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A G6P1E ISOMERASE OF SUGAR METABOLISM IS INVOLVED IN THE FLOWER COLORS OF *Dianthus Chinensis*

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ABSTRACT

Dianthus chinensis L. is indigenous to northern China, Korea, Mongolia, Kazakhstan, and southeastern Russia. It is widely cultivated in urban landscapes. Its flower has a great variety of colors and color schemes. Sugars control and induce anthocyanin synthesis and accumulation in plants. In sugar metabolism, many enzymes are specific for their substrate's α or β anomer. Gaining and characterizing genes involved in sugar metabolism and flower color will be beneficial in clarifying the role of sugar in the flower colors of *D. chinensis*. Glucose-6-phosphate-1-epimerase (G6P1E, EC 5.1.3.15) catalyzes the α or β change of glucose-6-phosphate at the branch point of glucose metabolism. *DchG6P1E1* (MZ292712) was isolated in *D. chinensis* and characterized using the tobacco rattle virus (TRV)-based virus-induced gene silencing (VIGS) system. Its cDNA full length is 1401 bp, including an open reading frame of 918 bp. In the *DchG6P1E1*-silenced flowers, the reducing purple was observed, as well as the anthocyanin content, reducing sugar content, G6P1E activity, and *DchG6P1E1* expression were significantly decreased. During the development of floral buds and among the three flower colors, the anthocyanin content, reduced sugar content, G6P1E activity, and *DchG6P1E1* expression rose dramatically, with pigments increasing in the petals. Among the organs, the flowers had the highest anthocyanin contents and reducing sugar. The highest levels of G6P1E activity and *DchG6P1E1* expression were in the roots. The anthocyanin content was positively related to the reducing sugar content at 0.05 levels by correlation analysis. In conclusion, *DchG6P1E1* is a root-enriched gene associated with flower colors in *D. chinensis*.

Keywords: *Dianthus chinensis,* virus-induced gene silencing, G6P1E, anthocyanin content, reducing sugar content

INTRODUCTION

Anthocyanin is the largest class of flavonoids and is responsible for the colors of flowers and other tissues. In most plants, anthocyanin synthesis and accumulation are controlled and induced by sugars [Hu et al. 2016, Sun et al. 2017, Luo et al. 2019b]. Simultaneous increases of the anthocyanin content and sugar content are observed in some plant species [Hiratsuka et al. 2001, Zhou et al. 2020]. Many enzymes involved in sugar metabolism are specific for their substrate's α or β anomer [Sierkstra et al. 1993, Graille et al. 2006]. Glucose-6-phosphate-1-epimerase (G6P1E, EC 5.1.3.15) catalyzes the equilibrium of the anomeric forms of D-glucose-6-phosphate at the branch point of glucose metabolism [Sierkstra et al. 1993, Graille et al. 2006]. Faster NAD(P)H generation reflects a higher velocity constant for the interconversion from α-D-glucose-6-phosphate to β-D-glucose-6-phosphate [Graille et al. 2006]. So far, many *G6PlE* sequences in plant species have been submitted to NCBI [Shimizu et al. 2017, Baek et al. 2018, Luo et al. 2019b, Ou et al.

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2019, Zhang et al. 2019]. In *Zea mays*, *G6PlE* was found to mediate the yield and correlate the traits to phosphorus availability [Luo et al. 2019a].

Dianthus chinensis is indigenous to northern China, Korea, Mongolia, Kazakhstan, and southeastern Russia [Lim 2014] and is widely cultivated in urban landscapes [Kantia and Kothari 2002, Fu et al. 2008, Liu et al. 2021]. Its richness in flower colors and color schemes suggests that the formation mechanism of flower color is complicated. Gaining genes related to the flower color will be facilitated to illustrate the formation mechanism of flower color. Using the tobacco rattle virus (TRV)-based virus-induced gene silencing (VIGS) system, we characterized three *DchCHSs* involved in the anthocyanin synthesis and the flower color in *D. chinensis* [Liu et al. 2021]. In this paper, *DchG6P1E1* (MZ 292712) was obtained in *D. chinensis* and characterized by VIGS. The levels of anthocyanin content, reducing sugar content, G6P1E activity, and *DchG6P1E1* expression were studied during floral bud development among three flower colors and in organs. The correlation between the anthocyanin content, reducing sugar content, and G6P1E activity was analyzed.

