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# Elucidation of Low-temperature Regulated Flavone Synthesis in *Dahlia Variabilis* and its Effects on Flower Color

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**Dahlia (*Dahlia variabilis*) flower colors are diverse and are determined by the accumulation of flavonoids. Cultivars with dark red flowers accumulate more anthocyanins in their petals. Flower color changes such as color fading often occur in some cultivars. In this study, low minimum temperature regulated flower color fading and flavonoid synthesis in dahlia 'Nessho' were investigated. The pigment contents and expression levels of flavonoid biosynthesis genes were investigated in detail under several growing environments in which color fading occurs. Flavones accumulate more in color-faded orange flowers than in dark red ray florets. The expression analysis of the anthocyanin synthesis pathway genes indicated that the upregulation of flavone synthase (*DvFNS*) gene expression correlated with the high accumulation of flavones in color-faded petals. *DvFNS* expression was also detected in young leaves, and the expression level was higher in winter than in summer. Seasonal changes in *DvFNS* expression in young leaves significantly correlated with color fading in petals. The change in *DvFNS* expression in young unexpanded leaves of relatively high-sensitive plants was significantly higher than that of low-sensitive plants before and after treatment under inductive conditions. In conclusion, low-temperature-inducible changes in the flavonoid accumulation in petals was suggested to reflect a change in *DvFNS* expression occurring in the meristem prior to flower bud formation. This temporal *DvFNS* expression in young unexpanded leaves of 'Nessho' dahlia could be an insight for the selection and breeding of non-color fading plants.**

**Key Words:** anthocyanin, dahlia, flavone synthase, seasonal color fading, young unexpanded leaves.

## Introduction

Dahlias (*Dahlia variabilis*) are popular floricultural crops that are used as cut flowers or garden plants. Various cultivars with widely diverse flower colors have been bred, i.e., black, purple, orange, pink, red, white, and yellow, while other cultivars exhibit variegated or bicolor phenotypes. The flower colors result from flavonoid accumulation, mainly consisting of anthocyanins, butein, and flavone glycosides and their derivatives (Halbwirth et al., 2008; Harborne et al., 1990; Nordström and Swain, 1953; Ohno et al., 2011a). The flavonoid biosynthetic genes in dahlia have been largely elucidated (Deguchi et al., 2013; Ohno et al., 2011a, b, 2013, 2022).

Seasonal change in flower color has been reported in several plant species, including dahlia. Environmental factors such as temperature play key roles in the regulation of anthocyanin biosynthesis and pigment accumulation in plants (Carbone et al., 2009; Jaakola, 2013). Fukuta and Nakayama (2008) reported that the colored area on the petals of the eustoma (*Eustoma grandiflorum*) bicolor cultivar remained for longer in the winter while the white areas were greatly reduced. Suzuki et al. (2010) reported that color fading occurred in dahlia when the minimum temperature was 10°C. The instability of dahlia flower colors has been the focus of several studies (Deguchi et al., 2013, 2015; Ohno et al., 2011a, b, 2016, 2022). In these studies, post-transcriptional gene silencing (PTGS) of chalcone synthase (*DvCHS*) or type II (cytochrome P450)

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flavone synthase (*DvFNS*) genes were shown to be involved in the instability of the flower color in dahlia. The latter was induced by tobacco streak virus ($TSV_{dahlia}$) infection and resulted in color-changing occurrence in dark red colored dahlia cultivars. Further, Ohno et al. (2018) reported that the instability of the flower color in the dahlia bicolor cultivar 'Yuino' reflected the PTGS induction for *DvCHS* at the whole plant level.

'Nessho' is a dahlia cultivar with dark red flowers that exhibits color fading from autumn through spring in response to a minimum temperature under 10°C (Fig. 1), at which time the ray florets turn orange (Okada et al., 2020; Suzuki et al., 2010). Color fading progresses quantitatively along with decrease in minimum temperature. The incipient stage of color-fading occurrence is slight discoloration on the whole ray floret on a capitulum in mid-autumn (Fig. 1b). After that, partial color-fading in ray florets occurs until early winter (Fig. 1c, d), and finally, all ray florets on a capitulum are an orange color from mid-winter (Fig. 1e). The L*a*b* chromaticity values range from L*28.1 ± 0.4, a*41.2 ± 0.3, b* 40.8 ± 0.4 for dark red flowers to L* 42.4 ± 0.7, a* 40.0 ± 0.9, b* 46.4 ± 0.5 for completely color-faded orange flowers (Okada et al., 2020). Deguchi et al. (2013) suggested that the color changing occurrence of dark red dahlia is the result of a change in flavonoid composition, particularly an increase in flavone contents and decrease in anthocyanin contents. If the color-fading phenomenon in 'Nessho' is caused by the same mechanism, we may be able to elucidate the mechanism of low-temperature regulated flavone synthesis. In chrysanthemums, prolonged high temperature inhibits flavone biosynthesis by directly regulating *CmFNS* and anthocyanin by suppressing the expression of *CmCHS*, *CmDFR*, *CmANS*, and *CmUFGT* (Zhou et al., 2021). However, to the best of our knowledge, no other study has reported on low-temperature regulated flavone synthesis.

