Gene expression and flavonol biosynthesis are induced by ultraviolet-B and salt stresses in *Reaumuria trigyna*

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Abstract

In plants, flavonoids play roles not only in development, but also in responses to biotic and abiotic stresses. We analyzed the transcriptome data of NaCl-treated *Reaumuria trigyna*, a small, highly haloduric desert shrub, focusing on the flavonoid biosynthetic pathway. We identified 118 unigenes annotated as genes encoding enzymes related to flavonoid biosynthesis, 68 of which were differentially expressed under NaCl treatment (39 upregulated, 29 downregulated). Of the 118 annotated unigenes, 47 were annotated as members of families related to the flavonol biosynthetic pathway (*e.g.*, F3H, FLS, and OMT). Of those 47 genes, about 70 % (32 unigenes) were upregulated under NaCl treatment. Experiments were conducted to monitor changes in gene expression and accumulation of total polyphenols, total flavonols, and antioxidant capacity under NaCl and ultraviolet-B (UV-B) radiation treatments. The expressions of genes related to the flavonol biosynthesis pathway (RtC4H, RtCHS, RtF3H3, RtF3L1, RtF3'O5'H, RtF3'H, RtOMT, and RtMYBF1) increased under NaCl and UV-B treatments. Treatments with NaCl and UV-B also increased the total flavonols content and antioxidant activity. The content of several flavonols including rutin, hyperoside, isorhamnetin-3-O-neohesperidoside, and myricetin increased in response to NaCl and UV-B stresses. Overall, our results show that the expression of genes related to flavonol biosynthesis as well as flavonol content increased in *R. trigyna* under NaCl and UV-B stresses.

Additional key words: antioxidants, differentially expressed genes, flavonoids, reactive oxygen species, transcriptome.

Introduction

Flavonoids are a diverse group of plant secondary metabolites that are widely distributed throughout the plant kingdom. In terrestrial plants, nine subclasses of flavonoids, *i.e.*, chalcones, flavones, flavonols, dihydroflavonols, flavandiols, anthocyanins, proanthocyanidins, isoflavonoids, and aurones have been categorized based on their chemical structures (Winkel-Shirley 2001, Martens et al. 2010). They are of a great pharmaceutical interest because they have health-promoting effects, including antioxidant, anticancer, anti-inflammatory, cardioprotective, and neuroprotective effects on humans and animals (Harborne and Williams 2000, Misra et al. 2010). In plant tissues, flavonoids function as pigments and seed coat components; act as hormone-like signals in pollen tube development, reproduction, and plant-microbe interactions; play protective roles against pests and pathogens; and regulate auxin transport during plant growth and development (Simmonds 2003, Kobayashi et al. 2004, Koes et al. 2005, Peer and Murphy 2007, Stracke et al. 2007, Buer et al. 2010, Misra et al. 2010, Hassan and Mathesius 2012, Pandey et al. 2012). Flavonoids are also produced as a protective response to environmental stresses. Flavonoid biosynthesis is upregulated under a wide range of abiotic stresses. For example, in *Arabidopsis thaliana*, phosphate, nitrogen, high irradiance, temperature, and drought stresses affect the expressions of genes related to the flavonoid synthesis pathway (Rowan et al. 2009, Catalá et al. 2011, Lei et al. 2011, Nakabayashi et al. 2014). Ultraviolet (UV) radiation is one of the strongest inducers of flavonoid accumulation, and many studies have shown that flavonoid synthesis can protect plants from UV-induced damage (Landry et al. 1995, Jansen et al. 2008, Wang et al. 2011, Hectors et al. 2014). For example,
the UV-induced flavonoid accumulation in *Betula pendula* (Zhang et al. 2011) and *Asparagus officinalis* (Eichholz et al. 2012) appear to protect plants from stress-induced reactive oxygen species (ROS). Emiliani et al. (2013) show that *Arabidopsis* plants over-accumulating flavonols showed reduced ultraviolet-B (UV-B) damage with lower DNA damage and lipid peroxidation.

