Plant Physiology and Biochemistry 96 (2015) 388–400

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

journal homepage: www.elsevier.com/locate/plaphy

Research article

Overexpression of a tomato flavanone 3-hydroxylase-like protein gene improves chilling tolerance in tobacco

Chen Meng [b,1], Song Zhang [a,1], Yong-Sheng Deng [c], Guo-Dong Wang [a], Fan-Ying Kong [a,*]

[a] State Key Laboratory of Crop Biology, College of Life Science, Shandong Agricultural University, Daizong Street, Tai'an, Shandong 271018, China
[b] Key Laboratory of Plant Cell Engineering and Germplasm Innovation, Ministry of Education, School of Life Sciences, Shandong University, Jinan 250100, China
[c] Key Laboratory of Cotton Breeding and Cultivation in Huang-Huai-Hai Plain, Ministry of Agricultural, Cotton Research Centre, Shandong Academy of Agricultural Science, Jinan 250100, China

A R T I C L E I N F O

Article history:
Received 17 June 2015
Received in revised form
14 August 2015
Accepted 24 August 2015
Available online 29 August 2015

Keywords:
Chilling stress
Flavonoids
Flavanone 3-hydroxylase-like protein
Reactive oxygen species
Tomato
Transgenic tobacco

A B S T R A C T

Flavonoids are secondary metabolites found in plants with a wide range of biological functions, such as stress protection. This study investigated the functions of a tomato (*Solanum lycopersicum*) flavanone 3-hydroxylase-like protein gene *SlF3HL* by using transgenic tobacco. The expression of the gene was up-regulated under chilling (4 °C), heat (42 °C), salt (NaCl) and oxidative (H_2O_2) stresses. The transgenic plants that displayed high *SlF3HL* mRNA and protein levels showed higher flavonoid content than the WT plants. Moreover, the expression of three flavonoid biosynthesis-related structural genes, namely, chalcone synthase (CHS), chalcone isomerase (CHI) and flavonol synthase (FLS) was also higher in the transgenic plants than in the WT plants. Under chilling stress, the transgenic plants showed not only faster seed germination, better survival and growth, but also lower malondialdehyde (MDA) accumulation, relative electrical conductivity (REC) and H_2O_2 and $O_2^{\cdot-}$ levels compared with WT plants. These results suggested that SlF3HL stimulated flavonoid biosynthesis in response to chilling stress.
© 2015 Elsevier Masson SAS. All rights reserved.

1. Introduction

Chilling stress is a major environmental factor that limits plant growth, productivity and geographical distribution. Chilling-sensitive plants, including important vegetable crops (e.g. sweet pepper, tomato and cucumber), suffer from chilling injury at temperatures below 10 °C–12 °C. Therefore, the response and adaptation mechanisms of plants to chilling stress should be understood. Chilling stress limits enzyme activities in the Calvin cycle, which consequently reduces the utilization of absorbed light energy for CO_2 assimilation and induces the overproduction of reactive oxygen species (ROS). ROS accumulation causes DNA and RNA damage, protein oxidation, membrane lipid peroxidation and, ultimately, cell death. Plants have evolved enzymatic and non-enzymatic antioxidant defence systems to scavenge ROS. The enzymatic system includes ascorbate peroxidase (APX), superoxide dismutase (SOD), catalase (CAT) and guaiacol peroxidase (GPX). The non-enzymatic system includes several plant secondary metabolites, such as ascorbic acid (AsA), glutathione (GSH) and flavonoids, which play important roles in protecting plants from environmentally induced oxidative stress, and also pollen tube germination, seed dormancy, and auxin transport (Brown et al., 2001; Debeaujon et al., 2000; Hichri et al., 2011).

Several studies have focused on the protective effects of flavonoids on plants exposed to abiotic stresses, such as low and high temperatures, high light, drought, salt, low nutrient availability and ultraviolet (UV) damage (Castellarin et al., 2007; Hernández et al., 2009; Park et al., 2007). Flavonoids possess antioxidant

Abbreviations: 4CL, 4-coumarate-CoA ligase; ANS, anthocyanidin synthase; APX, ascorbate peroxidase; C4H, cinnamate4-hydroxylase; CHI, chalcone isomerase; CHS, chalcone synthase; DAB, 3,3′-diaminobenzidine; DFR, dihydroflavonol 4-reductase; F3H, flavanone 3-hydroxylase; F3′H, flavonoid 3′-hydroxylase; FLS, flavonol synthase; GPX, guaiacol peroxidase; H_2O_2, hydrogen peroxide; HPLC, high performance liquid chromatography; K, kaempferol; MDA, malondialdehyde; NAR, naringenin; NBT, nitroblue tetrazolium; $O_2^{\cdot-}$, superoxide radical; PAL, phenylalanine ammonia lyase; PFD, photon flux density; Q, quercetin; qRT-PCR, quantitative real-time polymerase chain reaction; REC, relative electronic conductance; ROS, reactive oxygen species; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; SOD, superoxide dismutase; WT, wild type.