Deguchi et al. (2015) reported that $TSV_{dahlia}$ infection caused color-changing occurrences in darker red dahlia cultivars; however, there is no report indicating any virus involvement in color-fading occurrences in 'Nessho' dahlia. Further, it is reported that the 'Nessho' cultivar consists of some plants with relatively high sensitivity to low-temperature conditions (RH-sensitive plants) (Okada et al., 2020). RH-sensitive plants quickly respond to changes in the minimum temperature under 10°C and exhibit color fading in their ray florets; thus, they are a good model for analyzing the regulation of the flavone synthesis pathway.

In the present study, we attempted to elucidate the regulation of the flavone synthesis pathway under low-temperature conditions that cause flower color fading in dahlia using vegetatively propagated RH-sensitive plants. We identified the possible flavonoid synthesis pathway gene for color-fading occurrence under low-temperature conditions. Further, we sought to identify whether or not the seasonal regulation in the flavonoid synthesis pathway spread over the whole plant. We examined this by analyzing the expression of the potential causal flavonoid synthesis pathway gene in leaves from summer through winter. Our results show a relationship between *DvFNS* gene expression in leaves, color-fading occurrence in ray florets, and the minimum temperature under experimental conditions.

## Materials and Methods

### *Plant materials*

A dahlia cultivar plant with relatively high sensitivity to low-temperature conditions (RH-sensitive plant) was vegetatively propagated, and the plantlets were main-


**Fig. 1.** Indexed classification of the degree of flower color fading in RH-sensitive plants under low minimum temperature conditions. (a) color fading index (CFI) 0: no color fading, (b) CFI 1: dark red ray florets and slight color fading on the disc florets, (c) CFI 2: orange ray florets occur, but with dominant red florets, (d) CFI 3: orange ray florets dominate the dark red florets, (e) CFI 4: all orange ray florets (Bar = 1 cm).

tained and used for the subsequent experiments according to our previous report (Okada et al., 2018). Petals and leaves were collected from greenhouse-grown plants in the experimental field of Shinshu University (Minamiminowa, Japan) and used in the subsequent analyses. Additionally, relatively low sensitivity plants (RL-sensitive plants) and RH-sensitive plants were selected and grown in an Okayama University experimental field according to our previous study (Okada et al., 2020). Plants used for gene expression analysis were grown in an incubator under controlled conditions.

*Pigment analysis*

The extraction of flavonoids was done as previously described by Deguchi et al. (2013). The setup of the High-performance liquid chromatography (HPLC) system, pump, detector, column, gradient program, and solvents was previously described by Hamauzu et al. (2018). The detection wavelength was 350 nm for flavones and 530 nm for anthocyanins. To make a standard curve, 1 mg of known pelargonidin, cyanidin, apigenin, and luteolin were eluted in 600 μL buffer (10% hydrochloric acid in 50% methanol) and five serial dilutions were made. Twenty microliters of each dilution were injected into the HPLC and pigment amounts were calculated from peak areas. Amounts of pigments were quantified as peak area-equivalents from 100 mg ray floret fresh weight. Three to four dark red ray florets and color-faded orange ray florets were sampled from distinct capitula and analyzed as biological replicates. Comparison of flavonoid amounts-equivalents of each flavonoid from the samples was carried out by analysis of variance (ANOVA), with pairwise multiple comparisons for the Tukey-Kramer test.

*Expression analysis for flavonoid synthesis pathway genes*

Expression analysis of flavonoid synthesis pathway genes was conducted using real-time reverse transcription (RT)-PCR. Unexpanded ray florets were sampled from distinct capitula. Developmental stages of the ray florets were defined as follows: stage 1, 10 mm length ray floret; stage 2, 20 mm length ray floret; stage 3, 30 mm length ray floret. Three red ray florets and four color-faded orange ray florets were analyzed as biological replicates. Total RNA (> 200 nucleotides) was extracted using NucleoSpin miRNA (Takara Bio Inc., Shiga, Japan), and purified with a high-salt solution for precipitation (Takara Bio Inc.) two times. The extracted total RNA was reverse-transcribed with ReverTra Ace® qPCR RT Master Mix with gDNA Remover (TOYOBO, Osaka, Japan), and 1 μL of RT product was used as a template for real-time RT-PCR. Real-time RT-PCR was performed with THUNDERBIRD® SYBR qPCR Mix (TOYOBO) according to the manufacturer's instructions using a Dice® Real-Time System Thermal Cycler (Takara Bio Inc.). The primers used were as reported by Ohno et al. (2011b). The PCR program was set at 95°C for 1 min, followed by 40 cycles of 95°C for 15 s, 60°C for 1 min, and subsequent dissociation steps. Expression profiles of *D. variabilis Anthocyanin synthase*, *DvANS*, *Dihydroflavonol 4-reductase*, *DvDFR*, *Flavone synthase DvFNS*, *Flavanone 3-hydroxylase*, *DvF3H*, and *Flavonoid 3'-hydroxylase DvF3'H* were analyzed. *DvActin* was used as an internal standard. Additionally, *DvFNS* expression was analyzed using ray florets that exhibited variegated coloration. Since 'Nessho' capitula often produce red and orange variegated ray florets, each red and orange area was separated from four distinct ray florets. Total RNA was extracted as mentioned above and used for the *DvFNS* expression analysis using the same primer sets and real-time RT-PCR conditions.

