The combination of R2R3-MYB gene AmRosea1 and hairy root culture is a useful tool for rapidly induction and production of anthocyanins in Antirrhinum majus L

Chunlan Piao1†, Jinguo Wu1† and Min-Long Cui1,2*

Abstract
Anthocyanins are the largest group of water-soluble pigments and beneficial for human health. Although most plants roots have the potential to express natural biosynthesis pathways required to produce specialized metabolites such as anthocyanins, the anthocyanin synthesis is specifically silenced in roots. To explore the molecular mechanism of absence and production ability of anthocyanin in the roots, investigated the effect of a bHLH gene AmDelila, and an R2R3-MYB gene AmRosea1, which are the master regulators of anthocyanin biosynthesis in Antirrhinum majus flowers, by expressing these genes in transformed hairy roots of A. majus. Co-ectopic expression of both AmDelila and AmRosea1 significantly upregulated the expression of the key target structural genes in the anthocyanin biosynthesis pathway. Furthermore, this resulted in strongly enhanced anthocyanin accumulation in transformed hairy roots. Ectopic expression of AmDelila alone did not gives rise to any significant anthocyanin accumulation, however, ectopic expression of AmRosea1 alone clearly upregulated expression of the main structural genes as well as greatly promoted anthocyanin accumulation in transformed hairy roots, where the contents reached 0.773–2.064 mg/g fresh weight. These results suggest that AmRosea1 plays a key role in the regulatory network in controlling the initiation of anthocyanin biosynthesis in roots, and the combination of AmRosea1 and hairy root culture is a powerful tool to study and production of anthocyanins in the roots of A. majus.

Keywords: Antirrhinum majus L, R2R3-MYB gene AmRosea1, Transformed hairy roots, Activation of anthocyanin biosynthesis, Anthocyanin content

Introduction
The plants including Antirrhinum majus are capable of biosynthesizing a wide variety of secondary metabolites including flavonoids, polysaccharides, fatty acids, vitamins, alkaloids, terpenoids and iridoid glycosides (Mehrotra et al. 2015; Jeziorek et al. 2018; Yousefian et al. 2020; Seo et al. 2020; Roy 2021). Among them, anthocyanins are flavonoids that a class of useful secondary metabolites play inhibit cancer cell proliferation and to serve as antioxidants promote human health (Tavsan and Kayali 2019; Kopustinskiene et al. 2020). The anthocyanins are found in the leaves, stems, flowers and fruits, however absence in the root of most plants. Moreover, despite the anthocyanin biosynthesis pathway in flowers, leaves and fruits of plants is well understood (Martin et al. 1991; Zhang et al. 2020), the molecular basis for the absence of anthocyanin accumulation in the roots remains unclear.

The plants are natural producers of many important pharmacologically active secondary components
including anthocyanins (Sharma et al. 2018). However, plant propagation and tissue culture to overproduction of some useful pharma molecules has limitations, especially where the synthesis of these molecules is affected by their complicated developmental regulation in the different cells, tissues, organs and ages in plants. Biotechnological approaches involving Agrobacterium-transformed tissue culture have the potential to overcome this. Agrobacterium rhizogenes has the ability to transfer its T-DNA from the root-inducing (Ri) plasmid to the host plant genome, thereby inducing the formation of hairy roots. The transformed hairy roots are rapidly and efficiently induced from explant tissues of the host plants and the culture procedure is very simple and can be maintained for a long period; the hairy roots show rapid growth rates and similar genetic characteristics to those of normal roots. Moreover, the hairy roots appear to produce the same spectrum of metabolites as do roots in planta, in addition to synthesizing novel compounds (Ritala et al. 2014; Mehrotra et al. 2015; Thakore and Srivastava 2017; Roy 2021). Therefore, hairy root culture represents a useful tool for studying molecular mechanisms of secondary metabolism, the molecular function of the genes involved and provide a reliable platform for production specific components by bioengineering (Gao et al. 2013; Ghorbani 2017).

Anthocyanins are flavonoid pigments. The biosynthetic pathway of anthocyanin is well studied and the main structural genes involved in this pathway such as chalcone synthase (CHS), chalcone isomerase (CHI), flavanone 3-hydroxylase (F3H), flavonoid 3’-hydroxylase (F3’H), dihydroflavonol 4-reductase (DFR), and anthocyanidin synthase (ANS) have been isolated and characterized in A. majus, Arabidopsis and petunia (Martin et al. 2001; Kitamura et al. 2004; Ai et al. 2016). The structural genes are regulated by transcription factors, including R2R3-MYB, basic helix-loop-helix (bHLH), and WD40 proteins (Ramsay and Glover 2005; Gonzalez et al. 2008; Albert et al. 2014). Among these, the R2R3-MYB genes play significant roles in regulate of anthocyanin biosynthesis process (Gao et al. 2013; Borevitz et al. 2000; Naing and Kim 2018).

