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Tyrosine Induces Anthocyanin Biosynthesis in *Arabidopsis thaliana*

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ABSTRACT

Anthocyanins are widely found in plants and are responsible for the purple coloration of plants. Anthocyanin biosynthesis is induced by environmental stresses, plant hormones, sugar, and so on. Tyrosine (Tyr) is the precursor of melanin that exits in both animals and plants. However, until now it has been unknown whether Tyr induces anthocyanin biosynthesis. In this study, the seedlings of *Arabidopsis thaliana* were treated with exogenous Tyr and then the anthocyanin accumulation was determined. The results showed that Tyr induced anthocyanin accumulation in *Arabidopsis thaliana* in a dose-dependent manner. Furthermore, the expression of the late anthocyanin biosynthetic genes including *DFR*, *LDOX*, and *UF3GT*, and the transcription factor genes *PAP*1, *PAP*2, and *EGL*3 was induced by Tyr. Taken together, these results demonstrated that Tyr is able to induce anthocyanin accumulation and suggested that Tyr up-regulates transcription factors *PAP*1, *PAP*2, and *EGL*3, which mediates the expression of the late anthocyanin biosynthetic genes and then induces anthocyanin biosynthesis.

KEYWORDS

***Arabidopsis*; Tyrosine; Anthocyanin**

1. Introduction

Anthocyanins are widely found in plants and are responsible for the purple coloration of plants. Anthocyanins are not only used as natural colorants [1] but also play an important role in plant defense against both biotic and abiotic stresses including protecting plants from damage by UV irradiation and acting as antimicrobial agents and feeding deterrents against pathogens and herbivores [1-3]. In addition, Anthocyanins can help humans prevent cardiovascular diseases, anti-platelet aggregation and enhance immune modulating activity [4,5].

Anthocyanin biosynthesis is induced by a series of endogenous developmental and environmental signals, such as sugar, light, and plant hormones [6-11]. The anthocyanin biosynthetic genes are divided into two groups, the early anthocyanin biosynthetic genes such aschalcone synthase (*CHS*) and chalcone isomerase (*CHI*), and the late anthocyanin biosynthetic genes inculding dihydroflavonol reductase (*DFR*), leucoanthocyandin dioxygenase (*LDOX*), and UDP-glucose: flavo-noid 3-O-glucosyltransferase (*UF3GT*) [12-14]. The WD-repeat/Myb/bHLH transcription complex including transcription factors PRODUCTION of ANTHOCYANIN PIGMENT1 (PAP1), PAP2, GLABRA3 (GL3), ENHANCER of GLABRA3 (EGL3), and TRANSPARENT TESTA GLABRA 1 (TTG1) mainly regulates the expression of the late anthocyanin biosynthetic genes [15].

Tyr is the precursor of melanin that exits in both animals and plants [16-18]. However, until now it has been unclear whether Tyr induces anthocyanin biosynthesis. In this study the seedlings of *Arabidopsis thaliana* were treated with exogenous Tyr for analysis of anthocyanin accumulation and the expression of the anthocyanin bio-

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synthesis genes and the WD-repeat/Myb/bHLH transcription factor genes to determine whether Tyr induces anthocyanin biosynthesis.

2. Materials and Methods

Arabidopsis thaliana ecotype Columbia (Col-0) was used in this study.

Seeds were surface-sterilized with 20% chlorine bleach containing 0.1% Triton X-100 for 10 min, washed five times with sterile water, plated on Murashige and Skoog medium supplemented with 1% sucrose (MS, pH 5.8), chilled at 4˚C for 3 d, and then transferred to a growth chamber and cultured under a 16 h light/8h dark photoperiod at 22˚C.

For measurement of anthocyanin content, 7-d-old seedlings grown on MS were transferred onto MS containing 3% sucrose supplemented without or with different concentrations of Tyr for an additional 7 d growth. For analysis of gene expressions, 7-d-old seedlings grown on MS were transferred onto MS without or with 2 mM Tyr for an additional 7 d growth.

The measurement of anthocyanin was performed as described by Deikman and Hammer [19]. The preweighed seedlings were placed into 1 mL extraction buffer (18% 1-propanol, 1% HCl, and 81% water), boiled for 3 min and then incubated in darkness overnight at room temperature. The absorbance of the supernatant was measured at 535 nm and 650 nm. The anthocyanin content was determined by the formula (A535-A650) g^{-1} fresh weight (FW). There were six samples per each treatment.