* Corresponding author. State Key Laboratory of Crop Biology, College of Life Sciences, Shandong Agricultural University, 61 Dai Zong Street, Tai'an, Shandong 271018, China.
E-mail address: fykong@sdau.edu.cn (F.-Y. Kong).
[1] Both these authors contributed equally to this work.

http://dx.doi.org/10.1016/j.plaphy.2015.08.019
0981-9428/© 2015 Elsevier Masson SAS. All rights reserved.

properties in vitro. For example, quercetin chelates transition metals, such as Fe^{2+}, to prevent the involvement of such metals in ROS-generating Fenton reaction. The observation that salt-tolerant species often accumulate more flavonoids than the salt-sensitive species suggests a link between flavonoids and salt stress tolerance (Liu et al., 2012). It has long been recognized that flavonoids accumulation was induced rapidly by abiotic stresses. For example, chilling stress increases flavonoid accumulation in grape, maize, red orange and apple (Christie et al., 1994; Mori et al., 2005; Piero et al., 2005; Ubi et al., 2006). In Arabidopsis, chilling stress significantly up-regulates chalcone synthase (CHS), chalcone isomerase (CHI) and flavanone 3-hydroxylase (F3H) to induce flavonoid accumulation (Zhang et al., 2010). In fact, flavonoid pathway-related genes are induced by stress treatment in various plants, such as potato, birch and rice; this finding suggests that these genes play important roles in plant stress tolerance (André et al., 2009; Lenka et al., 2011; Liu et al., 2013; Morales et al., 2010). The overexpression of key flavonoid genes in transgenic plants induces flavonoid accumulation, which is often accompanied by increased antioxidant ability and stress tolerance (Mitsunami et al., 2014; Lukaszewicz et al., 2004; Reddy et al., 2007). By contrast, the down-regulation of flavonoid genes in mutants reduces antioxidant ability and stress tolerance (Oosten et al., 2013). Therefore, the antioxidant capacity of flavonoids could influence their protective effects against stress.

Flavonoid biosynthesis is mainly controlled by structural genes in the highly conserved and well characterized phenylpropanoid pathway and regulatory genes. The structural genes encode multiple enzymes, such as phenylalanine ammonia lyase (PAL), cinnamate4-hydroxylase (C4H), 4-coumarate-CoA ligase (4CL), CHS, CHI, F3H, flavonoid 3′-hydroxylase (F3′H), flavonol synthase (FLS), and dihydroflavonol 4-reductase (DFR, Fig. 1). F3H catalyzes the transition of flavanones to dihydroflavonols, which serve as intermediates for the biosynthesis of flavan 3-ols, flavonols, and

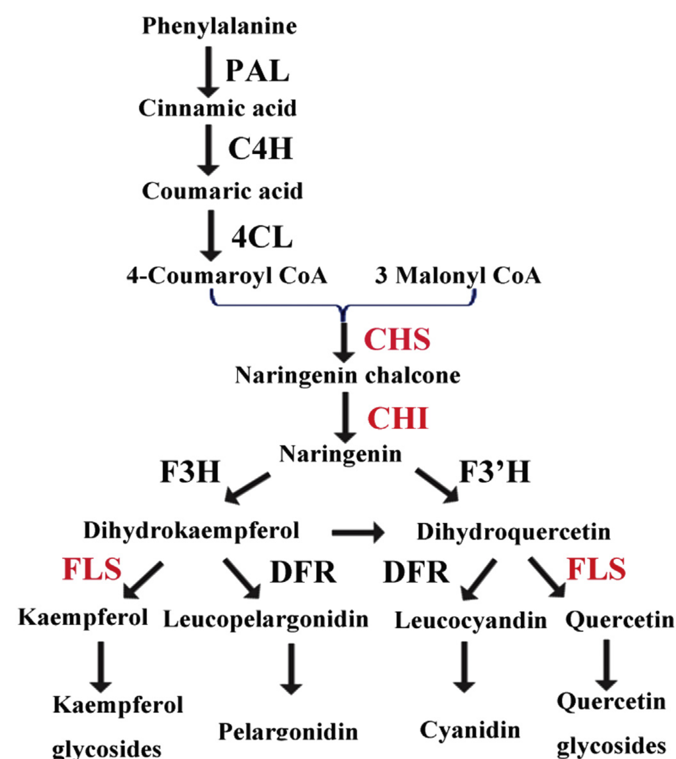

Fig. 1. The flavonoid branch of the phenylpropanoid pathway.