Antirrhinum majus is a medicinal plant as well as has been used particularly as a model system for the molecular analysis of floral pigmentation (Martin et al. 1991; Saqqallah et al. 2018; Seo et al. 2020). In A. majus, the structural genes encoding the enzymes of anthocyanin biosynthetic pathway have been well characterized and identified genetically, namely, AmCHS, AmF3H, AmF3’H, AmDFR, and AmAS (Additional file 1: Fig. S1) (Martin et al. 1985, 1991; Sommer and Saedler 1986). These genes are divided into two groups, early biosynthetic genes (EBGs), including AmCHS, AmCHI, AmF3H, and AmF3’H; and late biosynthetic genes (LBGs), including AmDFR, AmANS, and AmUFGT (Pelletier et al. 1997; Winkel-Shirley 2001). In Antirrhinum, each of the two groups has been found to be mainly co-regulated by different regulators, including an bHLH transcription factor, AmDelila (AmDEL), and three R2R3-MYB transcription factors, namely, AmRosea1(AmROS1), AmRosea2 (AmROS2), and AmVenosa (AmVE) (Almeida et al. 1989; Schwinn et al. 2006; Shang et al. 2011). In flowers of A. majus, AmDelila affects pigmentation in the corolla tube; AmRosea1 affects the pattern and intensity of pigmentation in the lobes and tubes; and AmVenosa affects pigmentation of the epidermis overlying the veins in the lobes and tubes. In addition, transcription factors are required for the activation of expression of the late biosynthetic genes, including AmDFR, AmAS and AmUFGT, in the corolla tube (Goodrich et al. 1992; Martin and Gerats 1993).

In the present study, we investigated the molecular mechanism of absence and production ability of anthocyanins in the transformed hairy roots of A. majus, and discuss the usefulness of combination of the R2R3-MYB gene AmRosea1 and hairy root culture method is a powerful tool to control of the secondary metabolic pathway and product of anthocyanins in the root of A. majus.

Materials and methods
Plant material and growth conditions
The seeds of Antirrhinum J1 7 were used in this study (provided by Lucy Copes and Professor Enrico Coen, John Innes Centre, UK). The seeds were surface-sterilized by brief rinsing in 70% (v/v) ethanol, followed by steri-

dized in a 2% (v/v) solution of sodium hypochlorite for 10 min and washed five times using sterilized water. The sterilized seeds were germinated on solid MS medium (Murashige and Skoog 1962) in a growth room at 25 °C, 16 h light/8 h dark photoperiod. Four-week-old seedlings were used for transformation.

Agrobacterium strains and plasmids
The plant expression binary vectors of pBI35S:ROS1-35S:DEL, pBI35S:ROS1, pBI35S:DEL, and pBI121 (Fig. 1) were introduced into Agrobacterium rhizogenes strain of AR1193 (Weidi Biotech Co. Ltd. Shanghai, China) by electroporation (Shen and Forde 1989), and used in this study.

A. rhizogenes-mediated transformation of A. majus
The transformation of A. majus was performed according to the methods of Senior et al. (1995) and Cui et al. (2001). The A. rhizogenes strains were grown in 5 mL of liquid LB medium containing 50 mg/L kanamycin and 100 mg/L rifampicin at 28 °C, shaking at 200 rpm for
24 h. The Agrobacterium cultures were diluted 40-fold with liquid MS medium before inoculation. About 1 cm long hypocotyl segments of A. majus were inoculated with the diluted Agrobacterium suspensions for 8–10 min, and transferred to a solid co-cultivation MS medium containing 1 mg/L zeatin, 0.1 mg/L NAA (1-Naphthaleneacetic acid), and 20 μM acetosyringone. About 50 hypocotyl segments for each treatment were used. After 3 days of co-cultivation, the infected hypocotyl segments were transferred to solid MS medium containing 50 mg/L kanamycin and 250 mg/L cefotaxime and induced transformed hairy roots. The obtained adventitious roots were transferred to fresh solid MS medium containing 250 mg/L of cefotaxime and selected transformed hairy roots were seen by visible coloration and harvested for PCR analysis. The selected hairy roots were maintained at 25 °C under a 16 h light/8 h dark photoperiod condition.

