Available online at www.sciencedirect.com

SciVerse ScienceDirect

South African Journal of Botany 78 (2012) 295–301

SOUTH AFRICAN JOURNAL OF BOTANY

www.elsevier.com/locate/sajb

Short communication

Overexpression of the acerola (*Malpighia glabra*) monodehydroascorbate reductase gene in transgenic tobacco plants results in increased ascorbate levels and enhanced tolerance to salt stress

H.A. Eltelib [1], Y. Fujikawa, M. Esaka *

Graduate School of Biosphere Science, Hiroshima University, 1-4-4 Kagamiyama, Higashi-Hiroshima 739-8528, Japan

Received 11 May 2011; received in revised form 19 July 2011; accepted 16 August 2011

Abstract

Monodehydroascorbate reductase (MDHAR, EC 1.6.5.4) is a key enzyme of the ascorbate (AsA)-glutathione cycle that maintains reduced pools of AsA and serves as an important antioxidative enzyme. Previously, we have cloned MDHAR cDNA from acerola (*Malpighia glabra*), a plant that accumulates abundant amount of AsA. In this study, MDHAR cDNA from acerola was introduced into tobacco plants using an *Agrobacterium*-mediated gene delivery system. Transgenic tobacco plants accumulated greater amounts of AsA and showed higher MDHAR activity than the control plants. Lipid peroxidation and chlorophyll degradation, which were stimulated in control plants, were restrained in transgenic plants subjected to salt stress. These results indicate that overexpression of acerola MDHAR imparts greater tolerance to salt stress.

Keywords: Acerola (*Malpighia glabra*); Ascorbic acid; Monodehydroascorbate reductase; Salt stress; Transgenic

1. Introduction

Unable to move from their natural environment, higher plants undergo many unfavorable conditions such as drought, salinity, and extreme temperatures. Salinity is a major environmental factor leading to the deterioration of agricultural land and reduction in crop productivity (Vaidyanathan et al., 2003). About one-third of the world's cultivated land is estimated to be affected by salinity (Kaya et al., 2002). In plants, salinity causes diverse adverse effects such as the production of reactive oxygen species (ROS). These interact with a number of cellular molecules and metabolites, thereby leading to various destructive processes and cellular damage (Ashraf, 2009). ROS can seriously damage chlorophyll, proteins, membrane lipids, and nucleic acids (Alscher et al., 1997).

Plants possess an antioxidant defense system, which protects them from ROS. This system includes ascorbate (AsA), glutathione and α-tocopherol in addition to antioxidative enzymes such as catalase, superoxide dismutase, peroxidases, and enzymes involved in the AsA-glutathione cycle. Among the antioxidants, AsA plays a central role in defense against oxidative stress (Smirnoff, 1996). We previously reported an increase in AsA levels and tolerance to oxidative stress in transgenic tobacco cells expressing L-galactono-1,4-lactone dehydrogenase (Tokunaga et al., 2005). The AsA-glutathione cycle includes enzymes such as ascorbate peroxidase, monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase and glutathione reductase. Ascorbate peroxidase simultaneously catalyzes the reduction of H_2O_2 and the oxidation of AsA with the generation of monodehydroascorbate

Abbreviations: AsA, ascorbic acid; DHA, dehydroascorbate; MDA, malondialdehyde; MDHAR, monodehydroascorbate reductase; *MgMDHAR*, *Malpighia glabra* MDHAR; NOS, nopaline synthase; ROS, reactive oxygen species; T-AsA, total ascorbic acid.

* Corresponding author. Tel.: +81 82 4247927; fax: +81 82 4247916.
E-mail address: mesaka@hiroshima-u.ac.jp (M. Esaka).
[1] Permanent address: Faculty of Agriculture, Alzaiem Alazhari University, Khartoum North 13311, Sudan.

0254-6299/$ - see front matter © 2011 SAAB. Published by Elsevier B.V. All rights reserved.
doi:10.1016/j.sajb.2011.08.005

(MDHA). MDHAR may be critical in maintaining proper AsA concentrations by directly reducing MDHA to AsA (Asada, 1999). The active AsA-glutathione cycle plays an important role in the efficient removal of excess ROS (Noctor and Foyer, 1998; Asada, 2006). Protective functions provided by AsA and related antioxidant enzymes against photooxidative stress in chloroplasts have also been reported (Noctor and Foyer, 1998). Many antioxidant genes have been engineered in plants to tolerate oxidative stress. Transgenic potato plants over expressing L-gulono-γ-lactone gene isolated from rat were found to withstand various abiotic stresses caused by methyl viologen, NaCl or mannitol (Hemavathi et al., 2010). Recently Li et al. (2010) reported that overexpression of chloroplastic MDHAR enhanced tolerance to temperature and methyl viologen-mediated oxidative stresses in tomato.

Acerola (*Malpighia glabra*) is a tropical plant that accumulates large concentrations of AsA. We showed previously that acerola exhibits a much greater expression of AsA-biosynthetic enzymes than the model plant *Arabidopsis thaliana* (Badejo et al., 2009a). We also cloned the acerola AsA-recycling gene *MgMDHAR* and showed that it is vital for maintaining a high ascorbate redox status during stress conditions (Eltelib et al., 2011). Here we successfully generated transgenic tobacco plants overexpressing *MgMDHAR* and evaluated the performance of transgenic plants under salt stress conditions.