MATERIAL AND METHODS

Plant materials and growth conditions

The seeds of *D. chinensis* cv. Pink were brought from www.ebay.co.uk. Seedlings were grown in growth chambers under 16 h light/8 h dark cycles with a day/night temperature regime of 22 °C/20 °C, respectively. The light intensity was 150 μmol m^{-2} s^{-1}. The seedlings with 4–6 leaves were transplanted in greenhouses at Inner Mongolia Agricultural University, Hohhot, China.

Flora buds at three stages (Fig. 1Aa) and four kinds of organs (Fig. 1Ca) were collected from purple flowering plants. Three kinds of flower colors were also collected (Fig. 1Ba). The collected plant materials were frozen in liquid nitrogen and stored at –80 °C.

DchG6P1E1 isolation, sequence analysis, and plasmid construction

Based on in-house RNA-seq data of *D. chinensis*, a unigene annotated as G6P1E, c26377_g1, was selected. The homologous sequence of *DchG6P1E1* was identified using the BLAST program on the website of www.ncbi.nlm.nih.gov. Amino acid sequence alignment was generated by DNAMAN 5.0. A phylogenetic tree was constructed via the neighbor-joining method in MEGA 11.

Total RNA was extracted with Trizol reagent (Invitrogen) from leaves and used to synthesize the first-strand cDNA. A 443 bp fragment of the *DchG6P1E1* gene was PCR-amplified from the cDNA using primers of *DchG6P1E1*-F1 / R1 (5'-AAGGCCAAGATGATTCCCGA-3' and 5'-TCCGACCAAGCCATATGTGA-3') designed by Primer 3. The amplified fragment was inserted into the pGEM-T Easy vector (Promega). After sequencing, the inserted fragment was excised from the plasmid by the *Eco*R I restriction enzyme and sub-cloned into the pTRV2 vector to generate pTRV2-*DchG6P1E1*. The resulting plasmid was sequenced to verify the correct insertion of the fragment.

Agrobacterium preparation and infection

Electrocompetent cells of *Agrobacterium tumefaciens* strain GV3101 were transformed with pTRV1, pTRV2, and pTRV2-*DchG6P1E1*, respectively. The transformed cells were selected on LB medium, including 50 mg L^{-1} kanamycin and 50 mg L^{-1} rifampicin. The positive colonies of pTRV1 and pTRV2 were verified by their primers [Liu et al. 2021], and the pTRV2-*DchG6P1E1* was confirmed with the primers of *DchG6P1E1*-F1 / R1. The positive colonies were cultured overnight at 28 °C in the LB medium containing appropriate antibiotics. After centrifugation, the *Agrobacterium* cells were incubated in the infiltration buffer to a final OD_{600} of 1.8 at 28 °C. The infiltration buffer included 10 mM MES, 150 μM acetosyringone, and 10 mM $MgCl_2$.

The bacteria containing pTRV1 were mixed with those containing pTRV2 (control) or pTRV2-*DchG6P1E1* in a 1:1 ratio. The mixed bacterial cultures were used to infect flora buds at stage 2 under a vacuum at –100 kPa for 20 min [Liu et al., 2021]. Then, the infected flora buds were inserted into plastic tubes with the nutrition buffer [Shang et al. 2007] under the same growth condition mentioned above. The control and the flowers with silencing phenotypes were collected, frozen in liquid nitrogen, and stored at –80 °C.

