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Research article

Regulation of flavanone 3-hydroxylase gene involved in the flavonoid biosynthesis pathway in response to UV-B radiation and drought stress in the desert plant, *Reaumuria soongorica*

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ABSTRACT

Flavonoid are known to have various functions in growth, development, reproduction, and also involved in diverse stress responses in plants. However, little is known about the roles of the key enzymes in the flavonoid biosynthetic pathway in response to environmental stress, such as UV-B radiation and drought. To understand this problem, we investigated the participation of flavanone 3-hydroxylase gene (F3H), a key enzyme in flavonoid biosynthetic pathway under UV-B radiation and drought stress in the desert plant *Reaumuria soongorica*. A novel cDNA sequence, named as *RsF3H*, was isolated from *R. soongorica*. The deduced amino acids showed high identities to other F3Hs. A phylogenetic analysis indicated that *RsF3H* appeared to be most homologous to F3H from *Malus domestica* (*MdF3H*). *RsF3H* protein structure contained all five conserved motifs for 2-oxoglutarate-dependent dioxygenases (2-ODDs) and an Arg-X-Ser motif, all of which were also found in other F3Hs. Quantitative real-time RT-PCR analysis showed that there was a rapid increase in gene expression of *RsF3H* under stress. Both UV-B radiation and drought stress induced an increase in *RsF3H* enzyme activity and the accumulation of the products in the flavonoid biosynthetic pathway (total flavonoid and anthocyanin). The antioxidant ability (inhibition of lipid oxidation) of total flavonoid was enhanced during this study. The results suggested that one explanation of the stress tolerance of *R. soongorica* may be a combination of an increase in *RsF3H* gene expression, *RsF3H* enzyme activity and the anti-oxidative ability of the metabolic end products in the flavonoid biosynthetic pathway in response to UV-B radiation and drought.

1. Introduction

Flavonoid, a class of low-weight phenolic compounds, are widely distributed in plants. Attributed to their structural diversity, flavonoid perform varied biological functions, such as acting as pigments to attract pollinators, as auxin transport regulators and as molecular signals for the interaction of plants with microorganisms [1]. Most importantly, flavonoid are involved in the responses to biotic and abiotic stress [2]. Over the years, flavonoid always have attracted the attention of many scientists. The flavonoid biosynthetic pathway has been thoroughly investigated [1]. A collection of mutant lines defective in flavonoid biosynthetic pathway have been identified in *Arabidopsis* on the basis of altered color of seed coat [3]. The majority of enzymes in the pathway have been identified from a number of different species [4–6]. Flavonoid biosynthetic genes, such as chalcone synthase (CHS), chalcone isomerase (CHI), flavone synthase II (FSII), flavonoid 3-hydroxylase (F3′H) and flavanone 3-hydroxylase (F3H), have been used to modify flower color [7–10]. F3H is one of the first three genes (CHS, CHI, F3H) encoding the early, unbranched segment of the flavonoid biosynthetic pathway. F3H is a key enzyme in the flavonoid biosynthetic pathway, catalyzing the 3-hydroxylation of (2*S*)-flavanones, such as naringenin to dihydroflavonols. The F3H gene has been cloned from many other species [5,8,11,12].

Abbreviations: F3H, flavanone 3-hydroxylase; 2-ODDs, 2-oxoglutarate-dependent dioxygenases; RACE, rapid amplification of cDNA ends; TDR, time domain reflectometry; ORF, open reading frame; TBA, thiobarbituric acid; MDA, malonaldehyde; qRT-PCR, quantitative real-time RT-PCR; ROS, reactive oxygen species; HPLC, high performance liquid chromatography.

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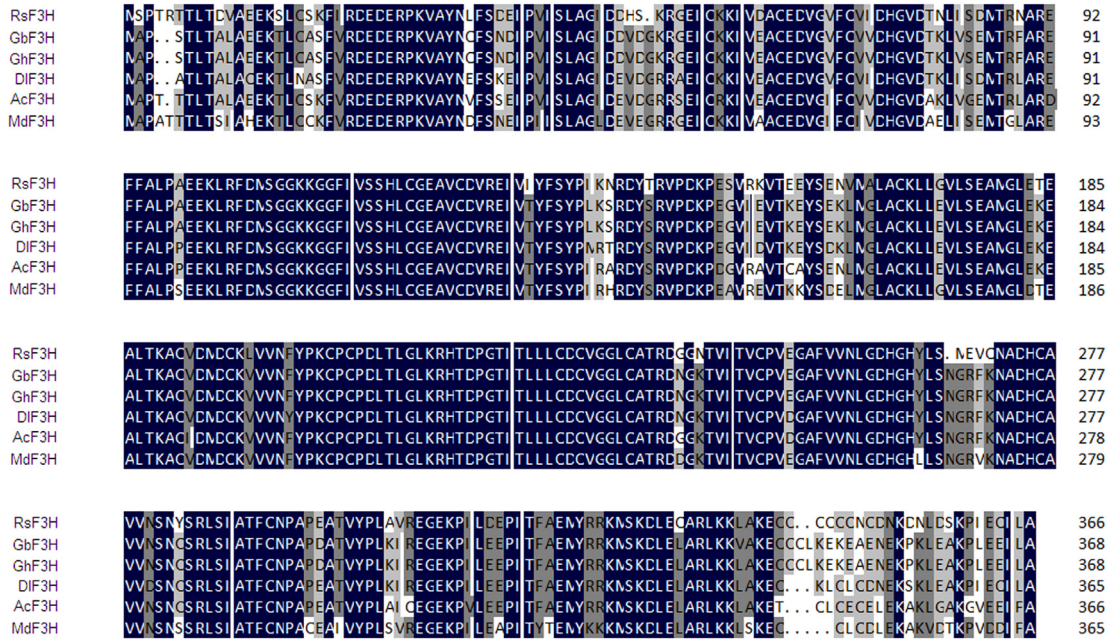