*DvFNS expression in leaves and its relationship with color fading occurrence in ray florets*

To confirm whether the seasonal changes in *DvFNS* expression occur only in ray florets but not in leaves, we analyzed the *DvFNS* expression in leaves at different maturation stages. The optimum leaf maturation stage for *DvFNS* expression analysis was decided using leaf blades sampled from RH-sensitive plants in February, when they produced completely color-faded orange ray florets. Unexpanded leaves and leaves on the 1st to 4th internodes were sampled from four distinct plants as biological replicates (Fig. 4a). Total RNA was extracted with a Get pure RNA Kit (Dojindo, Kumamoto, Japan). Extracted total RNA was reverse transcribed as described above, and 1 μL of RT product was used as a template for real-time RT-PCR. Real-time RT-PCR was performed with THUNDERBIRD® SYBR qPCR Mix (TOYOBO) according to the manufacturer's instructions using a StepOne™ Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). The primers used were as reported by Ohno et al. (2011b). The PCR conditions and internal standard were as previously described.

*DvFNS* expression in unexpanded leaves was analyzed from weeks 32 to 53 using 210 vegetatively propagated RH-sensitive plants. Total RNA extraction, reverse transcription, and real-time RT-PCR were conducted as previously described. Three to six unexpanded leaves sampled from distinct plants were analyzed as biological replicates. In conjunction with the *DvFNS* expression analysis, the color fading occurrence in ray florets was analyzed for the same plants. The extent of color fading on the nearly fully open terminal capitulum was evaluated according to the color fading index (CFI) (Fig. 1; Okada et al., 2020): CFI 0, color fading was not observed; CFI 1, slight color fading was observed, but the color change was not completely orange; CFI 2, color-faded ray florets exhibited a completely orange color, but red ray florets dominated the capitulum; CFI 3, color-faded ray florets exhibited orange color and orange ray florets dominated the capitulum; CFI 4, all

ray florets were completely orange. Pearson's correlation coefficients were analyzed for *DvFNS* expression level in leaves, CFI values and average weekly minimum temperature.

*Confirmation of the difference in DvFNS expression levels between RH-sensitive plants and RL-sensitive plants*

*DvFNS* expressions in young unexpanded leaves were compared between eight strains of RL-sensitive plants and three strains of RH-sensitive plants. Plants were approximately 20 cm in height with more than three internodes and were pinched on the second internode to allow new lateral shoot development. After pinching, the plants were transferred to an incubator set at acclimation conditions (25°C/20°C day and night temperature and 14 hours day length) to homogenize the *DvFNS* gene expression levels in all plants. Young unexpanded leaves were sampled 14 days after the onset of the acclimation condition. The conditions of the incubator were changed to inductive conditions of 20°C/9°C day and night temperatures and 10 hours day length, thus mimicking winter conditions to induce *DvFNS* gene expression. The second sampling was done 14 days after the onset of the inductive condition. The samples were used to obtain total RNAs. Total RNA extraction, reverse transcription, and real-time RT-PCR were conducted as described above.

## Results

*Pigment analysis of ray florets*

To clarify the difference in pigments between color-faded orange ray florets and original dark red ray florets, aglycones of anthocyanins and flavones were extracted from ray florets and analyzed by HPLC. The four analyzed aglycones (two anthocyanidins and two flavones) were detected differently in color-faded orange and dark red ray florets. The color-faded orange ray florets accumulated 7 to 10-fold higher amounts of flavones (apigenin and luteolin glycosides) than the dark red florets (Fig. 2). The dark red ray florets accumulated up to 9-fold higher amounts of anthocyanins (cyanidin and pelargonidin) contents compared to the color-faded orange ray florets. The accumulation of anthocyanidins in the ray florets was inversely proportional to that of the flavone glycosides.

