

Research Paper

Different response of GTP cyclohydrolase I gene from grape under abiotic stresses

Nadia Eslami-Bojnourdi, Raheem Haddad*, Ghasem-Ali Garoosi, and Reza Heidari-Japelaghi

Received: February 25, 2023 Accepted: June 5, 2023

Department of Biotechnology Engineering, Faculty of Agricultural and Natural Resources, Imam Khomeini International University, Qazvin, Iran

*Corresponding author; Email: r.haddad@eng.ikiu.ac.ir

Abstract

Folates are a vital necessity for the retention of normal cellular activity. In contrast to humans, other organisms including plants gain folate coenzymes via *de novo* synthesis. GTP cyclohydrolase I (gtpch I, EC 3.5.4.16) catalyzes the first step of the biosynthesis of tetrahydrofolate (FH₄) in plants by the conversion of GTP to dihydroneopterin triphosphate and formic acid. In this research, the expression pattern of the *Vvgtpch I* gene was assayed in different organs of the grape by the semi-quantitative RT-PCR. The analyses demonstrated that the *Vvgtpch I* gene was expressed in all grape organs. The highest amounts of expression were obtained in berry and leaf, whereas the lowest amount of *Vvgtpch I* transcript was related to the cluster. The response of *Vvgtpch I* gene to abiotic stresses was also investigated under the alkali and cold stresses by the semi-quantitative RT-PCR. Under the alkali stress, the transcript level of *Vvgtpch I* gene decreased considerably. Similar to the alkali stress, the transcript level of *Vvgtpch I* gene decreased under cold stress as well. To analyze the *Vvgtpch I* gene expression under oxidative stress, different treatments were applied such as chemical inducers, heavy metals, and plant growth regulators to trigger the production of reactive oxygen species. The *Vvgtpch I* showed a strong increase and a moderate increase in the transcript amount with Cu²⁺ and H₂O₂, respectively. Whereas, its transcript level was relatively down-regulated by the heavy metals and hormonal treatments, and almost disappeared by diamide.

Keywords: folate, gene expression, oxidative stress, reactive oxygen species, abiotic stress

How to cite: Eslami-Bojnourdi N, Haddad R, Garoosi GA, Heidari-Japelaghi R. 2023. Different response of GTP cyclohydrolase I gene from grape under abiotic stresses. J Plant Physiol and Breed. 13(2): 131-143.

Abbreviations: ABA: abscisic acid; EST: expressed sequence tag; FH₄: tetrahydrofolate; gtpch I: GTP cyclohydrolase I; PGR: plant growth regulator; ROS: reactive oxygen species; RT-PCR: reverse transcription-PCR; SA: salicylic acid.

Introduction

GTP cyclohydrolase I (gtpch I, EC 3.5.4.16) is involved in the conversion of GTP to dihydroneopterin triphosphate and formic acid via a complicated series of reactions. This reaction is the first step in the biosynthesis of tetrahydrofolate (FH₄) and tetrahydrobiopterin (BH₄) in plants, and some microorganisms,

and mammals, respectively (Blau and van Spronsen 2014). Folates belong to an extensive family of polyglutamates known as vital cofactors for biosynthesis of purines, pyrimidines, pantothenate, thymidylate, and in the metabolism of some amino acids, such as methionine, serine, and glycine (Blancquaert *et al.* 2014). Deficiency of folate in the diet

results in neural tube defects in infants (including spina bifida and anencephaly) (Imbard *et al.* 2013), increased risk of vascular disease, some cancers, and cerebral folate deficiency syndromes in childhood (Aune *et al.* 2011; Perez-Duenas *et al.* 2011). However, folic acid fortification of grain products has caused a considerable reduction in the occurrence of neural tube defects, some childhood cancers, and stroke (Strobbe and van Der Straeten 2017).

Humans cannot make folate in their bodies and must get it from food sources including crops, animal-based foods, or nutritional supplements. The staple crops such as wheat (10 to 91 $\mu\text{g}/100$ g FW), maize (33 to 129 $\mu\text{g}/100$ g FW), rice (11 to 111 $\mu\text{g}/100$ g FW), and soybean (344.06–685.81 $\mu\text{g}/100$ g FW) contain high levels of folates (Ashokkumar *et al.* 2018; Liang *et al.* 2020; Shahid *et al.* 2020; Agyenim-Boateng *et al.* 2023a). However, folate intake in humans often is lower than the prescribed rates of 400 $\mu\text{g}/\text{day}$ for adults and 600 $\mu\text{g}/\text{day}$ for pregnant women (Agyenim-Boateng *et al.* 2023b). Biofortification through conventional breeding and/or plant genetic engineering is a sustainable approach to enriching crops with folates (Ashokkumar *et al.* 2020).