The gene expressions were analyzed by quantitative real-time PCR (qPCR). The total RNA was isolated using TRIzol Reagent (Invitrogen, USA). After incubated with DNase (Thermo Fisher Scientific, USA) for 30 min at 37˚C and then for 10 min at 65˚C to remove genomic DNA, the RNA sample was quantified by a spectrophotometer. The reverse transcription was synthesized using ReverTra Ace qPCR RT Kit (Toyobo, Japan). The qPCR was carried out using SYBR qPCR Mix (Toyobo, Japan) with the ABI 7300 Sequence Detection System (Applied Biosystems, USA) following the manufacturer's instructions. The primers of genes tested in qPCR were as follows: *CHS*, 5'-CGCATCACCAACAGTGAACAC-3' and 5'-TCCTCCGTCAGATGCATGTG-3'; *CHI*, 5'-CCGGTTCATCGATCCTCTTC-3' and 5'-ATCCCGGTTTCAGGGATACTATC-3'; *DFR*, 5'-CCTTATCACCGCGCTCTCT-3' and 5'-TGTCCTTGTCTTATGATCGAGTAATGC-3'; *LDOX*, 5'-TCAATTTGGCCTAAGACACCAAGT-3' and 5'-TCGCTAGCAAACGAAGACACTT-3'; *UF3GT*, 5'-CAACTGGTTTTCCGTTTCTGGTT-3' and 5'-GCTTCCTCGACGGTTGATACAC-3'; *PAP*1, 5'-GACATTACGCCCATTCCTACAAC-3' and 5'-TCGAGGTCGAGGCTTATAAACATT-3'; *PAP*2, 5'-GAGGAAAGGTGCATGGACTG-3' and 5'-ATCGCATCAGCTTCTTGGT-3'; *GL*3, 5'-TCGGTTCGTTTGGTAATGAGG-3' and 5'-GCTTGCAATTGACGGTTAAGC-3'; *EGL*3, 5'-GGAAGACGATTCAAGCAGCA-3' and 5'-GGATTCAGCGAGGGAGAGAG-3'; *TTG*1, 5'-GTCATGAACCTCTTTATCAT-3' and 5'-ATGGATAATTCAGCTCCAGA-3'. *ACTIN*2 was used as an internal control and was amplified with primers 5'-AGCACTTGCACCAAGCAGCATG-3' and 5'-ACGATTCCTGGACCTGCCTCATC-3'. Gene expression for each sample was calculated on three analytical replicates, and the relative expression was quantified using the $2^{-\Delta\Delta Ct}$ method.

All the experiments were repeated at least three times. Data in the figures are expressed as means ± SE of three biological replicates for qPCR and six biological replicates for anthocyanin measurement.

3. Results and Discussion

In order to investigate whether Tyr induces anthocyanin biosynthesis in plants, the *Arabidopsis thaliana* seedlings were treated with exogenous Tyr. Upon Tyr treatment, pigmentation appeared in back of leaves and it was more obvious when treated with high concentrations of Tyr (**Figure 1(a)**). The content of anthocyanin was increased in a dose-dependent manner of Tyr (**Figure 1(b)**). These results indicated that Tyr is able to induce anthocyanin accumulation.

To investigate the molecular mechanism for Tyr-induced anthocyanin biosynthesis, the expression of anthocyanin biosynthetic genes including *CHI*, *CHS*, *DFR*, *LDOX*, and *UF3GT* was analyzed by qPCR. Upon Tyr treatment, the expression of *CHI* and *CHS* was comparable to control without Tyr treatment, however, the expression of *DFR*, *LDOX*, and *UF3GT* was significantly increased (**Figure 2**). Since *DFR*, *LDOX*, and *UF3GT* were classified as the late anthocyanin biosynthetic genes [13,15], we concluded that Tyr induces anthocyanin accumulation mainly by up-regulating the late anthocyanin biosynthetic genes.

Since WD-repeat/Myb/bHLH transcription complexes including transcription factors *PAP*1, *PAP*2, *GL*3, *EGL*3, and *TTG*1 mediates the expression of late anthocyanin biosynthetic genes [15], we further analyzed the expression of these transcription factors genes. As shown in **Figure 3**, the expression of *PAP*1, *PAP*2, and *EGL*3 was increased in the presence of Tyr, however, the expression of *GL*3 and *TTG*1 was not obviously induced by Tyr. These results suggested that the expression of late anthocyanin biosynthetic genes induced by Tyr might be mediated by transcription factors *PAP*1, *PAP*2, and *EGL*3.

Accumulation of anthocyanins in plants is stimulated by diverse developmental signals, sugar, plant hormone, and environmental stresses [6-11]. Jasmonate (JA), a kind of plant hormones, induces anthocyanin accumula-