*Quantitative real-time RT-PCR analysis of flavonoid biosynthetic pathway genes*

To determine the regulation of flavonoid biosynthesis genes in the 'Nessho' dahlia cultivar, the level of expression of major structural genes in the anthocyanin synthetic pathway was analyzed. The expression levels of anthocyanin genes *DvANS*, *DvDFR*, *DvF3H*, and *DvF3'H* did not differ between the color-faded orange ray florets and dark red ray florets. However, higher *DvFNS* expression was detected in color-faded orange

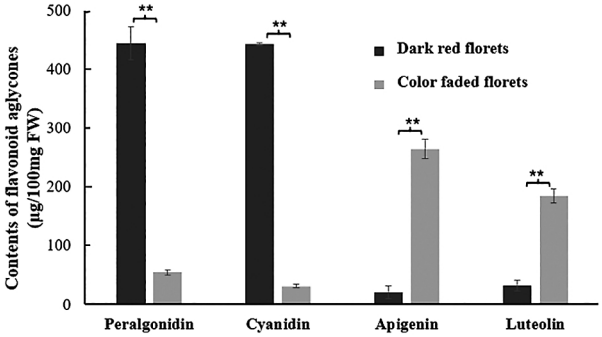


**Fig. 2.** Total content of flavonoid aglycones in the dark red and color-faded ray florets of RH-sensitive plants. The vertical bars represent the mean ± SE (n = 3). ** indicates significant differences for *t*-test $P < 0.01$.

ray florets at all analyzed developmental stages (Fig. 3a). There was a distinct difference in the level of *DvFNS* expression between the three developmental stages with the expression levels being greatest in stage 3 ray florets. *DvFLS* expression was undetectable in all three developmental stages in both the dark red and the orange-colored ray florets. The expression levels of *DvANS*, *DvDFR*, *DvF3H*, and *DvF3'H* were not different across the ray florets' developmental stages, although they were consistently higher in the 3rd stage.

Comparison of the gene expression profiles for red and orange variegated ray florets showed that the expression level of *DvFNS* was significantly different between the red and the orange regions ($P < 0.05$). There were also small, but significant, differences in the expression of *DvCHS1*, *DvCHS2*, and *DvCHI* (Fig. 3b).

*DvFNS expression in leaves and its relationship with color-fading occurrences in ray florets*

Leaves at five maturation stages; unexpanded leaves, leaves at the 1st, 2nd, 3rd, and 4th nodes of RH-sensitive plants were used to evaluate whether *DvFNS* expression change occurred in the flower stage and also in the leaves in response to minimum low-temperature. *DvFNS* expression levels were greatest in the young unexpanded leaves (Fig. 4a). The expression levels declined up to 5-fold and more in the leaves above the 1st, 2nd, 3rd, and 4th nodes. *DvFNS* expression levels in young unexpanded leaves of RH-sensitive plants were compared between the winter (February) and summer (June) seasons. There was higher *DvFNS* expression during winter compared to summer for the same plants as shown in Figure 4b.

Further, *DvFNS* expression in young unexpanded leaves was analyzed for 210 RH-sensitive plants and compared to the CFI of the capitulum of the same plants from August to December, 2015. During the months of August and September, *DvFNS* expression in young unexpanded leaves was low, as was the corresponding CFI (Fig. 5). From October through November, *DvFNS* expression levels gradually increased, as did the