Polymerase chain reaction analysis
Genomic DNA was extracted from a non-transformed root, transformed hairy roots of pBI21, pBI35S:ROS1-35S:DEL, pBI35S:ROS1 and pBI35S:DEL, respectively, according to the CTAB (Hexadecyl trimethyl ammonium Bromide) method (Rogers and Bendich 1985). The primer sets of NptII, AmROS1, and AmDEL were used for amplification (Table 1). PCR analysis was performed by using an ABI 2720 PCR machine, with 20 μL of reaction mixtures containing 50 ng genome DNA, 10 pmol of each primer, and 1 unit of Taq polymerase (Takara, Dalian, Japan). The following PCR conditions were used: an initial denaturation step at 94 °C for 3 min; followed by 35 cycles each at 94 °C for 1 min, 58–62 °C for 1 min, and 72 °C for 2 min; and a final extension step at 72 °C for 10 min. Amplified DNA bands were analyzed by using 1.0% (w/v) agarose gel electrophoresis at 100 V for 30 min, followed by staining with ethidium bromide and observation under UV illumination.

Expression analysis of the genes involved in the anthocyanin biosynthetic pathway in hairy roots
Total RNA was extracted from 0.1g samples of a non-transformed root and the transformed hairy roots with pBI21, pBI35S:ROS1-35S:DEL, pBI35S:ROS1 and pBI35S:DEL, respectively, use the SV Total RNA Isolation System and RNase-free DNase (Promega, Beijing, USA). First-strand cDNA was synthesized from 2 μg of total RNA using a Superscript III First Strand cDNA Synthesis Kit (Invitrogen, Shanghai, USA). Semi-quantitative RT-PCR analyses were carried out using AmROS1, AmDEL, AmCHS, AmF3H, AmDFR, and AmANS genespecific primers (Table 1) and the ubiquitin genes of A. majus (AmUBI) as positive control. The PCR conditions are: a preliminary denaturation step at 95 °C for 5 min; followed by 30 cycles of denaturation at 94 °C for 30 s, annealing at 58–62 °C for 1 min and extension at 72 °C for 1 min; and a final extension step at 72 °C for 10 min. The RT-PCR experiments were repeated at least two times independently, and the PCR products were confirmed by sequencing.

Quantification of anthocyanins
A hairy root transformed with pBI21 as a control and five independent hairy roots transformed with pBI35S:ROS1 were ground to a fine powder in liquid nitrogen, and 100 mg of the powder was extracted with 1 mL acidic methanol (1% hydrochloric acid, w/v) at room temperature for 12 h with moderate shaking. After centrifugation at 12,000 rpm for 10 min, 800 mL of the supernatant was added to 4 mL of acidic methanol. The absorbance at 530 and 657 nm was determined using a spectrophotometer (UV757CRT, Shanghai precision and scientific instrument Co. Ltd., China), and the relative level of anthocyanin was calculated using the equation...
ODA530–(0.25 X ODA657) (Rabino and Mancinelli 1986). Each sample was tested three times. Error bars indicate the standard deviation (SD) values of the average anthocyanin contents.

Results

Expression analysis of AmDelila and AmRosea1 in A. majus

Despite the leaves of A. majus exhibited red coloration in the abaxial parts when grown under light conditions, the roots did not display any color accumulation, regardless of light conditions (Fig. 2A). To determine the molecular mechanism responsible for the absence of anthocyanin accumulation in the roots, investigated the expression of the AmDelila and AmRosea1 in the roots, red-colored leaves, and flowers of A. majus JI 7 (Fig. 2A), by using semi-quantitative RT-PCR analysis (Fig. 2B). The visible expression of both AmDelila and AmRosea1 were detected in the flowers and leaves, however, the AmDelila and AmRosea1 expression were not observed in the roots (Fig. 2B). Therefore, absence of anthocyanin pigmentation in the roots of A. majus may be affected by silence of AmDelila- or AmRosea1-like regulatory genes.

Simultaneous expression of AmRosea1 and AmDelila promotes anthocyanin accumulation in transformed hairy roots of A. majus

To test hypothesis, we used the A. rhizogenes strains AR1193/pBI35S:ROS1-35S:DEL with harboring both Delila and Rosea1 genes under the control of the 3SS promoter, respectively, and AR1193/pBI121 (3SS:GUS) as a negative control to transform A. majus JI 7. After 3 weeks of infection, transformed non-pigmented hairy roots were emerged from the wounded end of hypocotyl segments co-cultivated with AR1193/pBI121 (data not shown). Despite of these hairy roots were similar to non-transformed root showed no any color accumulation, these roots revealed rapid elongation when maintained under 16 h light/8 h dark conditions (Fig. 3A, B). In contrast, highly pigmented hairy roots emerged from hypocotyl segments transformed with AR1193/pBI35S:ROS1-35S:DEL (Fig. 3C, D). These roots appeared later than non-pigmented hairy roots (Fig. 3C), and the elongation was slower than for non-pigmented hairy roots when maintained under 16 h light/8 h dark condition (Fig. 3D).