The *Vvgtpch I* gene with chromosomal position 1 was submitted in NCBI GenBank database under RefSeq accession number XM_002269229. The *Vvgtpch I* genomic

sequence with 4,964 bp length contains two exons and an intron. Also, its ORF is 1,338 nucleotides in length encoding a protein with 445 amino acid residues. The computed molecular mass and the predicted isoelectric point of the deduced polypeptide are 48.65 kDa and 6.43, respectively. The *Vvgtpch I* gene expression has been analyzed in various plants under different abiotic stress conditions. Changsong and Diqiu (2010) assayed the transcript expression pattern in mature pollen of *Arabidopsis* using Affymetrix GeneChips containing ~24,000 genes. They showed that *gtpch I* gene is down-regulated in the mature pollen of *Arabidopsis* under cold stress. Moreover, analysis of folates in seedlings, inflorescence, and dry seeds of *Arabidopsis* indicated stable levels in the wild-type, mutant, and overexpressor line, upon heat and drought stress and normal conditions, indicating no induction of FH_4 biosynthesis genes, especially *gtpch I* gene (Navarrete *et al.* 2012). Furthermore, it has been reported that *gtpch I* gene from the grape was differently induced under different abiotic stress conditions. Its transcript level decreased significantly under drought, salt, and heat whereas it was increased under heavy metal treatments (Eslami-Bojnourdi *et al.* 2017).

The *gtpch I* has been a suitable gene for metabolic engineering because it is imagined to be the rate-limiting step controlling flux into the folate pathway (Wakeel *et al.* 2018). In rice

(*Oryza sativa*), folate biofortification of seeds obtained 100 times above wild type via overexpression of two *Arabidopsis thaliana* genes coding for *gtpch* I and aminodeoxychorismate synthase (Storozhenko *et al.* 2007). Nunes *et al.* (2009) produced transgenic lettuce lines containing a synthetic codon-optimized *gtpch* I gene based on the native red jungle fowl (*Gallus gallus*) gene. Immunoblotting analyses affirmed the presence of the *gtpch* I in the transgenic lines. Another example of plant genetic engineering was reported in tomato fruit in which the aminodeoxychorismate synthase gene was overexpressed through the gene from *Arabidopsis*. Compared with the wild-type, the transgenic fruit contained an average of 19-fold more p-ABA, without any enhancement in the folate amount (Diaz de la Garza *et al.* 2007). In contrast, Ramirez Rivera *et al.* (2016) introduced *gtpch* I gene from *Arabidopsis* into three common bean cultivars by particle bombardment and enhanced the folate levels in common bean via engineering the pteridine branch required for their biosynthesis. Recently, De Lepeleire *et al.* (2018) have indicated that the introduction of downstream genes in mitochondrial folate biosynthesis (HPPK/DHPS and/or FPGS) results in the enhancement of folate to appropriate levels (12-fold) and ensures folate stability upon long-term storage of potato (*Solanum tuberosum*) tubers.

Here, we analyzed the transcript level of the *Vvgtpch* I gene from the grape (*Vitis vinifera* L. cv. Askari) under non-stress and stress conditions by the semi-quantitative RT-PCR. The aim was to show that the *Vvgtpch* I gene differentially expresses in different organs of grape. Moreover, we wanted to reveal that *Vvgtpch* I gene is differentially induced upon abiotic/oxidative stress conditions.

Materials and Methods

Plant materials

Different organs of grape (*V. vinifera* L. cv. Askari) in clouding berries, leaves, petioles, clusters, roots, and seeds were prepared from the Grape Research Station, Takistan, Qazvin, Iran, during the 2018 field season. After immediate freezing in liquid nitrogen, all samples were stored at -80 °C until use. By gently breaking the berries in liquid nitrogen, seeds were separated from the berries at the veraison stage, then pericarp and seed portions were separately stored at -80 °C.

Total RNA extraction and first-strand cDNA synthesis

Total RNA was extracted from different grape organs as described by Heidari-Japelaghi *et al.* (2011). First-strand cDNA was synthesized with 2 µg of total RNA treated with DNase I (Fermentas) as a template and 1 µg Oligo (dT)₁₈ primer (Qiagen) for 5 min at 70 °C.

After incubation of the reaction mixture with RevertAidTM M-MuLV Reverse Transcriptase (200 u/μl, Fermentas) for 60 min at 42 °C, the reaction was stopped by heating the mixture at 70 °C for 10 min.

Abiotic stress treatments

To study the response of *Vvgtpch I* gene to various abiotic stresses, the cuttings were treated with abiotic stimuli in three independent replicates. For the alkali stress, the cuttings were treated with various concentrations of NaHCO₃, including 25, 50, 75, 100, 125, and 150 mM (Gong *et al.* 2013). The cuttings were gently exposed to different concentrations of NaHCO₃ during 5–7 days and then the youngest fully expanded leaves were harvested after almost seven days. Control and alkali-treated cuttings were incubated under a 16 h light period with a photon fluence rate of 120 μM quanta m⁻² s⁻¹ at 21 °C and 50% humidity in a greenhouse. Cold stress was also accomplished by exposing the cuttings to a temperature of 4 °C for 6, 12, 18, 24, 48, and 72 h under a 16 h light period, a photon fluence rate of 120 μM quanta m⁻² s⁻¹, and 50% humidity in a growth chamber (Grouc, Iran).