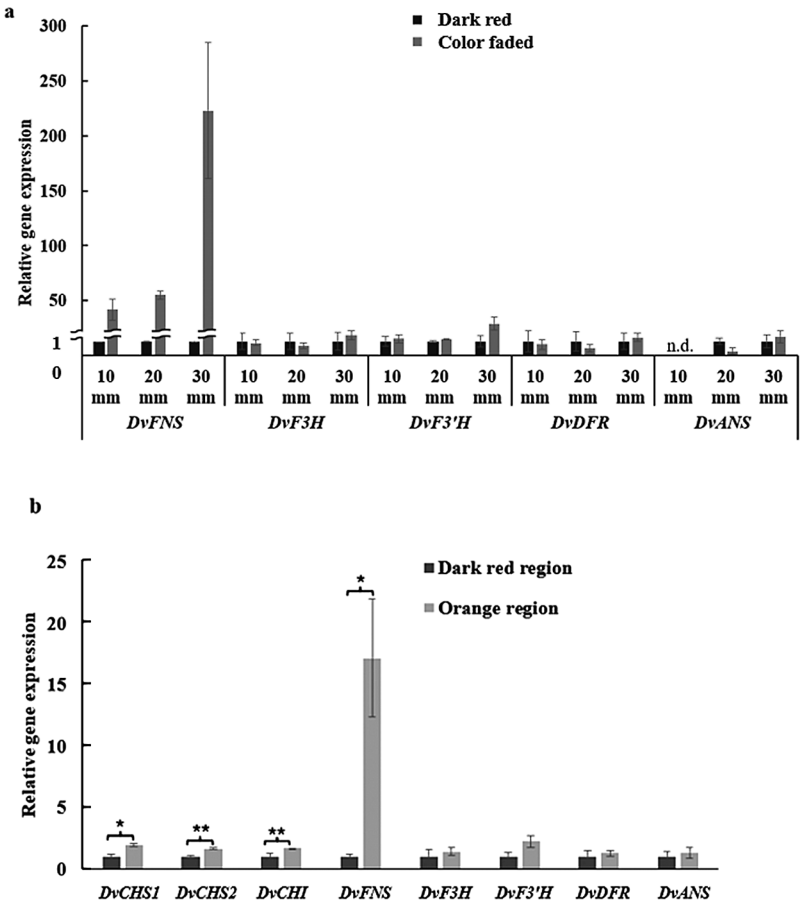


**Fig. 3.** Gene expression profiles for structural genes. (a) Profiles for five flavonoid biosynthesis genes (*DvFNS*, *DvF3H*, *DvF3'H*, *DvDFR*, and *DvANS*) in the anthocyanin biosynthetic pathway at three developmental stages (10 mm, 20 mm, and 30 mm indicate the length of ray florets) of dark red and orange flowers. (b) Profiles for major structural genes; *DvCHS1*, *DvCHS2*, *DvCHI*, *DvFNS*, *DvF3H*, *DvF3'H*, *DvDFR*, and *DvANS* expressed in the red and orange regions of variegated ray florets. Vertical bars show ± SE of the means (n = 3). * and ** indicate significant differences for *t*-test at 5% and 1% significance levels, respectively. Gene expression level in dark red florets in (a) and the dark red (b) region is set at a constant 1.

CFI values. In December, the *DvFNS* expression levels were highest, and this correlated with high CFIs (3 to 4), indicating extreme color fading from the dark red color. The pattern of expression correlated with the color fading indices.

To ascertain the relationship between temperature, *DvFNS* expression and seasonal flower fading in 'Nessho', a correlation analysis was done. As the level of *DvFNS* expression increased, the flower CFI increased (Fig. 6a), indicating a positive correlation ($r = 0.775$) between *DvFNS* expression and degree of color fading. The correlation between the flower color fading index and average weekly minimum temperature indicated an inverse relationship such that as the average weekly minimum temperature increased, the color fading occurrence decreased (Fig. 6b). Similarly, as the average weekly minimum temperature increased, there was a reduced level of *DvFNS* gene expression, and hence a negative regression ($R^2 = 0.744$; Fig. 6c).

A comparison between the change in *DvFNS* expression levels in young unexpanded leaves of RL- and RH-sensitive plants before and after inductive conditions indicated a significant difference at the 95% confidence level. The relative expression levels in RH-sensitive plants were higher than in RL-sensitive plants (Fig. 7).

## Discussion

Flower color change in response to changes in environmental conditions, mainly temperature, light intensity and daylength is a common phenomenon reported in a variety of plants. Color intensity in plant organs is determined by the accumulation of flavonoids, particularly anthocyanins and flavones (Harborne et al., 1990; Mizuno et al., 2015; Nordström and Swain, 1953; Tanaka et al., 2008). Prevailing environmental conditions influence flavonoid accumulation, as in the case of anthocyanin accumulation in *Arabidopsis thaliana* (Rowan et al., 2009). In many plant species, the accumulation of flavonoids in plant organs decreases under high temperature conditions. In grapes, high night temperatures reduced anthocyanin accumulation in the grape skin the quality of berries (Mori et al., 2005). In

the 'Nessho' red dahlia cultivar, high summer temperatures result in the production of red flowers whereas low winter temperature results in orange flowers (Okada et al., 2020). Color changing in *D. variabilis* dark red cultivars results from the accumulation of more flavones and fewer anthocyanins (Deguchi et al., 2013; Thill et al., 2012). Similarly, the accumulation of more flavones instead of anthocyanins led to paler violet *Antirrhinum majus* flowers (Luo et al., 1991). In our study, the red ray florets of 'Nessho' accumulated more pelargonidin and cyanidin-based anthocyanins, while the color-changed orange ray florets accumulated more apigenin and luteolin, both of which are flavones (Fig. 2). This finding corresponds with earlier reports on the dahlia 'Kokucho', including the original red and its purple mutants (Deguchi et al., 2015). Both anthocyanins and flavones are produced in the general flavonoid pathway, with flavones being a product of the branch pathway from chalcones. A control of gene expression and enzymes encoding their activities are crucial to the balance at branch points (Davies et al., 2003). This is a result of substrate competition by the enzymatic activities at these branch points (Deguchi et al., 2015; Takos et al., 2006; Thill et al., 2012). When *DvFNS* was silenced in black dahlia cultivars, the competition between anthocyanin synthesis and flavone synthesis was disrupted leading to high accumulation of anthocyanins (Deguchi et al., 2013).