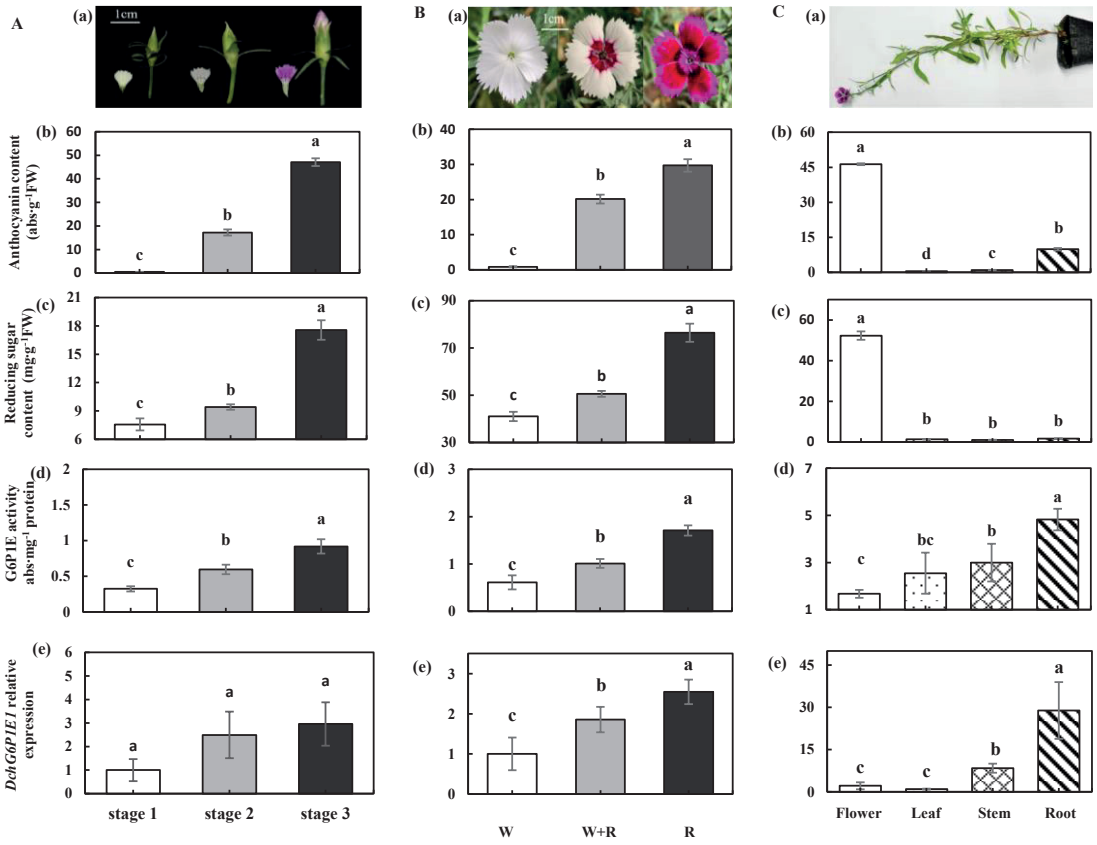

Fig. 1. The contents of anthocyanin and reducing sugar, G6P1E activity, and *DchG6P1E1* expression at three stages of flower buds, in different flower colors and different organs in *D. chinensis*. A. Flower buds; B. Flower colors, white (W), white with red (W + R) and red (R); C. Different organs. The different lowercase letter represents significant difference ($P < 0.05$) according to Duncan's multiple range tests

Measurements of anthocyanin content and reducing sugar content

The anthocyanin content was measured based on Rabino and Mancinelli [1986]. The collected plant materials were ground and extracted by 1% HCl in methanol (v/v) with gentle shaking at 4 °C for 24 h. Four hundred milliliters of distilled water and four hundred milliliters of chloroform were added to the extraction. After centrifugation, the absorbance of the supernatant was measured at 530 and 657 nm with a Spectrophotometer UV-1800 (SHIMADZU, Japan). The sample's anthocyanin content per 1 g fresh weight was determined by $A_{530} - 0.25\ A_{657}$.

The reduced sugar content was measured based on Wood and Bhat [1988]. The collected plant materials were ground and extracted by distilled water. The extraction was mixed with 2 mL DNS reagent (Beijing Solarbio Science & Technology Co., Ltd, China) and placed in a boiling water bath for 5 min. After being cooled at room temperature, the mixture was assayed at 540 nm. The absorbance values were translated into glucose equivalent using a standard graph obtained by plotting glucose (0.1–3.0 mg) against absorbance.

G6P1E activity assay

Based on the methods [Graille et al. 2006], the G6P1E activity was assayed with minor modifications. The collected plant materials were ground and extracted in the buffer (50 mM imidazole/hydrochloric acid, 50 mM KCl, 8 mM $MgSO_4$, pH 7.6) at 25 °C. After centrifugation, the supernatant was mixed with 1.5 mM α-D-glucose (Sigma-Aldrich, Co.), 2 mM

Fig. 2. Alignment of the putative protein sequence of *DchG6P1E1* with its homologs from other plant species. The accession numbers of the amino acid sequences are as follows: *Ipomoea nil (Ini)* XP019161630.1; *Spinacia oleracea (Sol)*, XP021866936.1; *Citrus clementine (Ccl)*, XP006446609.1; *Chenopodium quinoa (Cqu)*, XP021728522.1; *Pyrus ussuriensis* × *Pyrus communis* (*Pus* × *Pco*), KAB2610169.1; *Malus domestica (Mdo)*, XP028953379.1; *Dianthus chinensis (Dch)*, MZ292712; *Beta vulgaris subsp. vulgaris (Bvu)*, XP010681853.1

$NADP^+$ (Coolaber Science & Technology, China), 2 mM ATP, 0.1 U mL^{-1} hexokinase and 30 U mL^{-1} 6-phosphoglucose dehydrogenase. The last three reagents were bought from Baiji (Hubei) Biotechnology Co. Ltd, China. The reaction system was incubated at 25 °C for 1 min, and 200 µL of 4 mol L^{-1} $HClO_4$

was added to terminate. And then, 200 μL of 4 mol L^{-1} KOH was added to neutralize. The G6P1E activity was determined by measuring the increase in absorbance at 340 nm caused by the generation of NADPH.