Oxidative stress treatments

To test whether the steady-state transcript levels may respond to oxidative stress, varied treatments were applied including chemical

inducers, heavy metals, and PGRs to induce the production of reactive oxygen species. The effectors were used to leaf slices through incubation in the effector solution after infiltration to ensure quick and homogenous application. Three to four youngest fully expanded leaves were removed from one-year-old cuttings and cut into 1 cm diameter leaf slices. After vacuum infiltration with deionized distilled water, the leaf slices were suspended in effector solutions (pH 5-6) including 10 mM H₂O₂, 1mM diamide, 100 μM CuSO₄, 100 μM CoCl₂, 100 μM CdCl₂, 100 μM AlCl₃, 100 μM abscisic acid (ABA), and 100 μM salicylic acid (SA). The leaf slices were incubated at 21 °C and a photon fluence rate of 120 μmol quanta m⁻² s⁻¹ for 4 h.

Semi-quantitative RT-PCR

The semi-quantitative RT-PCR amplifications were performed with 100 ng of the reverse transcription product in a final volume of 20 μl containing specific primers (10 pmol) for the coding sequence of *Vvgtpch I* gene (Table 1). In parallel, the grape actin gene (*Vvact*) as an internal control was also amplified in all samples. PCR reactions were carried out in a programmable thermal cycler with the following temperature parameters: the initial denaturation for 3 min at 94 °C, followed by 30 cycles of 30 s at 94 °C, 1 min at 58 °C, 1 min at 72 °C, and the final extension for 5 min

at 72 °C. A reaction without a template was used as a negative control for each set of RT-PCR amplifications and reactions were carried out in triplicates. RT-PCR products were detected by the agarose gel electrophoresis and quantified using ImageJ software (W.S.

Rasband; 1997–2007; National Institutes of Health; <http://rsb.info.nih.gov/ij>). The normalization of signal intensities was accomplished concerning *Vvact* gene from the same sample.

Table 1. Nucleotide sequence of oligonucleotide primers used for semi-quantitative RT-PCR designed by Oligo7 software.

Primer name	Sequence (5'-3')	Melting temperature (°C)	Size of amplicon (bp)
Semi-quantitative RT-PCR			
Vvgtpch IF	ATGGGCGTCTTGGACGAGG	61.5	1,338
Vvgtpch IR	TCAAGAAGTTGGAGTGTTTTGAA	58.6	
VvactF	GTTAGCAACTGGGATGATATGG	66.8	530
VvactR	AGCACCAATCGTGATGACTTG	69.6	

Statistical analysis

The data were evaluated statistically by SPSS ver. 16 (SPSS Inc., Chicago, IL, USA), and comparisons between the treatment means were made by the Duncan multiple range test at the probability level of $p \leq 0.05$. The data were expressed as mean \pm standard deviation of three biological replicates.

Results and Discussion

Expression profile of *Vvgtpch I* in different organs

The number of expressed sequence tags (ESTs) coding for *Vvgtpch I* in various organs was accounted for among the whole grape ESTs recorded on the NCBI GenBank database. Around 13 ESTs were detected for *Vvgtpch I* in different grape organs including flower (6 hits), leaf (3 hits), root (3 hits), and berry (1 hit). However, no EST coding for

Vvgtpch I was found in clusters, petioles, and seeds in the NCBI GenBank database. Based on the number of identified ESTs, the *Vvgtpch I* gene probably appeared to be transcribed at a low level in different organs. Similarly, analysis of RNA-Seq mRNA baseline using the Expression Atlas Database revealed that the *Vvgtpch I* gene may express at medium or low levels in different cultivars or in various organs at different developmental stages (Figure 1a).

The analyses based on semi-quantitative RT-PCR demonstrated that the *Vvgtpch I* gene was expressed in all grape organs. The highest levels of expression were observed in berries and leaves, whereas the lowest amount of *Vvgtpch I* transcript was related to the clusters. Petioles, roots, and seed organs harbored almost similar transcript levels (Figure 1b). Waller *et al.* (2010) reported that changes in

transcript abundance of *gtpch* I gene during fruit development of tomato were maximally expressed at the mature green stage, and declined at the breaker, red, and red ripe stages. Similarly, transcripts for *gtpch* I were not detectable after the early phases of fruit

ripening in tomato. The mRNA level of *gtpch* I was highest in unripe (mature green and breaker stage) fruits. As the ripening progressed, the mRNA level declined aggressively and was scarcely detectable when the fruit reached full color and was softening