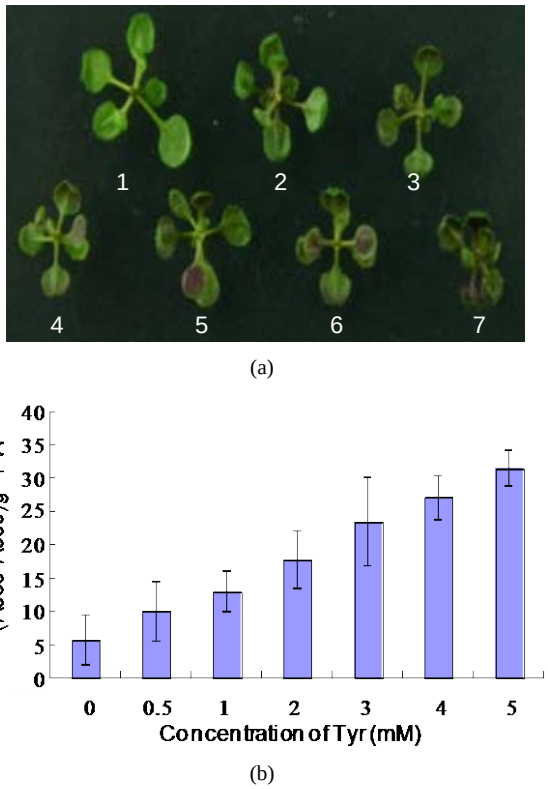

Figure 1. The phenotype of *Arabidopsis thaliana* seedlings (Col-0) treated with different concentrations of Tyr for 7 days (a) and the corresponding anthocyanin content (b). The number 1, 2, 3, 4, 5, 6, and 7 in (a) represent treatment with 0, 0.5, 1, 2, 3, 4, 5 mM Tyr, respectively. Error bars in (b) represent SE. Tyr, tyrosine.

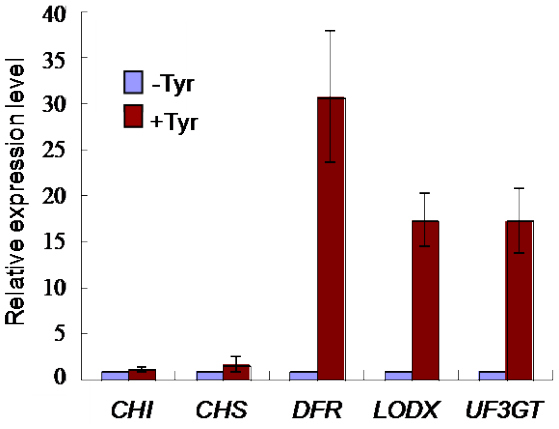

Figure 2. Relative expression level of anthocyanin biosynthesis genes including *CHI*, *CHS*, *DFR*, *LDOX*, and *UF3GT* in *Arabidopsis thaliana* seedlings (Col-0) treated with 2 mM Tyr to control without Tyr treatment. Error bars represent SE. Tyr, tyrosine.

tion and up-regulates the expression of late anthocyanin biosynthetic genes *DFR*, *LDOX*, and *UF3GT*, and transcription factors *PAP*1, *PAP*2, and *GL*3 [20]. In this study, we also found that Tyr induces the expression of

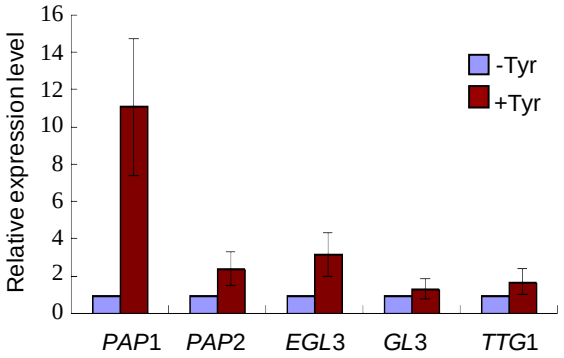

Figure 3. Relative expression level of the WD-repeat/MYB/bHLH transcription factors *PAP*1, *PAP*2, *EGL*3, *GL*3, and *TTG1* in *Arabidopsis thaliana* seedlings (Col-0) treated with 2 mM Tyr to control without Tyr treatment. Error bars represent SE. Tyr, tyrosine.

late anthocyanin biosynthetic genes *DFR*, *LDOX*, and *UF3GT*, and transcription factors *PAP*1 and *PAP*2 (Figures 2 and 3). However, the expression of transcription factor *GL*3 was not induced by Tyr (Figure 3). Instead, Tyr induces the expression of transcription factor *EGL*3 (Figure 3). Therefore, Tyr and JA modulate the expression of late anthocyanin biosynthetic genes by mediating the same Myb transcription factors *PAP*1 and *PAP*2, but the different bHLH transcription factors *EGL*3 and *GL*3.

4. Conclusion

This study demonstrated that the exogenous Tyr could induce the anthocyanin accumulation and the expression of late anthocyanin biosynthetic genes including *DFR*, *LDOX*, and *UF3GT*, and the transcription factor genes *PAP*1, *PAP*2, and *EGL*3 in *Arabidopsis thaliana* seedlings, and suggested that Tyr up-regulates transcription factors *PAP*1, *PAP*2, and *EGL*3, which mediates the expression of the late anthocyanin biosynthetic genes and then induces anthocyanin biosynthesis.

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