The flavonoid biosynthesis pathway has been extensively studied in several model plants including *Antirrhinum majus*, *Petunia hybrid*, and *Arabidopsis thaliana*. Many of the genes encoding biosynthetic enzymes and transcription factors involved in the pathway are well documented (Beld et al. 1989, Routaboul et al. 2006, Hichri et al. 2011). The transcriptional regulation of structural genes tightly controls flavonoid biosynthesis during plant development and in response to biotic and abiotic stresses (Lepiniec et al. 2006). In grape berries, the key flavonol biosynthesis gene *flavonol synthase 1* (FLS1) was shown to be upregulated by UV-B, leading to increased flavonol content in the skin. Changes in flavonol and anthocyanin composition could be largely explained by the transcript amounts of *flavonoid 3'-hydroxylase* (*F3H*), *flavonoid 3',5'-hydroxylase* (*F3'5'H*), and *O-methyltransferase* (OMT) (Martínez-Lüscher et al. 2014). In wheat, drought stress increased the expression of *chalcone synthase* (CHS), *flavanone 3-hydroxylase* (*F3H*), *FLS*, and *dihydroflavonol reductase* (DFR), resulting in increased flavonoid accumulation (Castellarin et al. 2007). To date, the effects of abiotic stress on the biosynthesis and degradation of flavonoids have been investigated mainly in crop and model plants. Comparatively few studies have focused on flavonoid biosynthesis in wild species such as *Betula pendula*, *Rhus typhina*, and *Salix alba* in such environments.

**Materials and methods**

**Plants, growth conditions, and treatments:** Seeds of *Reaumuria trigyna* Maxim. (family Tamaricaceae) were collected from the eastern Alxa-Western Ordos area in Inner Mongolia. Seeds were sterilized in 10 % (m/v) sodium hypochlorite for 15 min, and then rinsed three times with sterilized double-distilled water. Seeds were germinated on Murashige and Skoog (MS) medium in the dark for 72 h, and then grown on the same medium at a temperature of 25 °C, a relative humidity of 70 %, and a 8-h dark period. This alternation continued for a total of 52 h. Seedlings were grown to at least 10 cm before being transferred to MS liquid medium and grown for 3 weeks.

Healthy seedlings of similar sizes were subjected to either NaCl or UV-B stress. For the NaCl treatment, seedlings were grown for 10 d in MS liquid medium supplemented with NaCl at a final concentrations of 100, 300, or 500 mM. For the UV-B treatments, seedlings were treated with white light supplemented with UV-B radiation (7.5 - 12.5 μmol m⁻² s⁻¹) for 12 h, followed by an 8-h dark period. This alternation continued for a total of 52 h, and samples were collected after 12, 24, and 36 h of UV-B treatment. The UV-B radiation was supplied by UV-B lamps (15 W, *GI5T8E*, *Sanyo*, Osaka, Japan), which emit UV radiation between 280 and 360 nm, with a peak at 306 nm. Therefore, the UV-B treatments included some UV-A radiation (315 - 360 nm). The distance between the UV-B lamps and the plants was about 2 cm. Seedlings were harvested after the NaCl and UV-B treatments. Control plants that were not subjected to UV-B and NaCl treatments were harvested at the same time.

**RNA extraction and first-strand cDNA synthesis:** Analyses of the abundance of gene transcripts related to flavonoid biosynthesis were performed as described previously (Dang et al. 2013). Frozen samples were ground to a fine powder in liquid nitrogen in a precooled mortar and pestle. Then, RNA was extracted using a *MiniBEST* plant RNA extraction kit (*Takara*, Kyoto, Japan), according to the manufacturer’s instructions. Before reverse transcription, the quality of the extracted RNA was checked by agarose gel electrophoresis and verified using an UV spectrometer (absorbance ratios A₂₆₀/A₂₃₀ 2.0 - 2.2 and A₂₆₀/A₂₈₀ > 2.0). First-strand cDNA was synthesized using *PrimeScript™ RT Master Mix* (*Takara*), according to the manufacturer’s instructions.
**KEGG pathway analysis of flavonoid biosynthesis and transcription factor identification:** Genes in the flavonoid biosynthesis pathway were identified by *Illumina* sequencing using a functional annotation file based on the candidate gene name. We used a basic local alignment search tool (BLAST) and the National Center for Biotechnology Information (NCBI) GenBank database (http://www.ncbi.nlm.nih.gov/BLAST). We also performed a BLAST search for *R. trigyna* transcription factors in the plant transcription factor database (http://planttfdb.cbi.pku.edu.cn/blast.php).