To verify the genes responsible for color change in the dahlia 'Nessho' cultivar, relative expression analysis was done for major structural genes of the flavonoid biosynthetic pathway, namely, *DvCHS1*, *DvCHS2*, *DvCHI*, *DvANS*, *DvFNS*, *DvFLS*, *DvDFR*, *DvF3H*, and *DvF3'H* in red and orange ray florets. The expression profiles of all genes after the first main branch point on the biosynthesis pathway (at the naringenin stage) were not significantly different between the red and the orange ray florets except for *DvFNS* (Fig. 3a). This result suggests that *DvFNS* expression may be a major contributing factor to the red to orange color change of the 'Nessho' ray florets. The four-fold change in *DvFNS* expression levels in orange faded sections compared to red regions with variegated ray florets (Fig. 3b) further confirmed the possible role of this gene in the flower color change in 'Nessho'. Expression levels of anthocyanin structural genes (*DvDFR*, *DvF3H*, and *DvF3'H*) did not vary between the red and orange

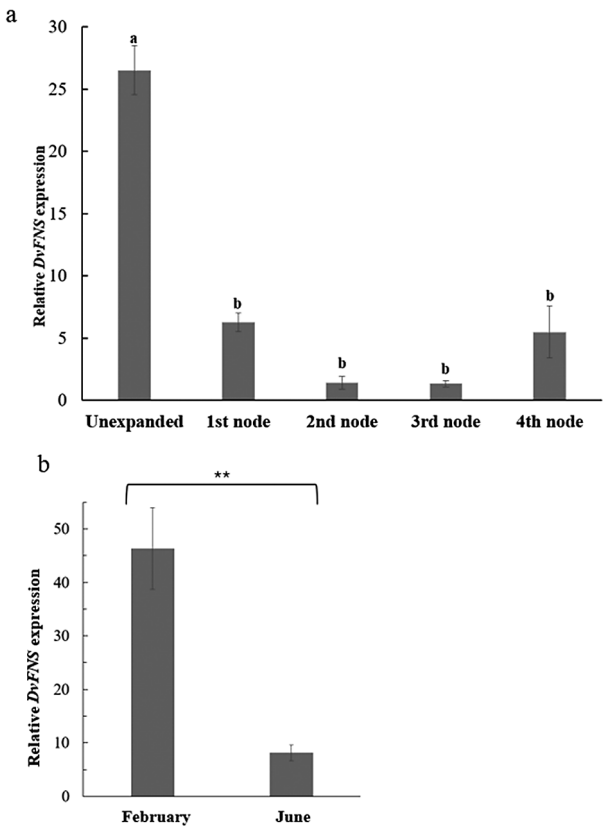


**Fig. 4.** *DvFNS* gene expression profiles (a) Profiles for five leaf developmental stages of RH-sensitive plants in winter (b) Profiles for RH-sensitive plants in winter (February) and in summer (June). Vertical bars show ± SE of the means (n = 3). a and b above error bars and ** indicate significant difference for the Tukey-Kramer test at 1% level.

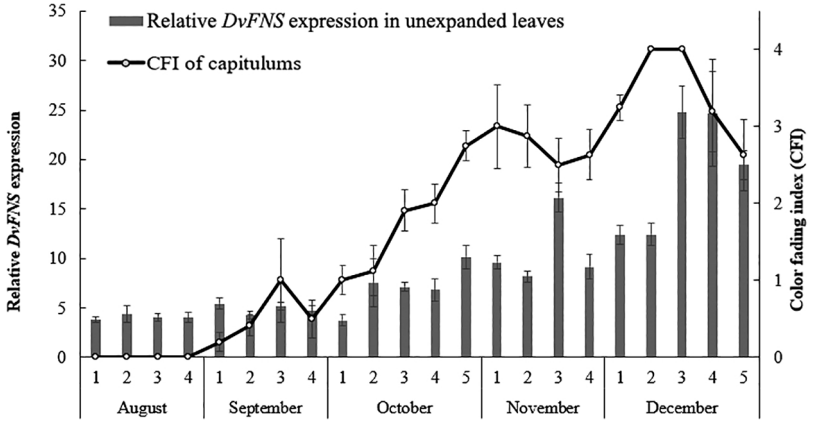


**Fig. 5.** *DvFNS* expression profiles for 210 RH-sensitive plants from August to December and their corresponding CFIs at the capitulum. Vertical bars show ± SE of the means (n = 3–12). The number above the month indicates the week number.