QRT-PCR analysis

Total RNA was prepared from the collected plant materials and was used to synthesize the first-strand cDNA. To avoid amplification of RNA from the silencing vectors, *DchG6P1E1*-specific primers (F2: 5'-TGTCTGGAAACCACCAAAAGC-3' and R2: 5'-AGTCATCCAACGACCACATCC-3') were designed outside the region used for VIGS. The size of the qRT-PCR product was 122 bp. *DchACTIN2* (MZ292711) was used as an internal reference gene. A fragment of 157 bp was amplified by *DchACTIN2*-specific primers (F: 5'-GGTTACGCCCTACCCCACG-3' and R: 5'-CGACATAAGCCAGCTTCTCCTT-3'). QRT-PCR was performed using Roche LightCycler 480 II (Switzerland). Twenty milliliters of the reaction system contained 10 μL cDNA, 5 μL 2 × SYBR Green qPCR Master Mix (Jiangsu Pubo Biotechnology Co. Ltd, China), and 0.1 μL of each forward and reverse primer. The thermal condition was conducted as follows: 95 ℃ for 10 min, 40 cycles of 95 ℃ for 15 s, and 60 ℃ for 30 s. The relative expression level of the gene was presented by $2^{-\Delta\Delta CT}$ [Livak and Schmittgen 2001].

Statistical analysis

All data were means of three replicates with standard deviations. Student's *t*-test ($P < 0.05$) and Duncan's multiple range test ($P < 0.05$) were used to identify the significant difference. The relations between the anthocyanin content, reducing sugar content, and G6P1E activity were assessed using Pearson's correlation. All statistical analyses were conducted in SPSS 24.0 (IBM, USA).

RESULTS

Sequence and homology comparison of *DchG6P1E1*

G6P1E catalyzes interconversion between *α*-D-glucose-6-phosphate and *β*-D-glucose-6-phosphate at the branch point of glucose metabolism. In order to clarify the role of sugar on flower colors, a unigene encoding putative G6P1E was identified in an in-house *D. chinensis* transcriptome database and re-named as *DchG6P1E1* (MZ292712). Its cDNA full length is 1401 bp with an open reading frame of 918 bp (from 196 bp to 1113 bp). The deduced amino acid sequence shared identities of 74.92%, 76.80%, 77.12%, 78.43%, 83.99%, 84.59% and 86.89% with the G6P1Es from *Ipomoea nil*, *Pyrus ussuriensis* × *Pyrus communis*, *Malus domestica*, *Citrus clementina*, *Spinacia oleracea*, *Chenopodium quinoa* and *Beta vulgaris* subsp. *Vulgaris*, respectively (Fig. 2).

The phylogenetic tree of the G6P1Es was constructed using the neighbor-joining method. The tree was divided into four subgroups, and the G6P1Es in the same subgroup were from the same family (Fig. 3). G6P1Es of *Malus domestica* (RXH69530.1), *Malus domestica* (XP028953379.1), *Pyrus ussuriensis* × *Pyrus communis* (KAB2610169.1), *Pyrus* × *bretschneideri* (XP009371762.1) and *Pyrus* × *bretschneideri* (XP009370357.1) from the *Rosaceae* family, were gathered in one group. The second group included G6P1Es from *Citrus unshiu* (GAY61338.1), *Citrus clementina* (XP006446609.1), and *Citrus sinensis* (XP006470230.1), which belongs to the *Rutaceae* family. *Beta vulgaris* subsp. *vulgaris* (XP010681853.1), *Chenopodium quinoa* (XP021728522.1), *Spinacia oleracea* (XP021866936.1), *and Spinacia oleracea* (KNA06251.1) were from the *Chenopodiaceae* family and their G6P1Es were clustered together. *DchG6P1E1* was selected in *D. chinensis* of the *Caryophyllaceae* family, and its G6P1E was in one group. It suggested that the G6P1Es might be family-specific.