**Gene expression analysis by quantitative PCR:** Based on the sequences of six unigenes in the flavonoid pathway, real-time PCR primers were designed by the Beijing Genomics Institute (BGI, Beijing, China; Table 1 Suppl). β-Actin was used as the internal control gene. The qPCR analyses were performed on the Qiagen Rotor-gene Q real-time PCR platform (Qiagen, Hilden, Germany) using a KAPA SYBR® FAST universal qPCR kit (Kapa Biosystems, Boston, MA, USA) to detect transcript abundance. The thermal cycling conditions were as follows: first denaturation at 95 °C for 30 s; then 40 cycles of denaturation at 95 °C for 5 s, and annealing and extension at 55 °C for 30 s. The relative expressions of the selected unigenes were calculated using the 2^(-ΔΔCt) comparative threshold cycle (ΔCt) method. All reactions were performed with three replicates, and data were analyzed using Rotor-gene Q series software.

**Measurements of total polyphenol content:** Total phenolics were extracted from freeze-dried samples (50 mg) in 1 cm³ of 80 % (v/v) ethanol solution at 4 °C for 2 h in the dark. The polyphenol extracts of *A. thaliana* and *R. trigyna* were named AtP and RtP, respectively. Then, HCl was added to a final concentration of 0.1 M and the mixture was incubated at 98 °C for 1 h to hydrolyze any conjugate forms of flavonoids (Bulgantuya et al. 2015). After hydrolysis, samples were evaporated to dryness and then resuspended in 1 cm³ of methanol. Quercetin diluted with ethanol was used to produce the calibration curve (y = 0.0377 x + 0.001, R² = 0.99758). A 0.5-cm³ aliquot of the standard solution or plant extract was mixed with 1.5 cm³ of 95 % (v/v) methanol, 2.8 cm³ of double distilled H₂O, 0.1 cm³ of 10 % (m/v) AlCl₃, and 1 M potassium acetate. After 30 min incubation at room temperature, absorbance was recorded at 415 nm using a SpectraMax® i3 plate reader. The total flavonol content of each sample was calculated as follows: total flavonols [mg g⁻¹] = y × 10 × 1/0.05, where y is content of quercetin in total flavonols according to calibration curve (Pandey et al. 2014).

**Antioxidant assay:** The free radical-scavenging ability of plant extracts was assayed using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and hydrogen peroxide spectrophotometric methods. The DPPH-scavenging activity was measured according to the method of Sanchez-Moreno (2002) with some modifications. A 2.8-cm³ aliquot of a methanolic solution of DPPH (0.1 mM) was mixed with 0.2 cm³ of extract and incubated in the dark for 30 min. The absorbance of the mixture was measured at 517 nm. Ascorbic acid (vitamin C) was used as the positive control. The radical-scavenging activities of the tested samples were evaluated by comparison with a control containing 2.8 cm³ of DPPH solution and 200 cm³ of methanol. The antioxidant activity of each sample was calculated according to the following formula: antioxidant activity [%] = [(Ac - As)/Ac] × 100, where Ac = is the absorbance of the reaction mixture with methanol and As = is the absorbance of the reaction mixture with plant extract. The IC₅₀ is defined as the amount of plant extract [μg cm⁻³] required to decrease DPPH absorbance at 517 nm to one-half of its original absorbance (Naik et al. 2003). H₂O₂-scavenging activity was measured according to the method of Liu (Liu 2005). The 10-cm³ reaction mixture consisted of 1 cm³ of plant extract (1 cm³ methanol as control), 3 cm³ of 2 mM FeSO₄, 3 cm³ of 6 mM salicylic acid, and 3 cm³ of 1 mM H₂O₂. The reaction mixture was incubated for 1 h at 37 °C and its absorbance was measured at 517 nm. The H₂O₂-scavenging activity of each sample was calculated using the same equation as mentioned above.