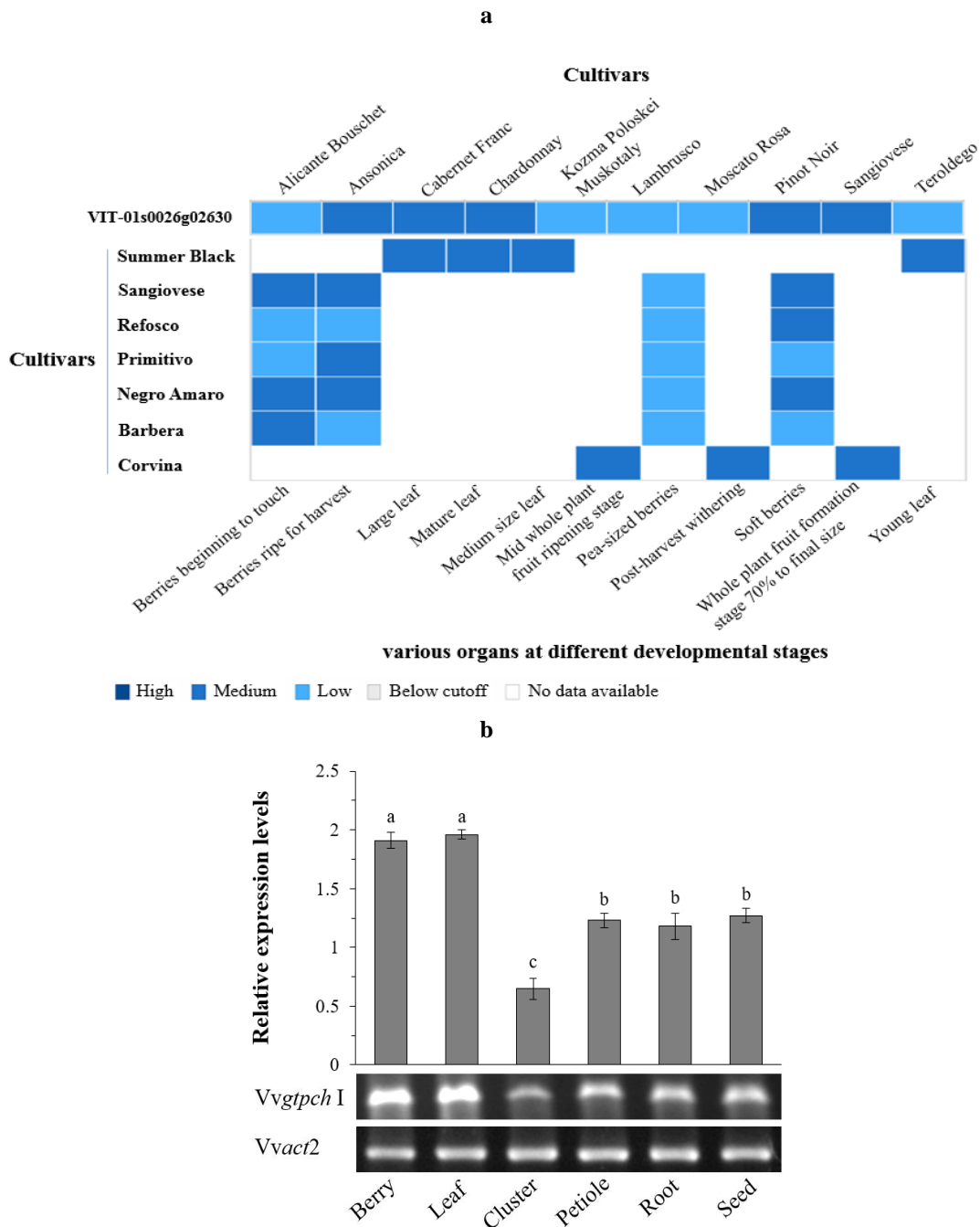


Figure 1. Expression of the *Vvgtpch* I gene in different grape organs. (a) Analysis of RNA-Seq mRNA baseline of the *Vvgtpch* I gene in different cultivars or various organs at different developmental stages using the Expression Atlas Database, (b) Analysis of expression pattern of the *Vvgtpch* I gene in different grape organs by the quantitative real-time-PCR and the semi-quantitative RT-PCR.

(red-ripe stage) (Basset *et al.* 2002). A differential expression profile of *gtpch* I mRNA took place overall seed development, whereas folate accumulation was reduced. The *gtpch* I transcripts were present in the embryo and maternal layers but not the endosperm (McIntosh *et al.* 2008).

Expression analysis of Vvgtpch I gene under abiotic stresses

Under abiotic stress conditions, plants have extended different mechanisms via a combination of metabolic, physiological, and morphological changes. These adaptive changes depend mostly on alterations in gene expression (Kamal *et al.* 2010). The salinity and alkalinity as two major abiotic stresses cause both hyperionic and hyperosmotic conditions, which finally lead to plant death. The tolerance to salinity and alkalinity is controlled by various genes involved in different molecular processes, such as selective ion uptake (Shi *et al.* 2000), accumulation of osmoprotectants and antioxidants (Blokhina *et al.* 2003), and expression of transcription factors (Baena-Gonzalez *et al.* 2007). Cold stress negatively influences the growth and development of plants via direct prohibition of metabolic reactions and, indirectly, via cold-induced osmotic, and oxidative stresses (Chinnusamy *et al.* 2007). The up-regulation of several genes

in response to cold stress, including cold-responsive genes encoding molecular chaperones, and several signal transduction and regulatory proteins was also reported (Mahajan and Tuteja 2005). Moreover, by transcriptomic analyses, the expression of *gtpch* I and II genes has been affected by biotic and abiotic stresses in pepper (*Capsicum annuum*) (Kim *et al.* 2019) and in wheat (Gupta *et al.* 2016; Diaz *et al.* 2019), respectively.