Silencing expression of *DchG6P1E1* in the flower by VIGS

The floral buds of *D. chinensis* with purple flowers at stage 2 were infected with a mixture of *Agrobacterium* transformed with pTRV2 (control) and with a mixture of *Agrobacterium* transformed with pTRV2-*DchG6P1E1*, respectively. Four days after infection, the phenotype of reducing purple was observed in the petals of pTRV2-*DchG6P1E1*-infected floral buds (Fig. 4A). Among the flowers infected with pTRV2-*DchG6P1E1*, 6.44% appeared the phenotype of reducing purple.

We collected the flowers of the control and the flowers with silencing phenotypes, respectively. In 1 g of the fresh petals, the anthocyanin content in the

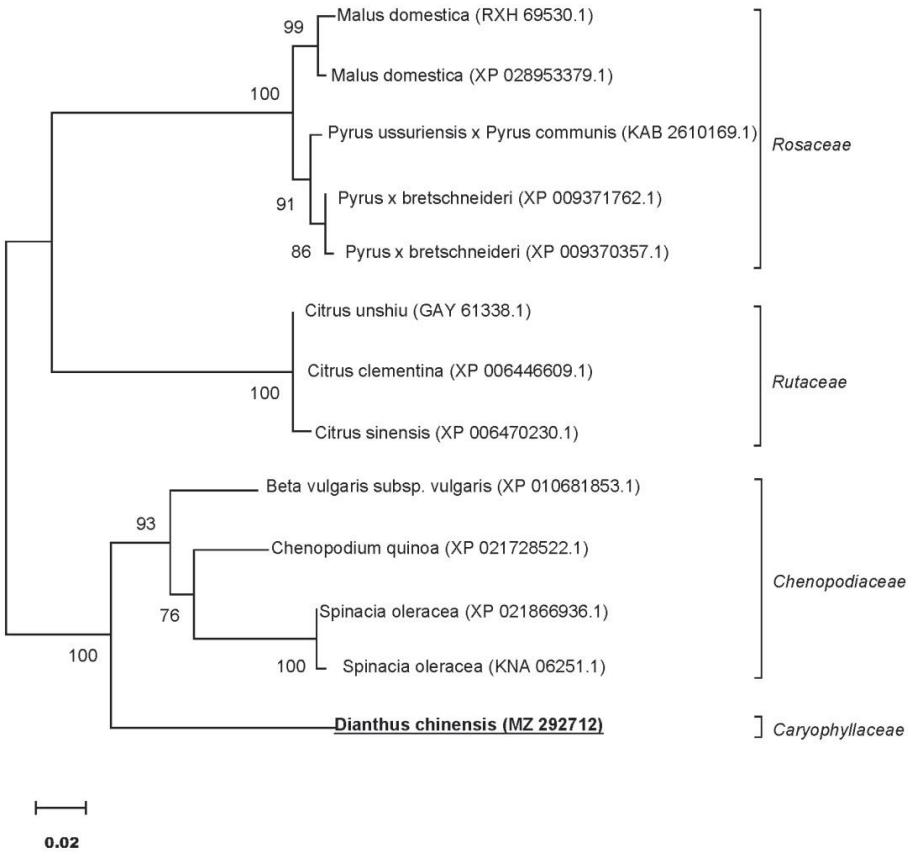

Fig. 3. Phylogenetic tree based on the amino acid sequences of G6P1Es from 13 plant species. The tree was constructed using MEGA 11 and a neighbor-joining method. The percentage of replicate trees where the associated taxa clustered together with 500 bootstrap replications is shown next to the branches

DchG6P1E1-silenced was 18.44 ±3.45 abs, which was dramatically lower than that in control (24.58 ±3.94 abs) (Fig. 4B). The reducing sugar content markedly dropped from 49.90 ±27.04 mg·g^{-1} in control to 35.39 ± 28.29 mg·g^{-1} in the *DchG6P1E1*-silenced (Fig. 4C). The G6P1E activity and the *DchG6P1E1* expression level were both significantly reduced (to 87.96% and 19.00%, respectively) in pale purple flowers of floral buds infected with pTRV2-*DchG6P1E1* (Fig. 4D-E).

Contents of anthocyanin and reducing sugar, G6P1E activity, and *DchG6P1E1* expression in floral buds

Floral buds at three stages were collected from purple flowering plants. The petals of buds at stage 1 and stage 2 had no pigment, and those at stage 3 had pigment (Fig. 1Aa). During the floral bud development, the anthocyanin content was significantly increased (Fig. 1Ab). The reducing sugar content showed the same pattern as the anthocyanin content (Fig. 1Ac). The G6P1E activity significantly rose from stage 1 to stage 3 (Fig. 1Ad). With the flower bud growth and the pigment accumulation in petals, the relative expression level of *DchG6P1E1* gradually increased (Fig. 1Ae). Compared with the *DchG6P1E1* expression at stage 1, expression levels of the gene were increased more than two times at stage 2 and stage 3.