Fig. 1. Alignment of *RsF3H* with F3H proteins from other species. Genbank accession numbers for the proteins in the alignment are as follows: *GbF3H* (ABL86673), *GhF3H* (ABM64799), *DlF3H* (AB048521), *AcF3H* (ACL54955) and *MdF3H* (AAX89398). The sequences were aligned using DNAMAN software.

Some recent studies have focused on the stress protection role of flavonoid, especially their involvement in responses to abiotic signals, including UV damage, temperature fluctuations and low availability of nutrients and water [13]. Flavonoid were found to accumulate rapidly and had a protective role when plants were exposed to UV-B radiation or drought [14,15]. It is reported that flavonoid are important UV-B shielding compounds due to their absorbance in this wavelength region and the significant increase in their concentrations in epidermal layers under UV radiation [16]. Flavonoid may also inhibit oxidative stress induced by environmental stress [17]. Up-regulation of the flavonoid pathway-related genes has been observed in potato, birch and rice after stress exposure [18–20]. These studies indicated that the flavonoid pathway may be involved in the response to stress. However, the roles of the key enzymes and the regulatory mechanism involved in the response to abiotic stresses in the flavonoid biosynthetic pathway are still poorly understood.

Reaumuria soongorica (Pall.) Maxim., a super-xerophytic desert semi-shrub, is a typical constructive and dominant species of desert vegetation community and is widely distributed in northwestern China [21]. As a resurrection plant, *R. soongorica* withers and enters a state of dormancy when desiccation. When precipitation occurs, it revives and will continue its life cycle [22]. It can survive in severe environments, showing tolerance to drought, salinity, extreme temperature fluctuations and UV irradiation. In recent years, several studies have focused on genetic diversity, the protection mechanism for photosynthetic components and metabolite changes [21]. Until now, few studies have been carried out on the effect of abiotic stress on the molecular regulation of primary and secondary metabolism in *R. soongorica*.

In the present paper, a gene (*RsF3H*) of the flavonoid biosynthetic pathway was isolated from *R. soongorica* using the rapid amplification of cDNA ends (RACE) method. The expression levels of the *RsF3H* gene, enzyme activity, the contents of total flavonoid and anthocyanin and the antioxidant ability of flavonoid were investigated under UV-B radiation and drought stress. The results of this study are expected to enable us to explore the stress-tolerance mechanism behind flavonoid participating in environmental stress responses in *R. soongorica*.

2. Results

2.1. Characterization of RsF3H

The full-length cDNA of *RsF3H* was 1409 bp and contained an 1101 bp ORF. The entire sequence has been deposited in the Genbank database with accession number JQ043380. It encoded a protein consisting of 366 amino acids with a theoretical molecular mass of 41.35 kDa and an isoelectric point of 5.01. The hydrophobicity profile of the *RsF3H* protein was predicted and the plot analysis showed that *RsF3H* was highly hydrophilic and had no predicted trans-membrane domain. Alignment of predicted amino acid sequences showed that the *RsF3H* protein contained five similar motifs for 2-ODDs [12], which were also found in other F3H proteins (Fig. 1). Among the five motifs, motifs 1, 2, 3 and 4 of *RsF3H* were absolutely conserved compared to the other plants (Fig. 1), but several amino acids had diverged in motif 5. Three prolines were conserved strictly in motifs 2 and 3, which were predicted to play crucial roles in the process of polypeptide folding. There were four amino acid residues (His76, His218, Asp220 and His275) for binding ferrous iron and an Arg285-X-Ser287 motif (RXS) that takes part in 2-oxoglutarate binding in *RsF3H* [12]. All of these were found in similar positions compared to their locations in F3Hs found in other plants (Fig. 1). All the conservation observed in these amino acids suggested that *RsF3H* protein have potential biological function. A phylogenetic analysis indicated that *RsF3H* appeared to be most homologous to F3H from *Malus domestica* (*MdF3H*) (Fig. 2).

2.2. Soil and leaf water content

The soil water content dropped dramatically from 34.6% to 4.94% during drought treatment. And the leaf water content decreased with the dropping of the soil water content (Fig. 3A). Leaf