Under the alkali stress, the transcript level of Vvgtpch I gene decreased considerably. The amount of transcript of Vvgtpch I gene gradually increased to 100 mM NaHCO₃ and then highly decreased upon very severe stress conditions (100 mM NaHCO₃) (Figure 2a). Similar to the alkali stress, the transcript level of Vvgtpch I gene decreased under cold stress. The amount of transcript of Vvgtpch I gene gradually increased to 18 h and then there was a highly dramatic decrease to 72 h of cold treatment. When treated plants were incubated for one week at 21 °C, the transcript level of Vvgtpch I gene increased to the control level (Figure 2b). We have already shown that the transcript level of Vvgtpch I gene decreased significantly under abiotic stress conditions such as drought, salt, and heat (Eslami-Bojnourdi *et al.* 2017). In addition, similar to our results, a down-regulation of *gtpch* I gene has also been found in the mature pollen of

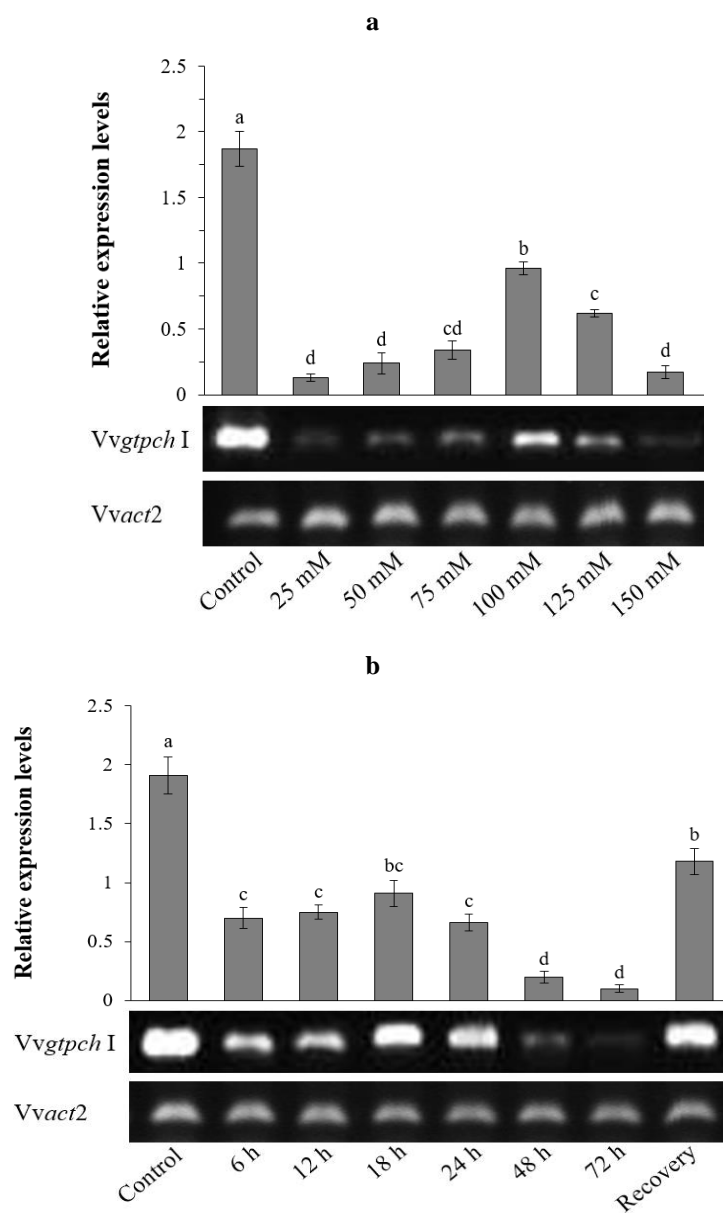


Figure 2. Expression of the *Vvgtpch I* gene under abiotic stresses by the semi-quantitative RT-PCR. (a) Expression of *Vvgtpch I* gene under alkali stress. The alkali stress was applied by exposing the cuttings to different concentrations NaHCO_3 , including 25, 50, 75, 100, 125, and 150 mM. (b) Expression analysis of *Vvgtpch I* gene upon low temperature and after recovery. The cold stress was applied by exposing the cuttings to a temperature of 4 °C for 6, 12, 18, 24, 48, and 72 h in a chamber growth. The experiments were carried out in three independent replicates. Bars with the same lowercase letter are significantly different ($p \leq 0.05$). Each histogram represents the mean \pm SD obtained from three independent PCR reactions.

Arabidopsis under cold stress (Changsong and Diqiu 2010) and *Arabidopsis* seeds under drought and heat stresses (Navarrete *et al.* 2012).

Expression of Vvgtpch I gene under oxidative stresses

The *Vvgtpch I* showed a strong increase and a moderate increase in the transcript amount

with Cu^{2+} and H_2O_2 , respectively. Whereas, its transcript level was relatively down-regulated by heavy metals and hormonal treatments, and almost disappeared by diamide (Figure 3). In contrast, by the semi-quantitative RT-PCR method, we already revealed that the transcripts amount of *Vvgtpch I* gene raised under H_2O_2 , CuSO_4 , CdCl_3 , and CoCl_2 treatments, while its expression decreased under AlCl_3 , ABA, and SA (Eslami-Bojnourdi *et al.* 2017). The H_2O_2 regulates *gtpch I* activity in a concentration-dependent manner. Up to 300 mmol L^{-1} activates *gtpch I* function,

but higher concentrations inhibit the enzyme activity (Chavan *et al.* 2009). This observation was similar to many other enzymes, where the low H_2O_2 concentrations are beneficial while higher concentrations are inhibitory (Schallreuter and Elwary 2007). Chavan *et al.* (2009) showed that 100 mM L^{-1} H_2O_2 increased the enzyme activity, whereas concentrations $>300 \text{ mM L}^{-1}$ decreased it. They also reported that *gtpch I* activity levels remained high even after the reduction of H_2O_2 .

