peroxidase genes in *Arabidopsis* during excess light stress, Plant Cell 9 (1997) 627–640.
[29] P.L. Conklin, R.L. Last, Differential accumulation of antioxidant mRNAs in *Arabidopsis thaliana* exposed to ozone, Plant Physiol. 109 (1995) 203–212.
[30] M.C. Criqui, E. Jamet, Y. Parmentier, J. Marbach, A. Durr, J. Fleck, Isolation and characterization of a plant cDNA showing homology to animal glutathione peroxidases, Plant Mol. Biol. 18 (1992) 623–627.