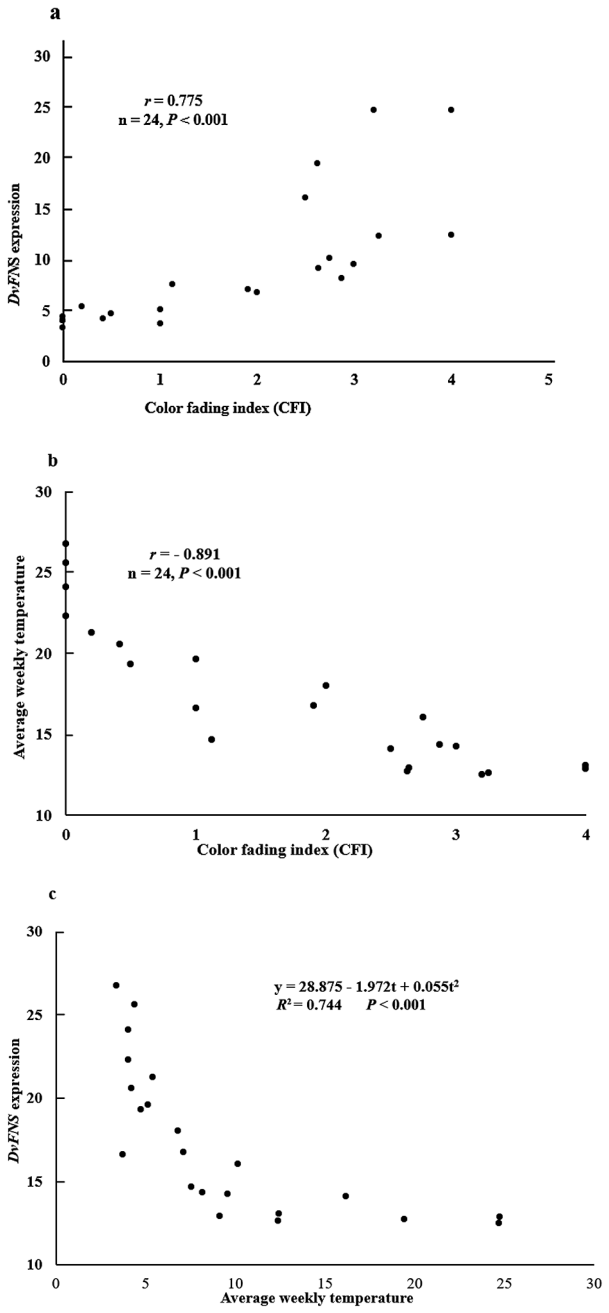


**Fig. 6.** Correlation between *DvFNS* expression, CFI, and weekly average temperature in RH-sensitive plants, (a) Relationship between *DvFNS* expression and the CFI, (b) Relationship between CFIs and weekly average temperature, (c) Relationship between *DvFNS* expression in young unexpanded leaves based on weekly average temperature.

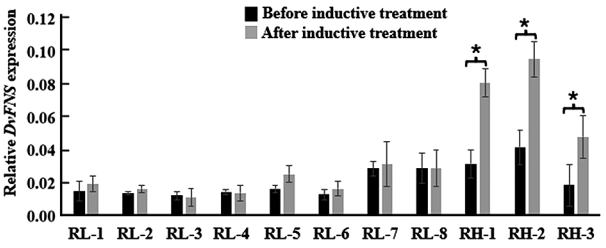


**Fig. 7.** *DvFNS* expression for several RL- and RH-sensitive plants before and after inductive conditions (20°C/9°C day and night temperatures and 10 hour day length. * indicates significant differences for the *t*-test at the 5% level. Vertical bars show ± SE of means (n = 4–8). Figure is made by adding new data to Muthamia et al. (2022).

regions. This concurs with a report in *Gerbera hybrida*, where lines lacking *FNSII* expression and FNSII activity accumulated more anthocyanin and no flavones, leading to a notable increase in color intensity (Martens and Mithöfer, 2005). In a similar manner, the majority of black dahlia cultivars accumulate more anthocyanins due to low FNSII activity, as well as *FNSII* expression (Thill et al., 2012). In *Lonicera japonica* and *L. macranthoides*, higher expression levels of *FNSII* were consistent with more flavone accumulation in flowers and flower buds, respectively (Wu et al., 2016).

Having demonstrated that *DvFNS* is the likely gene responsible for the color change in 'Nessho' ray florets, we tried to evaluate whether the expression change occurred only in the ray florets or also in leaves. The results for five leaf developmental stages (Fig. 4a) showed that *DvFNS* expression was elevated in the young unexpanded leaves as compared to leaves on the 1st–4th nodes in RH-sensitive plants. This indicates temporal expression of structural genes in the flavonoid biosynthetic pathway in this cultivar. This concurs with a report by Yuan et al. (2013), in which temporal expression profiles of some putative structural genes in various tissues (leaves, stems, stamen, pistil, and petals) revealed a strong correlation with the formation of the red pigment in tulip petals. Temporal expression of *CHS* genes in the dahlia bi-color cultivar 'yuino' was characterized by Ohno et al. (2011b) and was detected in all petal developmental stages except in the young stage, with lower expression in white flower parts than in red parts.