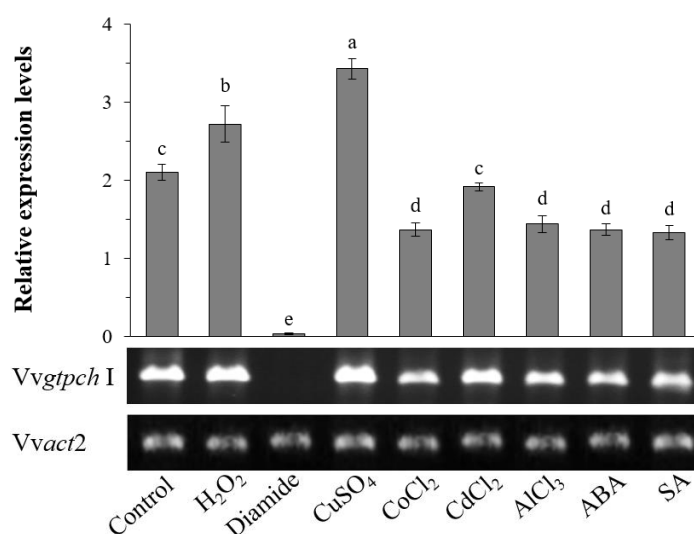


Figure 3. Expression of the *Vvgtpch I* gene under oxidative stressors by the semi-quantitative RT-PCR. Leaf slices were incubated in the presence of H_2O_2 (10 mM), diamide (1 mM), CuSO_4 (100 μM), CoCl_2 (100 μM), CdCl_2 (100 μM), AlCl_3 (100 μM), ABA (100 μM), and SA (100 μM) as mediators of the oxidative stress for 4 h before total RNA extraction. The experiments were carried out in three independent replicates. Bars with the same lowercase letter are significantly different ($p \leq 0.05$). Each histogram represents the mean \pm SD obtained from three independent PCR reactions.

Conclusion

The *Vvgtpch I* gene was found to be differentially excited under abiotic stress conditions probably due to the presence of diverse putative regulatory elements in its promoter region. The transcript level of

Vvgtpch I gene decreased under abiotic stresses including alkali and cold. Moreover, under oxidative stresses, the expression level of this gene enhanced under H_2O_2 and CuSO_4 , while it decreased under other heavy metals and plant growth regulators (ABA and SA).

The analysis of the promoter region of grape *gtpch* I demonstrated that there are several potential *cis*-acting elements responsive to environmental signals, including heat, heavy metals, light, and plant hormones (auxin and salicylic acid) (data not published). Collectively, due to the presence of different potential *cis*-acting elements in its promoter region, it was observed that the *Vvgtpch* I gene was down-regulated under abiotic stress

conditions but it was up-regulated in the presence of H₂O₂. Thus, it is concluded that the *Vvgtpch* I gene does not play a role in increasing the tolerance of plants to abiotic stress conditions and probably has a role in the regulation of cell redox.

Conflict of interest

There is no potential conflict of interest by the authors.

References

- Agyenim-Boateng KG, Zhang S, Shohag MJI, Shaibu AS, Li J, Li B, Sun J. 2023a. Folate biofortification in soybean: challenges and prospects. *Agronomy*. 13(1): 241.
- Agyenim-Boateng KG, Zhang S, Zhang S, Khattak AN, Shaibu AS, Abdelghany AM, Qi J, Azam M, Ma C, Feng Y, *et al.* 2023b. The nutritional composition of the vegetable soybean (Maodou) and its potential in combatting malnutrition. *Front Nutr*. 9: 1034115.
- Ashokkumar K, Govindaraj M, Karthikeyan A, Shobhana VG, Warkentin TD. 2020. Genomics-integrated breeding for carotenoids and folates in staple cereal grains to reduce malnutrition. *Front Genet*. 11: 414.
- Ashokkumar K, Sivakumar P, Saradhadevi M. 2018. Identification and determination of naturally occurring folates in grains of rice (*Oryza sativa* L.) by UPLC-MS/MS analysis. *Nat Prod Res*. 32(14): 1733–1737.
- Aune D, Deneo-Pellegrini H, Ronco AL, Boffetta P, Acosta G, Mendilaharsu M, De Stefani E. 2011. Dietary folate intake and the risk of 11 types of cancer: a case–control study in Uruguay. *Ann Oncol*. 22(2): 444–451.
- Baena-Gonzalez E, Rolland F, Thevelein JM, Sheen J. 2007. A central integrator of transcription networks in plant stress and energy signalling. *Nature*. 448(7156): 938–942.
- Basset G, Quinlivan EP, Ziemak MJ, Diaz De La Garza R, Fischer M, Schiffmann S, Bacher A, Gregory JF, Hanson AD. 2002. Folate synthesis in plants: the first step of the pterin branch is mediated by a unique bimodular GTP cyclohydrolase I. *Proc Natl Acad Sci USA*. 99(19): 12489–12494.
- Blancquaert D, De Steur H, Gellynck X, Van Der Straeten D. 2014. Present and future of folate biofortification of crop plants. *J Exp Bot*. 65(4): 895–906.
- Blau N, van Spronsen FJ. 2014. Disorders of phenylalanine and tetrahydrobiopterin metabolism. In: Blau N, Duran M, Gibson K, Dionisi Vici C (eds). *Physician's guide to the diagnosis, treatment, and follow-up of inherited metabolic diseases*. Springer: Berlin, pp. 3–21.
- Blokhina O, Virolainen E, Fagerstedt KV. 2003. Antioxidants, oxidative damage and oxygen deprivation stress. *Ann Bot*. 91(2): 179–194.
- Changsong Z, Diqiu YU. 2010. Analysis of the cold-responsive transcriptome in the mature pollen of *Arabidopsis*. *J Plant Biol*. 53: 400–416.