anthocyanidins (Holton and Cornish, 1995). It is classified as a 2-oxoglutarate-dependent dioxygenase (2-ODD) whose activity requires the presence of 2-oxoglutarate, ferrous iron (Fe^{2+}), molecular oxygen, and ascorbate. Since it was first cloned in *Antirrhinum majus* (Martin et al., 1991), F3H have been isolated and characterized in more than 50 plant species including *Perilla frutescenes*, *Zea mays*, *Daucus carota*, *Ginkgo biloba*, and *Persea americana*.

In the present study, we isolated a tomato (*Solanum lycopersicum*) F3H-like protein gene (*SlF3HL*). This protein is a member of the 2-ODD superfamily characterized by a 2OG-FeII-Oxy domain. It shares approximately 67% amino acid identity with Arabidopsis downy mildew resistant 6 (DMR6), which encodes a 2-ODD superfamily member with unknown functions. The expression of DMR6 is associated with stress. DMR6 mutation enhances the expression of a subset of defence-associated genes, suggesting its roles in plant defence (Damme et al., 2008). The expression of *SlF3HL* is also stress associated. In particular, *SlF3HL* overexpression enhances the chilling tolerance of transgenic tobacco plants.

2. Materials and methods

2.1. Plant material, growth conditions and treatments

Tomato seeds (*S. lycopersicum* cv. Zhongshu 4, provided by the Chinese Academy of Agricultural Sciences) were allowed to germinate on Murashige and Skoog (MS) medium at 25 °C for 2 weeks. Sprouted seedlings were transplanted into sterilized soil with nutrient solution and grown at 25 °C/20 °C (day/night) with a 16/8 h photoperiod, 500 μmol m^{-2} s^{-1}–600 μmol m^{-2} s^{-1} photon flux density (PFD) and 50%–60% relative humidity in a greenhouse. Two-month-old plants were used for subsequent abiotic stress assays in a growth chamber (GXZ-260C). For chilling treatment, the plants were exposed to 4 °C in the illuminated incubation chamber with a PFD of approximately 200 μmol m^{-2} s^{-1} for 0, 3, 6, 9, 12 and 24 h. For heat treatment, the whole plants in pots were placed in the illuminated incubation chamber at 42 °C for 0, 3, 6, 12 and 24 h. For oxidative treatment, the plant leaves were sprayed with 20 mM L^{-1} H_2O_2. Salt stress was induced by completely immersing the plant roots in 200 mM L^{-1} NaCl solution for 0, 3, 6, 12, 24, 48 and 72 h. For hormonal treatments, plant leaves were sprayed with 100 μM L^{-1} abscisic acid (ABA) and 100 μM L^{-1} jasmonic acid (JA) for 0, 1, 3, 6, 12 and 24 h. The treated plant leaves were harvested at an appropriate time, frozen in liquid nitrogen and then stored at −80 °C.

Tobacco seeds (*Nicotiana tabacum* cv. NC89) of the wild-type (WT) and transgenic plants were surface-sterilized and sown on MS medium for germination. The seedlings were transferred into fresh soil at the four-leaf stage and maintained under the same greenhouse conditions. When the sixth leaf was fully expanded (at about 2 months), the WT and transgenic tobacco plants were used for chilling stress treatment (for investigation of MDA content and REC, the plants were treated for 24 h. For investigation of other physiological parameters, the plants were treated for 12 h). The whole plants were exposed to low temperatures (4 °C) in an illuminated incubation chamber (GXZ-260C) at a PFD of 200 μmol m^{-2} s^{-1}. All the treated samples were immediately frozen in liquid nitrogen and then stored at −80 °C for later use.

2.2. RNA gel blot analyses

Total RNA was isolated from tomato leaves using the total RNA isolation system (Tiangen, Beijing). The total RNA (20 μg) was separated in a 1.2% agarose formaldehyde gel, transferred to a nylon membrane and then fixed on the membrane by cross-linking with UV light. Pre-hybridization was performed at 65 °C for 12 h. The 3′

Download English Version:

https://daneshyari.com/en/article/8354795

Download Persian Version:

https://daneshyari.com/article/8354795

Daneshyari.com