To analyse the mechanism for the AmDelila/AmRosea1 effect, we looked at the expression of target structural genes of the anthocyanin biosynthetic pathway. We investigated the expression patterning of the main structural genes, AmCHS, AmF3H, AmDFR, and AmANS, from hairy roots transformed with pBI35S:ROS1-35S:DEL and a negative hairy root transformed with AR1193/pBI121 (P121), by using semi-quantitative RT-PCR analysis (Fig. 3E). Except of AmF3H, the expressions of AmCHS, AmDFR and AmANS were dramatically upregulated in the hairy roots transformed with pBI35S:ROS1-35S:DEL, and that the expression pattern correlated with the anthocyanin accumulations in the hairy roots (Fig. 3D, E). In contrast we did not see any significant expression of AmDFR and AmANS in negative control hairy roots of P121 (Fig. 3B). Our results indicate that the combination of AmDelila and AmRosea1 are able to activate the expression of the structural genes involved in the anthocyanin biosynthetic pathway to enhanced anthocyanin accumulation in the hairy roots of A. majus.

Fig. 2 Comparison of colorations in the roots, leaves, and flowers of A. majus JI 7 and RT-PCR analysis of AmRosea1 and AmDelila expression. A Six-week-old seedling, mature leaves and flower of A. majus; B Semi-quantitative RT-PCR-based expression analysis of AmRosea1 (AmROS1) and AmDelila (AmDEL) in the roots, leaves, and flowers. Scale bar = 1 cm. ad, adaxial of leaf; ab, abaxial of leaf.
Ectopic expression of *AmRosea1* alone activates anthocyanin synthesis in transformed hairy roots

To investigate the contribution of each gene, AR1193/pBI35S:DEL, and the negative control AR1193/pBI121 were transformed into 3-week-old hypocotyls of *A. majus* JI 7, respectively. After 3 weeks of infection, a lot of independent hairy roots emerged from hypocotyl segments transformed with pBI35S:DEL (Fig. 4A). These hairy roots were maintained for 6 weeks under 16 h light/8 h dark condition, but remained similarly non-pigmented to the hairy roots of AR1193/pBI121 (P121). These results indicate that *AmDelila* alone does not appear to have the ability to stimulate anthocyanin synthesis in roots of *A. majus*.

Next, investigate the ability of *AmRosea1*, the AR1193/pBI35S:ROS1 transformed into 3-week-old hypocotyls of *A. majus* JI 7. After 3 weeks of infection, a lot of independent hairy roots emerged from hypocotyl segments transformed with pBI35S:ROS1 and many hairy roots showed pale or purple red pigmentation (Fig. 4B). Among these, some pale-red colored hairy roots (PRC) and deep-red colored hairy roots (DRC) were excised and transferred to the MS + 200 mg/L of cefotaxime. The culture was kept at 25 °C under a 16 h light/8 h dark photoperiod. (Fig. 4C, D). To get more examples, we further chose two pale-colored hairy root lines PRC1 and PRC2, three purple red colored hairy root lines DRC1, DRC2 and DRC3) and a negative control hairy root P121 to analyse the expression levels of *AmCHS*, *AmF3H*, *AmDFR*, and *AmANS* by semi-quantitative RT-PCR (Fig. 6). Clear expression of *AmCHS*, *AmF3H*, *AmDFR*, and *AmANS* were detected from five independent hairy roots. The expression levels of structural genes in DCR1, DCR2 and DCR3 lines were stronger than in PRC1 and PRC2 lines. Also, the expression patterns of *AmDFR* and *AmANS* coincided with the expression of
Moreover, the expression levels of AmDFR and AmANS seem to be harmony with anthocyanin accumulation in the hairy roots, (Fig. 6, Additional file 2: Fig. S2). Our findings suggest that AmRos1 alone is able to stimulate structural gene expression in the anthocyanin biosynthetic pathway, and thereby induce anthocyanin accumulation in the hairy roots of *A. majus*.

**Discussion**

The regulation of anthocyanin biosynthesis has been well examined in the above ground parts of plants such as flowers, leaves, seeds and fruits, in various species, but little has hitherto been known about underground organs such as roots, except in tuberous roots of sweet potato and potato (Liu et al. 2016; Strygina et al. 2019).