**Qualitative and quantitative analysis of flavonoids:** Freeze-dried samples were extracted in a 90 % (v/v) methanol solution (0.5 g sample + 10 cm³ of methanol) using sonication at 4 °C for 40 min. The mixture was centrifuged at 13 000 g for 10 min and passed through a 0.22-µm filter, after which the volume was adjusted to 2 cm³ with methanol solution. Rutin, hyperoside, myricetin, dihydromyricetin, tixifolin, quercetin, isorhamnetin-3-O-neohesperidoside, isorhamnetin, and kaempferidide (MUST, Chengdu, China) standard solutions were prepared.
by successive dilution of stock solutions (Table 2 Suppl). All samples were analyzed using an Agilent 1290 ultra-performance liquid chromatograph and an Agilent 6460 triple quadrupole mass spectrometer (UPLC-QQQ-MS) (Agilent Technologies, Singapore) with an Agilent Eclipse Plus C 18 column (50 × 2.1 mm i.d.; 1.8 μm particle size) at 30 °C. The mobile phase was 0.1 % (m/v) formic acid in H2O (solvent A) and MeOH (solvent B). The elution program was as follows: 0.5 min, 10 % B; 1.0 min, 20 %; 3.0 min, 32 %; 5.5 min, 35 %; 7.8 min, 40 %; 8.5 min, 75 %; 9.5 min, 76 %; 9.6 min, 100 %; 12 min, 100 %, then post time 1 min. The injection volume was 2 μL and the flow rate was 0.4 cm³ min⁻¹. Multiple reaction monitoring was used for detection and qualitative analysis in the negative ion mode. The ion source parameters were set as follows: capillary, 4 000 V; gas temperature of 350 °C; gas flow of 11 dm³ min⁻¹; nebulizer of 45 psi. All experiments were repeated three times.

Statistics: The experiments were repeated three times independently. Data were analyzed with the SPSS software using one-way analysis of variance (ANOVA). The significance of differences between treatments was analyzed by Tukey's test.

Results

In a previous study that analyzed transcriptomic data using KEGG enrichment analysis, an unexpected finding was that the flavonoid biosynthesis pathways were very active under NaCl stress (Dang et al., 2013). However, specific genes were not analyzed in that study. In this study, we analyzed the flavonoid synthesis pathway in the transcriptome data of *R. trigyna* under NaCl stress. In total, 118 unigenes encoding enzymes in the flavonoid biosynthesis pathway were identified, of which 68 were DEGs with 39 upregulated and 29 downregulated (Fig. 1). Within the general polyphenolic pathway, 11, 9, and 23 unigenes were identified, belonging to phenylalanine ammonia-lyase (PAL), cinnamate 4-hydroxylase (C4H), and 4-coumaroyl-CoA ligase (4CL) gene families, respectively. An additional 11 unigenes were annotated as CHS and 6 as chalcone isomerase (CHI). Six unigenes belonged to DFR and LDOX gene families, which are involved in anthocyanin biosynthesis. Five

![Flavonoid Pathway Diagram](image)