Contents of anthocyanin and reducing sugar, G6P1E activity, and *DchG6P1E1* expression in flowers

We selected the flowers with apparent differences in pigments. The colors were white (W), white with a red center (W+R), and red (R) (Fig. 1Ba). The anthocyanin content in the red was 2.74 times as much as that in the W+R and was 96.08 times as much as

that in the white (Fig. 1Bb). In 1 g of fresh petals, the reducing sugar content was 41.04 mg in the white, 50.60 mg in the W+R, and 76.45 mg in the red (Fig. 1Bc). The anthocyanin content and reducing sugar content differed significantly between any two flower colors. We also investigated the G6P1E activity and its gene expression level (Fig. 1Bd–e). The enzyme activity's change pattern was consistent with its gene expression. With pigments increase in petals, the G6P1E activities and *DchG6P1E1* expressions were significantly increased.

Contents of anthocyanin and reducing sugar, G6P1E activity, and *DchG6P1E1* expression in organs

Flowers, leaves, stems, and roots of purple flowering plants were collected when flowers opened on the first day (Fig. 1Ca). The highest anthocyanin content was in the flowers, and the lowest was in the leaves (Fig. 1Cb). The anthocyanin content in the roots was significantly lower than that in the flowers and significantly higher than in the stems. The difference in anthocyanin content was noticeable between the two organs. The reducing sugar content in the flowers was the highest and was remarkably higher than those in the other three organs (Fig. 1Cc). No difference was made among the other organs. The enzyme activity and *DchG6P1E1* expression in the roots were the highest, far higher than those in the other organs (Fig. 1Cd–e). The enzyme activity in the flowers was the lowest. It was close to that in the leaves but significantly lower than in the stems (Fig. 1Cd). The lowest level of *DchG6P1E1* expression was in the leaves. It was close to that in the flowers but significantly lower than in the stems (Fig. 1Ce).

The correlation among the anthocyanin content, reducing sugar content, and G6P1E activity

The anthocyanin content, reducing sugar content, and G6P1E activity at three stages of flower buds in three kinds of flower colors and four organs were used to analyze the correlation (Fig. 5). The anthocyanin content was markedly correlated to the reducing sugar content ($P < 0.05$) with the correlation coefficient of 0.706 (Fig. 5A). The G6P1E activity was negatively related to the anthocyanin content (–0.127) and to the reducing sugar content (–0.321) (Fig. 5B–C). Both relationships were statistically insignificant.