In the present study, transformed hairy roots of *A. majus* proves to be an excellent model to investigate the regulatory mechanisms of anthocyanin biosynthesis and how anthocyanin synthesis can be regulated in the root by transcription factors AmRosea1 and AmDelila. Previous studies have demonstrated that the bHLH gene, AmDelila, and the R2R3-MYB gene, AmRosea1, AmRosea2 and AmVenosa are transcription factors and mainly involved in the control of anthocyanin biosynthesis in *A. majus* flowers (Goodrich et al. 1992; Schwinn et al. 2006; Shang et al. 2011). AmDelila results in loss of pigmentation only in the corolla tube, whereas AmRosea1 promotes strong, intense red corolla pigmentation in the adaxial and abaxial epidermis of flowers (Goodrich et al. 1992; Schwinn et al. 2006). In the present study, we have detected that
ectopic expression of *AmDelila* alone seems to be insufficient to affect the anthocyanin biosynthetic pathway, and therefore does not promote anthocyanin accumulation in transformed hairy roots of *A. majus* (Fig. 4A). In contrast, ectopic expression of *AmRosea1* alone clearly up-regulated expression of key structural target genes that are involved in the anthocyanin biosynthetic pathway (Fig. 6), thereby significantly promoting anthocyanin accumulation in transformed hairy roots (Fig. 4D, Additional file 2: Fig. S2). Similar results have been also detected in transformed hairy roots of both *Antirrhinum* and cotton when ectopic expression of *Rosea1* like R2R3-MYB gene *RLC1* (Gao et al. 2013). In this study, we also examined the levels of gene expression involved in the anthocyanin biosynthetic pathway and analysed anthocyanin accumulation from transformed roots of *A. majus* using *A. rhizogenes* AR1193/35S:ROSI. Although the anthocyanin content showed variations in the independent hairy roots, but still generate about 1.948, 1.959 and 2.064 mg/g FW high anthocyanin amounts were detected in the deep colour hairy roots (Fig. 5). This result suggests that hairy root induced by combination of *AmROS1* and *A. rhizogenes*-mediated transformation could prove to be an alternative approach for the production of anthocyanin compounds.

R2R3-MYB transcription factors are involved in regulation of tissue-specific anthocyanin accumulation in various plants, for example, *StAN1*, *StMYB1* and *StMYB113* in potato (Liu et al. 2016); *LhMYB6* and *LhMYB12* in lily (Yamagishi et al. 2010); *Rosea1* and *Venosa* in *A. majus* (Schwinn et al. 2006; Shang et al. 2011); and *AtMYB75* and *AtMYB90* in *A. thaliana* (Borevitz et al. 2000). Also, two adjacent R2R3-MYB genes, *VvMYBA1* and *VvMYBA2* control the skin colour from red to white in grapes (Walker et al. 2007). In this study, we found that the structural genes *AmCHS*, *AmDFR* and *AmANS* were greatly upregulated in the transformed hairy roots of

![Fig. 5](image)

**Fig. 5** Analysis of total anthocyanin concentrations from transformed hairy roots with AR1193/pBI35S:ROSI and a negative control hairy root transformed with AR1193/pBI121. Total anthocyanin was extracted from two pale colored hairy roots PRC1 (Fig. 4C) and PRC2 (Additional file 2: Fig. S2), three deep colored hairy roots DRC1 (Additional file 2: Fig. S2), DRC2 (Additional file 2: Fig. S2) and DRC3 (Fig. 4D) and a control hairy root P121 (Fig. 2B), respectively. The anthocyanin content was measured using a UV spectrometer. Means of three replicates with error bars indicating standard error (±SD)

![Fig. 6](image)

**Fig. 6** Comparison of the expression level of *AmCHS*, *AmF3H*, *AmDFR*, and *AmANS* between a negative control hairy root transformed with AR1193/pBI121 and five independent hairy roots transformed with AR1193/pBI35S:ROSI by semi-quantitative RT-PCR analysis. P121, A negative control hairy roots transformed with AR1193/pBI121 (Fig. 2B); PRC1, 2 and DRC1-3, Five hairy roots transformed with AR1193/pBI35S:ROSI (Fig. 4C, D and Additional file 2: Fig. S2A, B, C)
pBI35S:ROS1 (Fig. 6) and that these hairy roots showed notable anthocyanin accumulation (Fig. 4D, Additional file 2: Fig. S2). Moreover, the expression levels of an EBG gene AmCHS, and the LBG genes AmDFR and AmANS were harmony with expression of AmRosea1. Previous study had been demonstrated that the R2R3-MYB gene AmRosea1 mostly involved in regulating LBGs AmDFR and AmANS expression in flower of A. majus (Schwinn et al. 2006). Therefore, the elevated expression of AmCHS might be the response of the metabolite feedback phenomenon induced by the up-regulation of LBGs. These results indicate that the genetic basis of root colour in A. majus is probably due to the silence of a common regulator of the AmRosea1 or AmRosea1-like genes.