Fig. 1. Several differentially expressed genes (DEGs) were annotated with the same gene name in the flavonoid pathway. ↑ indicates number of upregulated DEGs, ↓ indicates number of downregulated DEGs. PAL - phenylalanine ammonia lyase; C4H - cinnamate 4-hydroxylase; 4CL - 4-coumaroyl CoA ligase; CHS - chalcone synthase; CHI - chalcone isomerase; F3H - flavanone-3-hydroxylase; F3'H - flavonoid-3′-hydroxylase; F3′5′H - flavonoid-3′,5′-hydroxylase; FNS - flavone synthase; IFS - isoflavone synthase; 12′H, isoflavone 2′-hydroxylase; IFR - isoflavone reductase; IOMT - isoflavone O-methyltransferase; DFR - dihydroflavonol 4-reductase; LDOX - leucoanthocyanidin dioxygenase; UFGT - UDP - flavonoid glucosyltransferase; CYP450 - cytochrome P450; OMT - O-methyltransferase.
unigenes (all downregulated DEGs) encoded isoflavone reductase (IFR), a key enzyme in isoflavonoid biosynthesis. The other 47 unigenes belonged to other families: F3H, F3′H, F3′5′H, FLS, OMT, UFGT, and some types of CYP450, e.g., 7-ethoxycoumarin O-deethylase (ECOD) and glutathione S-transferase (GST). These families are also related to the flavonol biosynthesis pathway. Of these 47 unigenes in other families, about 70% (32 unigenes) were upregulated DEGs under NaCl treatment (Table 3 Suppl and Table 5 Suppl). A total of 512 unigenes were annotated as transcription regulators in four families: MYB, WD40, bHLH, and WRKY. We identified 13 transcription factors that were putatively related to flavonoid biosynthesis in R. trigyna. These unigenes showed 70 - 80% similarity to corresponding transcription factors that are known to play important roles in regulating the flavonoid biosynthesis pathway in Vitis vinifera, Arabidopsis thaliana, and Catharanthus roseus (Table 4 Suppl).

Based on the results of transcriptome analysis, we selected nine DEGs (RtC4H, RtCHS, RtF3H3, RtFLS1, RtFLS2, RtF3′5′H, RtF3′H, RtOMT, and the transcription factor RtMYBF1) showing high expression for further analysis (Fig. 2). The expressions of these genes increased under NaCl stress and UV-B stress. The transcript amounts of RtC4H, RtF3′5′H, RtCHS, RtOMT, and RtFLS1 increased gradually as the NaCl concentration increased from 100 mM to 500 mM. The expression of RtF3H3, RtFLS2, RtF3′H, and RtMYBF1 increased as the NaCl concentration increased from 100 mM to 300 mM and then decreased under 500 mM NaCl. Under UV-B stress, the transcription of RtC4H, RtOMT, RtF3′5′H, RtMYBF1, RtFLS1, and RtFLS2 peaked after 12-h UV-B exposure and decreased with longer exposure. The transcriptions of RtF3′H, RtCHS, and RtF3H increased from 12 to 24 h of UV-B exposure and decreased at 36 h.

Fig. 2. Expression analysis of nine differentially expressed genes (DEGs) of the flavonol synthesis pathway under NaCl or UV-B stress in Reaumuria trigyna. Fold change represents the relative fold-increase in expression as compared to respective control. Control was set to 1. Means ± SDs (n = 3). Different letters indicate significant differences from control (P < 0.05).

Fig. 3. Antioxidant assay of extracts from Reaumuria trigyna and Arabidopsis thaliana: Vc - vitamin C (positive control); AtF and AtP - flavonol and polyphenol extracts from A. thaliana, RtF and RtP - flavonol and polyphenol extracts from R. trigyna.
accumulation in transcription of flavonol biosynthesis pathways were significantly increased under NaCl treatment. Genes unigenes in flavonoid biosynthesis pathways differed significantly between NaCl-treated and control plants. Approximately 70% of the unigenes in flavonoid synthesis pathways were upregulated under NaCl treatment. Genes in flavonol biosynthesis pathways can be upregulated by abiotic stresses, leading to increased flavonol accumulation. For example, drought stress increases the transcription of CHS, F3H, and FLS and flavonol accumulation in V. vinifera (Martínez-Lüscher et al. 2014).

The antioxidant potentials of polyphenol and flavonol extracts were compared between Arabidopsis thaliana and R. trigyna with DPPH as the model free radical. The antioxidant activity is expressed as IC_{50} values (Fig. 3). The flavonol and polyphenol extracts of R. trigyna showed lower IC_{50} values than those of extracts from Arabidopsis thaliana. The flavonol extracts of R. trigyna had the highest antioxidant activity (IC_{50}=8). The polyphenol and flavonol content increased in the seedlings of R. trigyna in response to NaCl and UV-B stresses (Fig. 4A,B). The antioxidant potential of flavonol extracts from R. trigyna subjected to NaCl and UV-B treatments was determined by measuring their ability to scavenge H_{2}O_{2} and DPPH. The ability of R. trigyna flavonol extracts to scavenge H_{2}O_{2} and DPPH was similar, and the antioxidant activity of flavonol extracts from R. trigyna plants subjected to NaCl and UV-B treatments was approximately two-fold that of the control R. trigyna plants (Fig. 4C,D).