- Chavan B, Beazley W, Wood JM, Rokos H, Ichinose H, Schallreuter KU. 2009. H₂O₂ increases de novo synthesis of (6R)-L-erythro-5,6,7,8-tetrahydrobiopterin via GTP cyclohydrolase I and its feedback regulatory protein in vitiligo. *J Inher Metab Dis*. 32: 86–94.
- Chinnusamy V, Zhu J, Zhu JK. 2007. Cold stress regulation of gene expression in plants. *Trends Plant Sci*. 12(10): 444–451.
- De Lepeleire J, Strobbe S, Verstraete J, Blancquaert D, Ambach L, Visser RGF, Stove C, van Der Straeten D. 2018. Folate biofortification of potato by tuber-specific expression of four folate biosynthesis genes. *Mol Plant*. 11(1): 175–188.
- Diaz ML, Soresi DS, Basualdo J, Cuppari SJ, Carrera A. 2019. Transcriptomic response of durum wheat to cold stress at reproductive stage. *Mol Biol Rep*. 46: 2427–2445.
- Diaz De la Garza RI, Gregory III JF, Hanson AD. 2007. Folate biofortification of tomato fruit. *Proc Natl Acad Sci USA*. 104(10): 4218–4222.
- Eslami-Bojnourdi N, Haddad R, Garousi GA, Navabpour S, 2017. Expression analysis of gtp cyclohydrolase I gene under abiotic stresses in grape (*Vitis vinifera* L.). *Mod Genet J*. 11(4): 539–546 (In Persian with English abstract).
- Gong B, Wen D, VandenLangenberg K, Wei M, Yang F, Shi Q, Wang X. 2013. Comparative effects of NaCl and NaHCO₃ stress on photosynthetic parameters, nutrient metabolism, and the antioxidant system in tomato leaves. *Sci Hortic*. 157: 1–12.
- Gupta S, Singh Y, Kumar H, Raj U, Rao AR, Varadwaj PK. 2016. Identification of novel abiotic stress proteins in *Triticum aestivum* through functional annotation of hypothetical proteins. *Interdiscip Sci Comput Life Sci*. 10: 205–220.
- Heidari-Japelaghi R, Haddad R, Garoosi GA. 2011. Rapid and efficient isolation of high quality nucleic acids from plant tissues rich in polyphenols and polysaccharides. *Mol Biotechnol*. 49: 129–137.
- Imbard A, Benoist JF, Blom HJ. 2013. Neural tube defects, folic acid and methylation. *Int J Environ Res Public Health*. 10(9): 4352–4389.
- Kamal AHM, Kim KH, Shin KH, Choi JS, Baik BK, Tsujimoto H, Heo HY, Park C-S, Woo S-H. 2010. Abiotic stress responsive proteins of wheat grain determined using proteomics technique. *Aust J Crop Sci*. 4(3): 196–208.
- Kim DH, Kang WH, Yeom SI, Kim BD. 2019. Isolation of putative pepper defense-related genes against the pathogen *Phytophthora capsici* using suppression subtractive hybridization/microarray and RNA-sequencing analyses. *Hortic Environ Biotechnol*. 60: 685–699.
- Liang Q, Wang K, Shariful I, Ye X, Zhang C. 2020. Folate content and retention in wheat grains and wheat-based foods: effects of storage, processing, and cooking methods. *Food Chem*. 333: 127459.
- Mahajan S, Tuteja N. 2005. Cold, salinity and drought stresses: an overview. *Arch Biochem Biophys*. 444(2): 139–158.
- McIntosh SR, Brushett D, Henry RJ, 2008. GTP cyclohydrolase 1 expression and folate accumulation in the developing wheat seed. *J Cereal Sci*. 48(2): 503–512.
- Navarrete O, van Daele J, Stove C, Lambert W, van Der Straeten D, Storozhenko S. 2012. A folate independent role for cytosolic HPPK/DHPS upon stress in *Arabidopsis thaliana*. *Phytochem*. 73: 23–33.
- Nunes AC, Kalkmann DC, Aragao FJ. 2009. Folate biofortification of lettuce by expression of a codon optimized chicken GTP cyclohydrolase I gene. *Transgenic Res*. 18(5): 661–667.