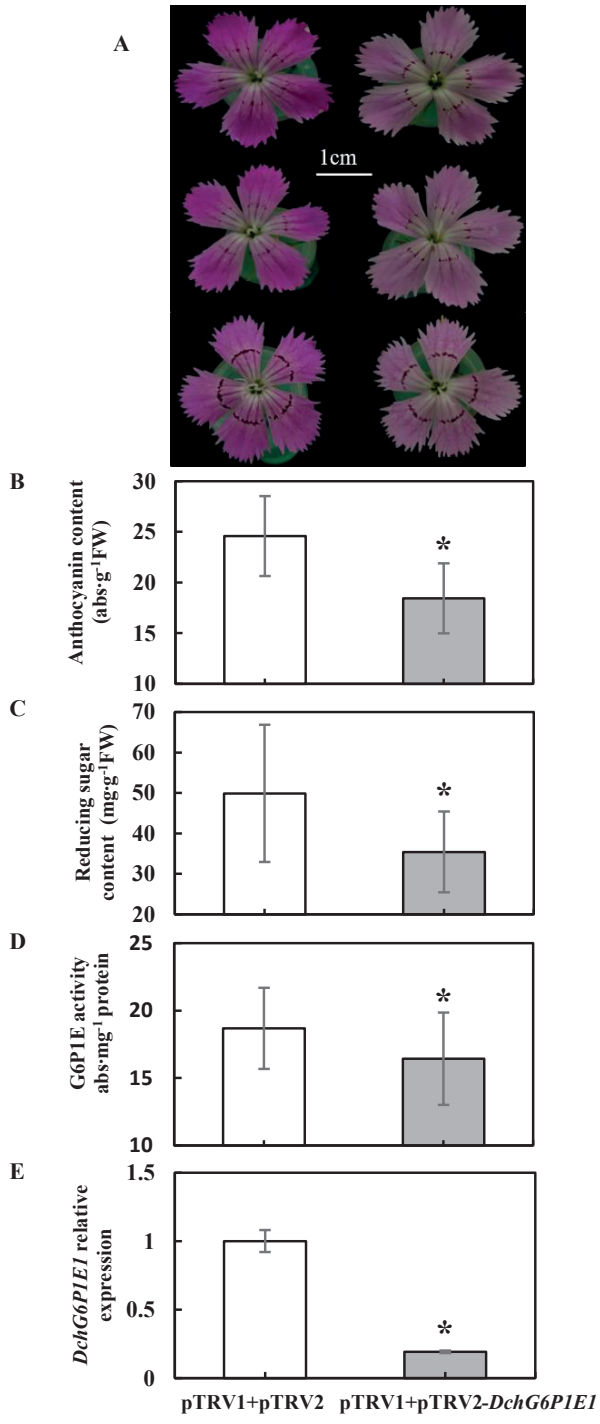

Fig. 4. The contents of anthocyanin and reducing sugar, G6P1E activity, and *DchG6P1E1* expression in the *DchG6P1E1*-silenced flowers of D. chinensis. Left: pTRV1 + pTRV2; Right: pTRV1 + pTRV2-DchG6P1E1. The * symbol represents a significant difference ($P < 0.05$) according to Student's *t*-test

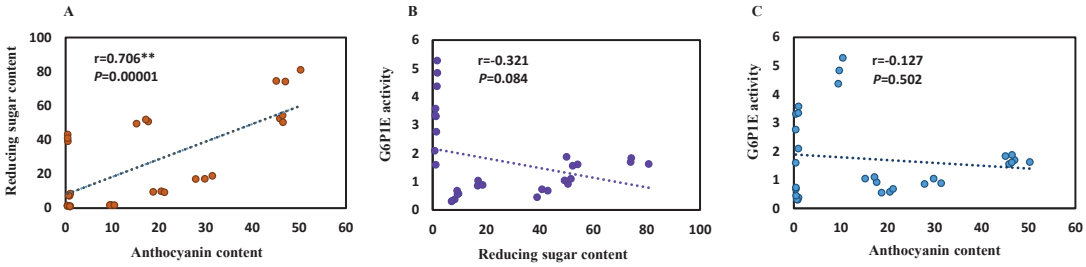

Fig. 5. Pearson's correlation analysis among the anthocyanin content, reducing sugar content, and G6P1E activity. The ** symbol represents a significant difference ($P < 0.01$) according to Student's *t*-test

DISCUSSION

Dianthus chinensis is a good ornamental plant in gardening [Kantia and Kothari 2002, Fu et al. 2008, Liu et al. 2021]. It is also a good plant material for studying anthocyanin synthesis and accumulation due to its rich flower colors and color schemes. At least three *DchCHSs* are related to flower colors in *D. chinensis* [Liu et al. 2021]. Compared with the number of *CHS*s related to flower colors in other plant species [Nakatsuka et al. 2003, Ohno et al. 2011, Suzuki et al. 2016], the number of *CHSs* in *D. chinensis* means that its anthocyanin synthesis and accumulation are much more complicated.

Sugar is essential in anthocyanin synthesis and accumulation [Hu et al. 2016, Sun et al. 2017, Luo et al. 2019b]. G6P1E belongs to the aldose-1-epimerase family and catalyzes the interconversion of α or β anomer of D-glucose-6-phosphate at the branch point of glucose metabolism [Graille et al. 2006]. So far, *G6PlEs* have been sequenced in many plants [Shimizu et al. 2017, Baek et al. 2018, Luo et al. 2019b, Ou et al. 2019, Zhang et al. 2019]. *DchG6P1E1* (MZ292712) was isolated from the in-house RNA-seq data of *D. chinensis,* and its cDNA full length is 1401 bp. The *DchG6P1E1* protein shared high identities with the G6P1Es of 7 plant species (Fig. 2). The G6P1Es of 13 plant species were used to build a phylogenetic tree (Fig. 3). These 13 plant species belong to 4 families, *Rosaceae*, *Rutaceae*, *Chenopodiaceae* and *Caryophyllaceae*, and their G6P1Es were divided into four subgroups. *D. chinensis* belongs to the *Caryophyllaceae* family, and its DchG6P1E1 belongs to one group. The result suggested that *DchG6P1E1* might be a family-specific gene.