Several studies have reported that bHLH transcription factors like AmDelila constitute a group of regulatory genes involved in anthocyanin biosynthesis in plants (Goodrich et al. 1992; Xie et al. 2012). For instance, the insertion of a transposon in such a gene altered the flower tube color in A. majus (Goodrich et al. 1992) and altered flower color in the morning glory (Park et al. 2007). In A. thaliana, anthocyanin synthesis is controlled by three bHLHs AtTT8, AtGL3 and AtEGL3. The AtTT8 mutant has a low proanthocyanidins content in the pale-yellow seed coat and the expression of AtTT8 is highly correlated with the pigment content in the seed coat in Arabidopsis (Nesi et al. 2000). In apple, MdbHLH3 expression is in response to low temperature, and then, MdbHLH3 directly regulates the expression of MdMYB1. MdbHLH3 and MdMYB1 which then work together to activate anthocyanin biosynthesis (Xie et al. 2012). In the present study, we found that ectopic expression of AmDelila alone insufficient to affect the expression of the main endogenous anthocyanin synthesis genes, and therefore, the transformed hairy roots were showed no any coloration (Fig. 4A). These results suggest that expression of AmDelila alone does not stimulate the initiation of anthocyanin biosynthesis in roots. By contrary, expression of AmRosea1 alone was able to activate AmDFR and AmANS expression and promoted anthocyanin synthesis, which was similar with co-expression of both AmRosea1 and AmDelila (Fig. 3; Fig. 6), indicating that AmRosea1 may directly or indirectly affects expression of bHLH transcription factor AmDelila in the root. These results strongly suggest that AmRosea1 or an AmRosea1-like R2R3-MYB gene plays a more important role than the bHLH gene AmDelila in regulating the initiation of anthocyanin biosynthesis in the root of A. majus.

In the present study, we investigated the action of transcription factors AmRosea1 and AmDelila on anthocyanin synthesis in root of A. majus. Our results clearly show that AmRosea1 alone is able to activate AmDFR and AmANS gene expression to enhance anthocyanin accumulation in the transformed hairy roots. These results excitingly suggest that AmRosea1 is a useful tool to uniquely induce anthocyanins in the root of A. majus.

**Abbreviations**

AmDFR, dihydroflavonol 4-reductase; AmANS, anthocyanidin synthase; AmCHI, chalcone isomerase; AmF3H, flavanone-3′-hydroxylase; AmF3′H, flavonoid 3′-hydroxylase; AmCHS, chalcone synthase; AmPAL, phenylalanine ammonia lyase; AmANS, anthocyanidin synthase; UFGT, flavonoid-3-O-glucosyl-transferase; PGR, Polymerase chain reaction; RT-PCR, Reverse transcription-polymerase chain reaction; NAA, 1-Naphthaleneacetic acid; CTAB, Hexadecyl trimethyl ammonium bromide.

**Supplementary Information**

The online version contains supplementary material available at https://doi.org/10.1186/s13568-021-01286-6.

**Additional file 1: Figure S1.** A simplified anthocyanin biosynthetic pathway in Antirrhinum. AmPAL, phenylalanine ammonia lyase; AmCHS, chalcone synthase; AmCHI, chalcone isomerase; AmF3H, flavanone-3′-hydroxylase; AmF3′H, flavonoid 3′-hydroxylase; AmDFR, dihydroflavonol 4-reductase; AmANS, anthocyanidin synthase.

**Additional file 2: Figure S2.** Transformed hairy roots with 1193/pBI35S:AmROS1. A Pale-red colored hairy root (PRC2); B Deep-red colored hairy root (DRC1); C Deep-red colored hairy root (DRC2). Scale bar = 1 cm.

**Acknowledgements**

We thank Lucy Copsey and Professor Enrico Coen in John Innes Centre (United Kingdom) for providing the Antirrhinum majus seeds. Also thank Dr. Desmond Bradley in John Innes Centre for critical comments.

**Authors’ contributions**

MLC designed the experiments. CP carried out for transformation, CP and JW performed RT-PCR. MLC wrote the manuscript. All authors read and approved the final manuscript.

**Funding**

This work was supported by the Scientific Research Funds of Zhejiang A & F University (No: 2018FR004), and the Science and Technology Department of Ningxia (No: 2019BFG2011).

**Availability of data and materials**

All data generated or analysed during this study are included in this published article and its Additional file 1, 2.

**Code availability**

Not applicable.

**Declarations**

**Ethical approval and consent to participate**

This article does not contain any studies with human participants or animals performed by any of the authors.

**Consent of publication**

Not applicable.

**Competing interests**

The authors declare no competing interests.