- Perez-Duenas B, Ormazabal A, Toma C, Torrico B, Cormand B, Serrano M, Sierra C, De Grandis E, Marfa MP, García-Cazorla A, *et al.* 2011. Cerebral folate deficiency syndromes in childhood clinical, analytical, and etiologic aspects. *Arch Neurol.* 68(5): 615–621.
- Ramirez Rivera NG, Garcia-Salinas C, Aragao FJ, De la Garza RII. 2016. Metabolic engineering of folate and its precursors in Mexican common bean (*Phaseolus vulgaris* L.). *Plant Biotechnol J.* 14(10): 2021–2032.
- Schallreuter KU, Elwary S. 2007. Hydrogen peroxide regulates the cholinergic signal in a concentration dependent manner. *Life Sci.* 80(24-25): 2221–2226.
- Shahid M, Lian T, Wan X, Jiang L, Han L, Zhang C, Liang Q. 2020. Folate monoglutamate in cereal grains: evaluation of extraction techniques and determination by LC-MS/MS. *J Food Compos Anal.* 91: 103510.
- Shi H, Ishitani M, Kim C, Zhu JK. 2000. The *Arabidopsis thaliana* salt tolerance gene SOS1 encodes a putative Na⁺/H⁺ antiporter. *Proc Natl Acad Sci USA.* 97(12): 6896–6901.
- Storozhenko S, De Brouwer V, Volckaert M, Navarrete O, Blancquaert D, Zhang GF, Lambert W, van Der Straeten D. 2007. Folate fortification of rice by metabolic engineering. *Nat Biotechnol.* 25(11): 1277–1279.
- Strobbe S, van Der Straeten D. 2017. Folate biofortification in food crops. *Curr Opin Biotechnol.* 44: 202–211.
- Wakeel A, Arif S, Bashir MA, Ahmad Z, Rehman HU, Kiran A, Ibrahim S, Khan MR. 2018. Perspectives of folate biofortification of cereal grains. *J Plant Nutr.* 41(19): 2507-2524.
- Waller JC, Akhtar TA, Lara-Nunez A, Gregory JF, McQuinn RP, Giovannoni JJ, Hanson AD. 2010. Developmental and feed forward control of the expression of folate biosynthesis genes in tomato Fruit. *Mol Plant.* 3(1): 66–77.

پاسخ متفاوت ژن *GTP* سیکلوهایدرولاز I تحت تنش‌های غیرزنده در انگور

نادیا اسلامی بجنوردی، رحیم حداد*، قاسمعلی گروسی و رضا حیدری جابلقی

گروه مهندسی بیوتکنولوژی، دانشکده کشاورزی و منابع طبیعی، دانشگاه بین‌المللی امام خمینی (ره)، قزوین

*مسئول مکاتبه؛ Email: r.haddad@eng.ikiu.ac.ir

چکیده

فولات یک نیاز ضروری برای حفظ فعالیت طبیعی سلول به شمار می‌رود. برخلاف انسان، بسیاری از موجودات زنده مانند گیاهان توانایی ساخت این کوآنزیم را دارند. آنزیم *GTP* سیکلوهایدرولاز I (*gtpch I*, EC 3.5.4.16) اولین مرحله بیوسنتز تتراهیدروفولات (*FH4*) در گیاهان را از طریق تبدیل *GTP* به دی‌هیدرونئوپترین تری‌فسفات و فرمیک اسید کاتالیز می‌کند. در این پژوهش، الگوی بیان ژن *Vvgtpch I* در بافت‌های مختلف انگور با روش RT-PCR نیمه کمی مورد بررسی قرار گرفت. نتایج نشان داد که ژن *Vvgtpch I* در تمام بافت‌های مورد مطالعه بیان می‌شود. بالاترین سطح بیان در بافت‌های حبه و برگ مشاهده شد. در حالی که بافت خوشه کمترین میزان سطح رونوشت را نشان داد. پاسخ ژن *Vvgtpch I* به تنش‌های غیرزنده مانند تنش‌های قلیایی و سرما با استفاده از روش RT-PCR نیمه کمی نیز مورد بررسی قرار گرفت. تحت تنش قلیایی، سطح رونوشت ژن *Vvgtpch I* به طور چشمگیری کاهش یافت. مشابه با تنش قلیایی، میزان بیان ژن *Vvgtpch I* نیز تحت تنش سرما با کاهش مواجه شد. به منظور بررسی بیان ژن *Vvgtpch I* تحت تنش اکسیداتیو، تیمارهای مختلفی شامل القاء‌کننده‌های شیمیایی، فلزات سنگین و تنظیم‌کننده‌های رشد گیاهی جهت القاء تولید گونه‌های فعال اکسیژن مورد استفاده قرار گرفتند. سطح رونوشت ژن *Vvgtpch I* تحت تیمار با Cu^{2+} و H_2O_2 به ترتیب با افزایش شدید و نسبتاً شدید روبرو شد. با وجود این، سطح رونوشت ژن *Vvgtpch I* تحت تیمار با فلزات سنگین و هورمون‌های گیاهی نسبتاً کاهش یافت و تحت تیمار با Diamide با کاهش بسیار شدید مواجه شد.

واژه‌های کلیدی: بیان ژن، تنش اکسیداتیو، تنش غیرزنده، فولات، گونه‌های فعال اکسیژن