VIGS is an efficient method to identify genes related to flower colors [Deng et al. 2014, Sui et al. 2018]. Using the established VIGS system [Liu et al. 2021], we studied the function of *DchG6P1E1* in *D. chinensis*. In the *DchG6P1E1*-silenced flowers, purples were reduced (Fig. 4Aa). The phenotypes were the same as those of silencing structural genes in anthocyanin synthesis [Sui et al. 2018, Donoso et al. 2021]. The silencing efficiency of *DchG6P1E1* was about 6.44%, which was lower than those of the *DchCHSs* [Liu et al. 2021]. At the same time, the anthocyanin content, reducing sugar content, G6P1E activity, and *DchG6P1E1* expression were significantly decreased in the *DchG6P1E1*-silenced flowers (Fig. 4B–E). With the floral bud development, the pigments in the petals were gradually accumulated, the G6P1E activity was significantly enhanced, and *DchG6P1E1* expression was up-regulated (Figs. 1Aa, d–e). Compared with *DchG6P1E1* expression at stage 1, the expression levels of the gene at stage 2 and stage 3 were increased more than two times (Fig. 1Ae). Among the flowers with apparent differences in pigments (Fig. 1Ba), the G6P1E activity and *DchG6P1E1* expression were significantly increased with pigments increase in petals (Fig. 1Bd–e). In purple flowering plants (Fig. 1Ca), the G6P1E activity and *DchG6P1E1* expression in the roots were far higher than in the other organs (Fig. 1Cd–e). In *Zea mays*, G6P1E mediates yield and correlates traits to phosphorus availability [Luo et al. 2019a]. In this paper, another function of *DchG6P1E1* was related to flower colors, and its expression is enriched in the roots of *D. chinensis.*

Sugar increases the anthocyanin content in plant species [Hu et al. 2016, Sun et al. 2017, Luo et al.

2019b]. During the floral bud development, the pigment in petals accumulated (Fig. 1Aa), and the anthocyanin and reducing sugar content significantly increased (Fig. 1Ab–c). Among the flowers with pronounced distinct colors (Fig. 1Ba), the contents of anthocyanin and reducing sugar in the red were significantly higher than those in the W+R, and the contents of anthocyanin and reducing sugar in the W+R were dramatically higher than those in the white (Fig. 1Bb–c). In purple flowering plants, the highest contents of anthocyanin and reducing sugar were in the flowers, followed by those in the roots (Fig. 1Cb–c). In our study, the anthocyanin content strongly correlated with the reducing sugar content by correlation analysis (Fig. 5A).

We were following enzyme entries of class 5.1.3. – at least ten of 44 enzymes acting on carbohydrates and derivatives catalyze the interconversion of α and β anomers of certain sugars [Bridge and Axelsen 2024]. Such enzymes are necessary in sugar metabolism [Graille et al. 2006]. The G6P1E enzyme mainly catalyzes α-D-glucose-6-phosphate to produce β-D-glucose-6-phosphate [Sierkstra et al. 1993], which is the substrate of the glucose-6-phosphate dehydrogenase (G6PD) in the pentose phosphate pathway (PPP). The catalyzation process of G6PD is the first step of the PPP (OPPP) oxidative phase. The OPPP is vital for the sugar regulation of nitrate transporter genes, which govern the availability of NO_3^- and NH_4^+ in the root [Lejay et al. 2008]. NO_3^- and NH_4^+ are transported from the root to the shoot, significantly influencing leaves' photosynthetic CO_2 assimilation capacity [Rascher et al., 2000; Jin et al., 2015; Feng et al., 2020; Raven, 2022]. In our study, the G6P1E activity was not related to the reducing sugar content by correlation analysis (Fig. 5B), but *DchG6P1E1* is involved in flower colors in *D. chinensis* (Fig. 4). Consequently, we deduce that the G6P1E in roots might indirectly influence the photosynthetic CO_2 assimilation capacity in leaves of *D. chinensis*, which results in the changes of sugar content and anthocyanin content. *G6PlE* is more sensitive to low-Pi treatment in roots than in leaves and is related to the yield in *Zea mays* [Luo et al. 2019a]. The detailed pathway of how *DchG6P1E1* influences anthocyanin synthesis and accumulation should be further studied.

CONCLUSIONS

Based on the TRV-based VIGS system and expression levels during floral buds development and among three flower colors, *DchG6P1E1* is related to flower colors in *D. chinensis*. Among the organs of *D. chinensis*, *DchG6P1E1* is highly expressed in the root.

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