**Author details**

1 College of Horticulture Science, Zhejiang A & F University, Linan 311300, China. 2 Institute of Virology and Biotechnology, Zhejiang Academy of Agricultural Sciences, Hangzhou 310021, China.
References

Ai TN, Naing AH, Anum M, Lim SH, Kim CK (2016) Sucrose-induced anthocyanin accumulation in vegetative tissue of Petunia plants requires anthocyanin regulatory transcription factors. Plant Sci 252:144–150

Albert NW, Davies KM, Lewis DH, Zhang H, Montefiori M, Brendolise C, Boase MR, Ngo H, Jameson PE, Schwinn KE (2014) A conserved network of transcriptional activators and repressors regulates anthocyanin pigmentation in eudicots. Plant Cell 26:962–980

Almeida J, Carpenter R, Robbins TP, Martin C, Coen ES (1989) Genetic interactions underlying flower colour patterns in Antirrhinum majus. Genes Dev 3:1758–1767

Boveitiz JO, Xia YJ, Blount J, Dixon RA, Lamb C (2000) Activation tagging identifies a conserved MYB regulator of phenylpropanoid biosynthesis. Plant Cell 12:2383–2393

Cui M, Takeyanagi K, Kamada H, Handa T (2001) Efficient shoot regeneration from hairy roots of Antirrhinum majus L. transformed by the rol type MAT vector system. Plant Cell Rep 20:55–59

Gao Z, Liu C, Zhang Y, Li Y, Ji H, Zhao X, Cui M (2013) The promoter structure and leucoanthocyanidin dioxygenase genes in Arabidopsis thaliana. Mol Genet Genomics 288:1–10

Ghorbani A (2017) Mechanisms of anti-diabetic effects of flavonoid rutin. Biomed Pharmacother 96:305–312

Gonzalez A, Zhao M, Leavitt JM, Lloyd AM (2008) Regulation of the anthocyanin biosynthetic pathway by the TIG/bHLH/MYb transcription factor complex in Arabidopsis seedlings. Plant J 53:814–827

Goodrich J, Carpenter R, Coen ES (1992) A common gene regulates pigmentation pattern in diverse plant species. Cell 68:955–964

Jeziorek M, Sykłowska-Baranek K, Pietrosiuk A (2018) Hairy root cultures for the production of anti-cancer naphthoquinone compounds. Curr Med Chem 25:4718–4739

Kimbara S, Itoh M, Sato T, Takada H, Kitao A, Kojima H, Inoue K, Akiyama K, Taga T (2017) Mechanisms of anti-diabetic effects of flavonoid rutin. Biomed Pharmacother 96:305–312

Kotapaska SP, Suplinska-Stefanska M, Domagalska M, Bialobok J, Sliwinska-Smoczyńska B (2021) Genetic control of anthocyanin accumulation in potato tubers. Plant Physiol Biochem 162:80–87

Kotapaska SP, Suplinska-Stefanska M, Domagalska M, Bialobok J, Sliwinska-Smoczyńska B (2021) Genetic control of anthocyanin accumulation in potato tubers. Plant Physiol Biochem 162:80–87

Kotapaska SP, Suplinska-Stefanska M, Domagalska M, Bialobok J, Sliwinska-Smoczyńska B (2021) Genetic control of anthocyanin accumulation in potato tubers. Plant Physiol Biochem 162:80–87

Kotapaska SP, Suplinska-Stefanska M, Domagalska M, Bialobok J, Sliwinska-Smoczyńska B (2021) Genetic control of anthocyanin accumulation in potato tubers. Plant Physiol Biochem 162:80–87

Kotapaska SP, Suplinska-Stefanska M, Domagalska M, Bialobok J, Sliwinska-Smoczyńska B (2021) Genetic control of anthocyanin accumulation in potato tubers. Plant Physiol Biochem 162:80–87

Kotapaska SP, Suplinska-Stefanska M, Domagalska M, Bialobok J, Sliwinska-Smoczyńska B (2021) Genetic control of anthocyanin accumulation in potato tubers. Plant Physiol Biochem 162:80–87

Kotapaska SP, Suplinska-Stefanska M, Domagalska M, Bialobok J, Sliwinska-Smoczyńska B (2021) Genetic control of anthocyanin accumulation in potato tubers. Plant Physiol Biochem 162:80–87

Kotapaska SP, Suplinska-Stefanska M, Domagalska M, Bialobok J, Sliwinska-Smoczyńska B (2021) Genetic control of anthocyanin accumulation in potato tubers. Plant Physiol Biochem 162:80–87

Kotapaska SP, Suplinska-Stefanska M, Domagalska M, Bialobok J, Sliwinska-Smoczyńska B (2021) Genetic control of anthocyanin accumulation in potato tubers. Plant Physiol Biochem 162:80–87