2. Materials and methods

2.1. Plant materials, plasmid construction and plant transformation

Tobacco plants (*Nicotiana tabacum* L.) were grown on MS (Murashige and Skoog, 1962) medium and maintained in a growth chamber at 25 °C under a 16/8-h light/dark photoperiod. The cDNA encoding *MgMDHAR* (accession number AB558587) was inserted into the *Bam*HI and *Sst*I sites of the binary vector pBI121 (Clontech, CA, USA). This replaced the *β*-glucuronidase reporter gene downstream of the CaMV 35S promoter and upstream of the NOS terminator to construct the expression vector pBI-*MgMDHAR* (Fig. 1A). This construct was delivered into *Agrobacterium tumefaciens* strain LBA4404 by electroporation and then into tobacco using the leaf disc method described previously (Badejo et al., 2008), resulting in *MgMDHAR*-transformed tobacco. T_0 transgenic plants were allowed to self-pollinate and T_1 seeds were collected and germinated on MS medium containing 100 μg mL^{-1} kanamycin. Non-transformed plants seeds were germinated on antibiotic-free MS medium. Transgenic and non-transformed plants were maintained at 25 °C under a 16/8-h light/dark photoperiod. Seven weeks later, the seedlings were transplanted into soil and maintained at 25 °C.

2.2. DNA isolation, PCR, and DNA blot analysis

Genomic DNA was extracted from tobacco leaves by the CTAB method described earlier (Eltelib et al., 2011). To detect the transgene, we performed PCR analysis using the sets of gene specific primers (5′-GGCTATTCGGCTATGACTGGGCAC-3′), and (5′-ATCACGGGTAGCCAACGCTATGTCC-3′) for the kanamycin resistance gene (*nptII*) and (5′-ATGGCAGAGAAGACTTTCA-3′) and (5′-GATCTTACAGGCAAAGGAGAGG-3′) for the *MgMDHAR* gene. The amplified DNA fragments corresponding to *nptII* (628 bp) and *MgMDHAR* (1304 bp) were detected by electrophoresis in 1% agarose gels. We performed DNA blot analysis as described previously (Eltelib et al., 2011).

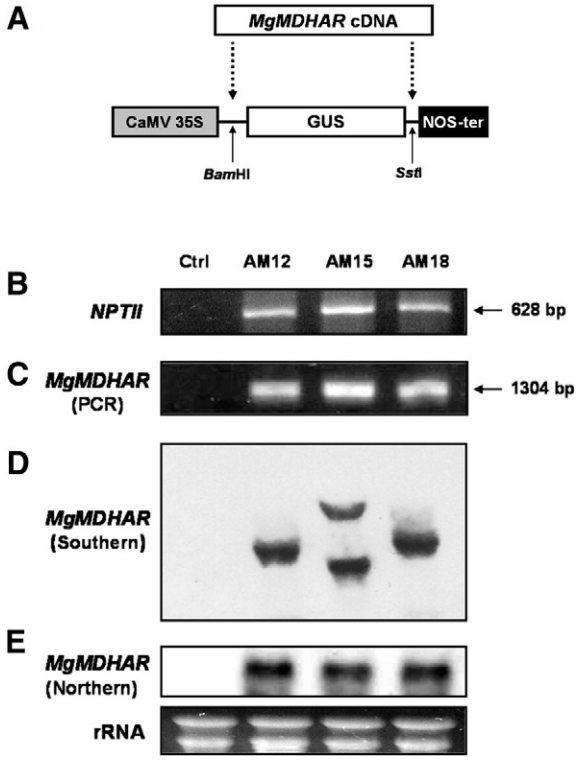

Fig. 1. Expression of *Malpighia glabra* MDHAR (*MgMDHAR*) in transgenic tobacco plants. (A) Schematic representation of *MgMDHAR* expression vector used to transform tobacco plants. CaMV 35S, cauliflower mosaic virus 35S promoter; GUS, β-glucuronidase reporter gene; Nos-ter, nopaline synthase terminator. (B and C) PCR using the kanamycin resistance gene *nptII* and *MgMDHAR* gene specific primers for detection of the transgene in the genome of the selected *MgMDHAR* transgenic lines. The size of the amplified products corresponds to the size of the cDNAs (*nptII*, 628 bp and *MgMDHAR*, 1304 bp). (D) DNA blot analysis for detection of *MgMDHAR* in the genome of *MgMDHAR*-transgenic lines. Genomic DNA (10 μg) from tobacco leaves were digested with *Eco*RI, fractionated on agarose gel, transferred to a nylon membrane and hybridized with digoxigenin-labeled cDNA probe of *MgMDHAR*. (E) RNA blot analysis for the mRNA expression levels of *MgMDHAR* in *MgMDHAR*-transgenic lines. Total RNA (10 μg) were extracted from tobacco leaves, and probed using *MgMDHAR* cDNA.

2.3. RNA isolation and RNA blot analysis

We confirmed the expression of *MgMDHAR* in the transgenic lines by RNA blot analysis (Eltelib et al., 2011). Total RNA was isolated from tobacco leaves as described previously (Badejo et al., 2009b), and subjected to RNA blot analysis.