We quantitatively analyzed nine flavonols by mass spectrometry to determine changes in their concentrations under NaCl and UV-B treatments. The content of four flavonol components (rutin, isorhamnetin-3-O-neohesperidoside, hyperoside, and myricetin) significantly increased (P < 0.05) under NaCl and/or UV-B stress (Fig. 5), whereas the content of the other five compounds was unchanged (data not shown). Among the flavonols, rutin and hyperoside showed dramatically increased content under both UV-B and NaCl stresses. The highest rutin content was at the 300 mM NaCl treatment [0.81 ± 0.036 mg g^{-1}(d.m.)], higher than the hyperoside content [0.032 ± 0.005 mg g^{-1}(d.m.)]. The isorhamnetin-3-O-neohesperidoside content was higher under NaCl stress than under UV-B stress. The maximum isorhamnetin-3-O-neohesperidoside content was in the 100 mM NaCl treatment [0.043 ± 0.005 mg g^{-1}(d.m.)]. The highest myricetin content was in the 24-h UV-B treatment [0.032 ± 0.005 mg g^{-1}(d.m.)].

**Discussion**

Many studies have focused on the molecular mechanism of flavonoid metabolism, with evidence of increased expression of several biosynthetic genes, including PAL, F3H, CHS, FLS, and DFR when plants are subjected to abiotic stresses (Fan et al. 2014, Ma et al. 2014). In this study on the highly halophytic shrub R. trigyna, the transcription of genes in the flavonoid biosynthesis pathway were significantly increased under NaCl treatment. The transcript of 57.63 % (68/118) of the unigenes in flavonoid biosynthesis pathways differed significantly between NaCl-treated and control plants. Approximately 70% of the unigenes in flavonoid synthesis pathways were upregulated under NaCl treatment. Genes in flavonol biosynthesis pathways can be upregulated by abiotic stresses, leading to increased flavonol accumulation. For example, drought stress increases the transcription of CHS, F3H, and FLS and flavonol accumulation in V. vinifera (Martínez-Lüscher et al. 2014).

In Fagopyrum tataricum, FtFLS was upregulated by salicylic acid and NaCl stress, and higher transcription of FtFLS was correlated with higher flavonol content (Li et al. 2013). In R. trigyna, the transcription of RtFLS1, RtFLS2, RtCHS, and RtF3H3 in the flavonol pathway increased under NaCl and UV-B stresses, and the total flavonol content also markedly increased under these stresses. These results indicated that both NaCl and UV-B treatments induced the expression of genes in the flavonol pathway, leading to increased flavonol content in R. trigyna.

Some transcription factors in the MYB family control the expression of genes encoding flavonol pathway enzymes. In Pinus taeda, PtMYB1 has been hypothesized to activate transcription by binding to the PAL promoter (Bomal et al. 2008). In another study, MYBPA1 and MYB3α/b appeared to regulate the expression of CHI and F3 5’H, both of which are involved in the flavonol
pathway (Matus et al. 2009). MYBF1 was shown to specifically activate FLS transcription, leading to flavonol production (Czemmel et al. 2009). A low expression of MYBF1 led to rapid decrease in FLS1 transcription and flavonol content (Czemmel et al. 2012). In R. trigyna, the expression of RtMYBF1, RtMYB5h and RtMYB1 increased under salt stress, together with the expression of flavonol structural genes and the activity of the flavonol biosynthetic pathway. These MYB TFs may regulate the flavonol pathway of R. trigyna in response to salt stress.