Kotapaska SP, Suplinska-Stefanska M, Domagalska M, Bialobok J, Sliwinska-Smoczyńska B (2021) Genetic control of anthocyanin accumulation in potato tubers. Plant Physiol Biochem 162:80–87

Kotapaska SP, Suplinska-Stefanska M, Domagalska M, Bialobok J, Sliwinska-Smoczyńska B (2021) Genetic control of anthocyanin accumulation in potato tubers. Plant Physiol Biochem 162:80–87

Kotapaska SP, Suplinska-Stefanska M, Domagalska M, Bialobok J, Sliwinska-Smoczyńska B (2021) Genetic control of anthocyanin accumulation in potato tubers. Plant Physiol Biochem 162:80–87

Kotapaska SP, Suplinska-Stefanska M, Domagalska M, Bialobok J, Sliwinska-Smoczyńska B (2021) Genetic control of anthocyanin accumulation in potato tubers. Plant Physiol Biochem 162:80–87

Kotapaska SP, Suplinska-Stefanska M, Domagalska M, Bialobok J, Sliwinska-Smoczyńska B (2021) Genetic control of anthocyanin accumulation in potato tubers. Plant Physiol Biochem 162:80–87

Kotapaska SP, Suplinska-Stefanska M, Domagalska M, Bialobok J, Sliwinska-Smoczyńska B (2021) Genetic control of anthocyanin accumulation in potato tubers. Plant Physiol Biochem 162:80–87

Kotapaska SP, Suplinska-Stefanska M, Domagalska M, Bialobok J, Sliwinska-Smoczyńska B (2021) Genetic control of anthocyanin accumulation in potato tubers. Plant Physiol Biochem 162:80–87

Kotapaska SP, Suplinska-Stefanska M, Domagalska M, Bialobok J, Sliwinska-Smoczyńska B (2021) Genetic control of anthocyanin accumulation in potato tubers. Plant Physiol Biochem 162:80–87

Kotapaska SP, Suplinska-Stefanska M, Domagalska M, Bialobok J, Sliwinska-Smoczyńska B (2021) Genetic control of anthocyanin accumulation in potato tubers. Plant Physiol Biochem 162:80–87

Kotapaska SP, Suplinska-Stefanska M, Domagalska M, Bialobok J, Sliwinska-Smoczyńska B (2021) Genetic control of anthocyanin accumulation in potato tubers. Plant Physiol Biochem 162:80–87

Kotapaska SP, Suplinska-Stefanska M, Domagalska M, Bialobok J, Sliwinska-Smoczyńska B (2021) Genetic control of anthocyanin accumulation in potato tubers. Plant Physiol Biochem 162:80–87

Kotapaska SP, Suplinska-Stefanska M, Domagalska M, Bialobok J, Sliwinska-Smoczyńska B (2021) Genetic control of anthocyanin accumulation in potato tubers. Plant Physiol Biochem 162:80–87

Kotapaska SP, Suplinska-Stefanska M, Domagalska M, Bialobok J, Sliwinska-Smoczyńska B (2021) Genetic control of anthocyanin accumulation in potato tubers. Plant Physiol Biochem 162:80–87

Kotapaska SP, Suplinska-Stefanska M, Domagalska M, Bialobok J, Sliwinska-Smoczyńska B (2021) Genetic control of anthocyanin accumulation in potato tubers. Plant Physiol Biochem 162:80–87

Kotapaska SP, Suplinska-Stefanska M, Domagalska M, Bialobok J, Sliwinska-Smoczyńska B (2021) Genetic control of anthocyanin accumulation in potato tubers. Plant Physiol Biochem 162:80–87

Kotapaska SP, Suplinska-Stefanska M, Domagalska M, Bialobok J, Sliwinska-Smoczyńska B (2021) Genetic control of anthocyanin accumulation in potato tubers. Plant Physiol Biochem 162:80–87

Kotapaska SP, Suplinska-Stefanska M, Domagalska M, Bialobok J, Sliwinska-Smoczyńska B (2021) Genetic control of anthocyanin accumulation in potato tubers. Plant Physiol Biochem 162:80–87

Kotapaska SP, Suplinska-Stefanska M, Domagalska M, Bialobok J, Sliwinska-Smoczyńska B (2021) Genetic control of anthocyanin accumulation in potato tubers. Plant Physiol Biochem 162:80–87

Kotapaska SP, Suplinska-Stefanska M, Domagalska M, Bialobok J, Sliwinska-Smoczyńska B (2021) Genetic control of anthocyanin accumulation in potato tubers. Plant Physiol Biochem 162:80–87

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.