To verify the relationship between *DvFNS* expression in young unexpanded leaves and seasonal flower color changes, gene expression profiles were compared in winter and summer. A five-fold higher *DvFNS* expression was observed in winter as compared to summer and this corresponded to flower color changes in the different seasons (Fig. 4b). Additionally, a comparison between *DvFNS* expression in young unexpanded leaves and the color fading index of the capitulum from August through December showed a similar trend (Fig. 5). This may indicate temporal *DvFNS* expression in young leaf tissues that corresponds with flavone accumulation in flowers, as in the case of apigenin accumulation and *FNSII* expression in *Gentiana triflora* 'Maciry' leaves (Nakatsuka et al., 2005). The positive correlation between *DvFNS* expression and the color changing index CFI (Fig. 6a) agrees with the reports of Deguchi et al. (2013), Deguchi et al. (2015), and Thill et al. (2012) who found that in purple and red dahlia cultivars a decline in the expression of *FNSII* resulted

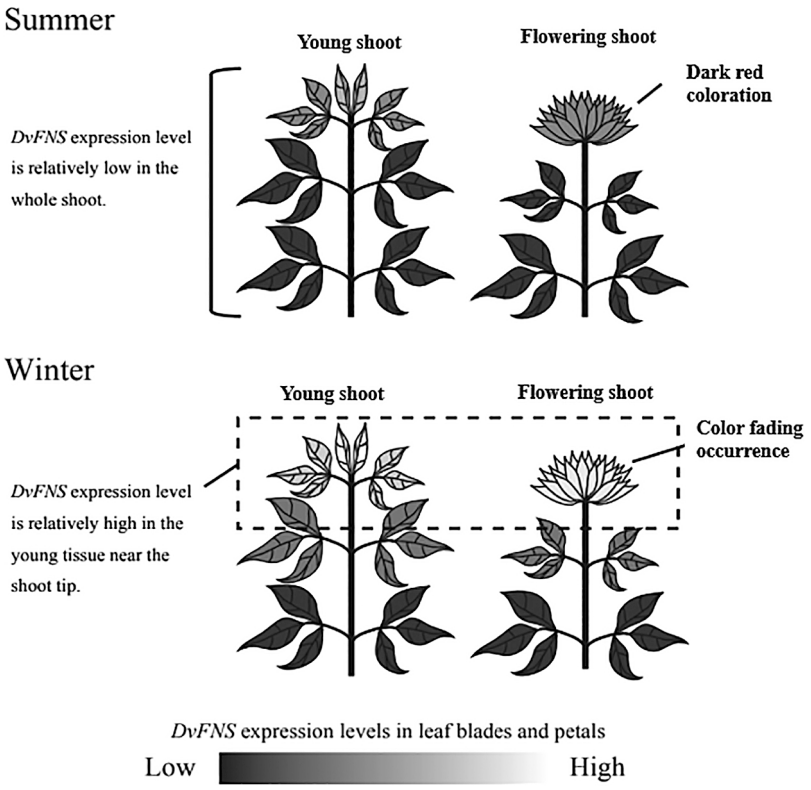


**Fig. 8.** Schematic representation of the *DvFNS* expression in an RH-sensitive plant in summer and winter.

in a deeper color in majority of the black dahlia cultivars and vice versa in the other colored cultivars such as white, yellow, orange, pink, and red. The negative correlations between CFI and average weekly temperature (Fig. 6b) and *DvFNS* expression in young unexpanded leaves and average weekly temperature further confirm the role of temperature in the expression of the *DvFNS* gene, accumulation of flavone and consequently flower color change in the 'Nessho' dahlia cultivar. Similarly, *Pohlia nutans* FNSI possesses hypothermic enzyme characteristics, retaining relatively high enzymatic activity in low-temperature environments in Antarctica. This led to high flavone accumulation in moss under low-temperature conditions (Wang et al., 2020).

Some 'Nessho' plants are more sensitive to color fading than others in similar environmental conditions. This is evidenced by the varying color-fading indices at the flowering stage and by the difference in gene expression at the young leaf stage (Fig. 7). Other factors may be involved in the difference in the expression of *DvFNS* in RL- and RH-sensitive cultivars at low-temperatures. In the future, identifying the genetic differences between RL- and RH-sensitive plants that cause different *DvFNS* expression levels will lead to the development of genetically uniform cultivars and high quality flower production.

*Conclusion*

In conclusion, our study demonstrates that *DvFNS* expression in the 'Nessho' dahlia cultivar plays an important role in the accumulation of flavones in ray florets. Seasonal temperature changes influence the expression of *DvFNS*, with low winter temperatures up-regulating the expression and high summer temperatures down-regulating it. This results in the production of faded orange flowers in winter. There is also a positive correlation between *DvFNS* expression in ray florets, in young unexpanded leaves, and color fading occurrence (Fig. 8). This is an important finding that can be used in the selection of non-fading lines at the seedling stage.

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