![Graph showing contents of four flavonol components under NaCl or UV-B stress](image)

**Fig. 5.** Contents of four flavonol components (rutin, isorhamnetin-3-O-neohesperidoside, hyperoside, and myricetin) under NaCl stress or UV-B stress. Means ± SDs (n = 3). Different letters indicate significant differences from control (P < 0.05).

There are at least 35 flavonol compounds in the model plant A. thaliana. The huge chemical diversity of flavonols is because of extensive modifications by enzymes (Saito et al. 2013). F3′H and F3′5′H, which catalyze the synthesis of quercetin and myricetin, respectively, are required to produce certain flavonol subclasses (Liu et al. 2014). In R. trigyna, the expression of RtF3′H and RtF3′5′H increased under salt and UV-B stresses, as did the myricetin content. Although the quercetin content did not change significantly, the content of quercetin glycosides (rutin, hyperoside and isorhamnetin-3-O-neohesperidoside) increased in response to NaCl and UV-B treatments in R. trigyna. UFGT catalyzes the glycosylation of flavonol skeletons, and OMT methylates quercetin to form isorhamnetin (Muzac et al. 2000, Jones et al. 2003). In R. trigyna, the expression of three RtUFGT genes and six RtOMT genes increased dramatically under salt stress. These genes encode products that may be responsible for the glycosylation of the flavonols that accumulated under the stress treatments. Flavonol glycosides are very strong free radical scavengers that are involved in the responses to many abiotic stresses (Hirano et al. 2001). Cold, desiccation, and UV-B can affect the rutin content and activity in Fagopyrum tataricum leaves (Suzuki et al. 2005). In another study, content of kaempferol and quercetin glycosides increase substantially in Glycine max and Catharanthus roseus treated with UV-B (Middleton and Teramura 1993, Ferreres et al. 2011). Thus, flavonol glycosides are key flavonoid compounds that protect R. trigyna plants from salt and UV-B stresses.

Plants growing at high altitudes must have mechanisms to prevent damage caused by high UV-B exposure. For example, maize lines growing at high altitudes develop mechanisms to prevent UV-B damage, such as the accumulation of flavonoids in leaves and silks. These compounds increase the antioxidant capacity of the plant and protect them from UV stress (Casati et al. 2005). R. trigyna grows in salinized desert regions where there is extraordinarily high UV-B radiation. Our results showed that the antioxidant activity of R. trigyna flavonol extracts...
was much higher than that of Arabidopsis flavonol extracts. Some plants accumulate more flavonoids when they are grown under stress conditions (Agati et al. 2012). In this work, R. trigyna plants showed increased amounts of flavonol transcripts, increased flavonol content, and increased antioxidant potential when they were grown under UV-B and NaCl stresses. The results showed that the expression of certain genes related to flavonol biosynthesis and the content of some flavonols increased under UV-B and NaCl stresses in R. trigyna, leading to increased antioxidant activity, which improved the tolerance of R. trigyna to these stresses. Although the expression of RfC4H, RfFLS2, and RfMYBF1 (which are related to flavonol biosynthesis) in R. trigyna showed some differences between the NaCl and UV-B treatments, the synthesis of flavonols is tightly regulated by many structural genes and transcription factors (Winkel-Shirley 2001). Thus, the total flavonol content and antioxidant activity did not differ markedly between the two stress treatments; that is, flavonol biosynthesis was similar in the NaCl-treated and UV-B-treated R. trigyna plants.

Some gene transcripts and compounds showed non-linear dose-response relationships with the NaCl or UV-B stresses. The causes of such non-monotonic responses remain unclear (Cvrčková et al. 2015.). However, it is likely that feedback promotion/inhibition, transcription factors and the factors that regulate them, as well as post-transcriptional regulation mechanisms contribute to the non-linear response curves of certain genes and compounds. In general, our results showed that both genes related to flavonol biosynthesis and the accumulation of certain flavonols was induced by UV-B and salt stress in R. trigyna. The results may be caused by these two stresses regulating the response elements of various inducible genes in flavonol biosynthetic pathway. More research is required to explore these ideas. The findings reported in the current study provide new information about the relationships between environmental factors and flavonoid accumulation in R. trigyna.

References


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