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Short Communication

Transposon-mediated mutation of CYP76AD3 affects betalain synthesis and produces variegated flowers in four o'clock (*Mirabilis jalapa*)

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ABSTRACT

The variegated flower colors of many plant species have been shown to result from the insertion or excision of transposable elements into genes that encode enzymes involved in anthocyanin synthesis. To date, however, it has not been established whether this phenomenon is responsible for the variegation produced by other pigments such as betalains. During betalain synthesis in red beet, the enzyme CYP76AD1 catalyzes the conversion of L-dihydroxyphenylalanine (DOPA) to *cyclo*-DOPA. RNA sequencing (RNA-seq) analysis indicated that the homologous gene in four o'clock (*Mirabilis jalapa*) is *CYP76AD3*. Here, we show that in four o'clock with red perianths, the *CYP76AD3* gene consists of one intron and two exons; however, in a mutant with a perianth showing red variegation on a yellow background, a transposable element, *dTmj1*, had been excised from the intron. This is the first report that a transposition event affecting a gene encoding an enzyme for betalain synthesis can result in a variegated flower phenotype.

Introduction

Flowering plants have three major classes of pigments in their flowers, namely, flavonoids, betalains and carotenoids (Tanaka et al., 2008). In most plants, the major orange, red, purple and blue colors in flowers are produced by anthocyanins, a class of flavonoids, while the yellow colors are produced by carotenoids and flavonoids (e.g. chalcones and aurones). However, in plants belonging to families in the order Caryophyllales (with the exception of the Caryophyllaceae and Molluginaceae), the red and yellow colors are produced by betalains, such as betacyanins and betaxanthins (Clement and Mabry, 1996; Strack et al., 2003). The biosynthetic pathways for betalains have not yet been fully elucidated, although enzymes catalyzing some of the reaction steps have been identified (Fig. 1) (Christinet et al., 2004; Sasaki et al., 2004, 2005, 2009; Hatlestad et al., 2012; Gandía-Herrero and García-Carmona, 2013). Recently, a critical step was identified in red beet (*Beta vulgaris*) in which CYP76AD1 catalyzes synthesis of *cyclo*-DOPA from L-DOPA (Hatlestad et al., 2012). However, the enzyme catalyzing L-tyrosine to L-DOPA (step I, Fig. 1) is not yet known.

Four o'clock (*Mirabilis jalapa*) has three basic flowers colors, namely red, yellow and white; however, some flowers can show variegation with red sectors or dots on a yellow or white background, or yellow sectors or dots on a white background. The enzyme that mediates *cyclo*-DOPA glucosylation (Step V in Fig. 1) was recently identified and its cDNA isolated (Sasaki et al., 2004, 2005). Additionally, the enzyme L-DOPA 4,5-dioxygenase (DOD) (step III in Fig. 1) was identified in *Portulaca grandiflora* using a reverse genetics approach (Christinet et al., 2004) and, subsequently, we characterized its biochemical properties using a recombinant protein derived from four o'clock DOD cDNA (Sasaki et al., 2009). Hatlestad et al. (2012) predicted that *CYP76AD3* was the four o'clock ortholog of *CYP76AD1* in red beet for synthesis of *cyclo*-DOPA from L-DOPA (Step II in Fig. 1). Currently, however, there is no genetic or biochemical evidence to confirm this prediction.

Variegated flower colors, in which colored sectors and dots appear on a differently colored background, may be the product

Abbreviations: DOD, L-DOPA 4,5-dioxygenase; L-DOPA, L-dihydroxyphenylalanine; ORF, open reading frame; PDA, photodiode array.

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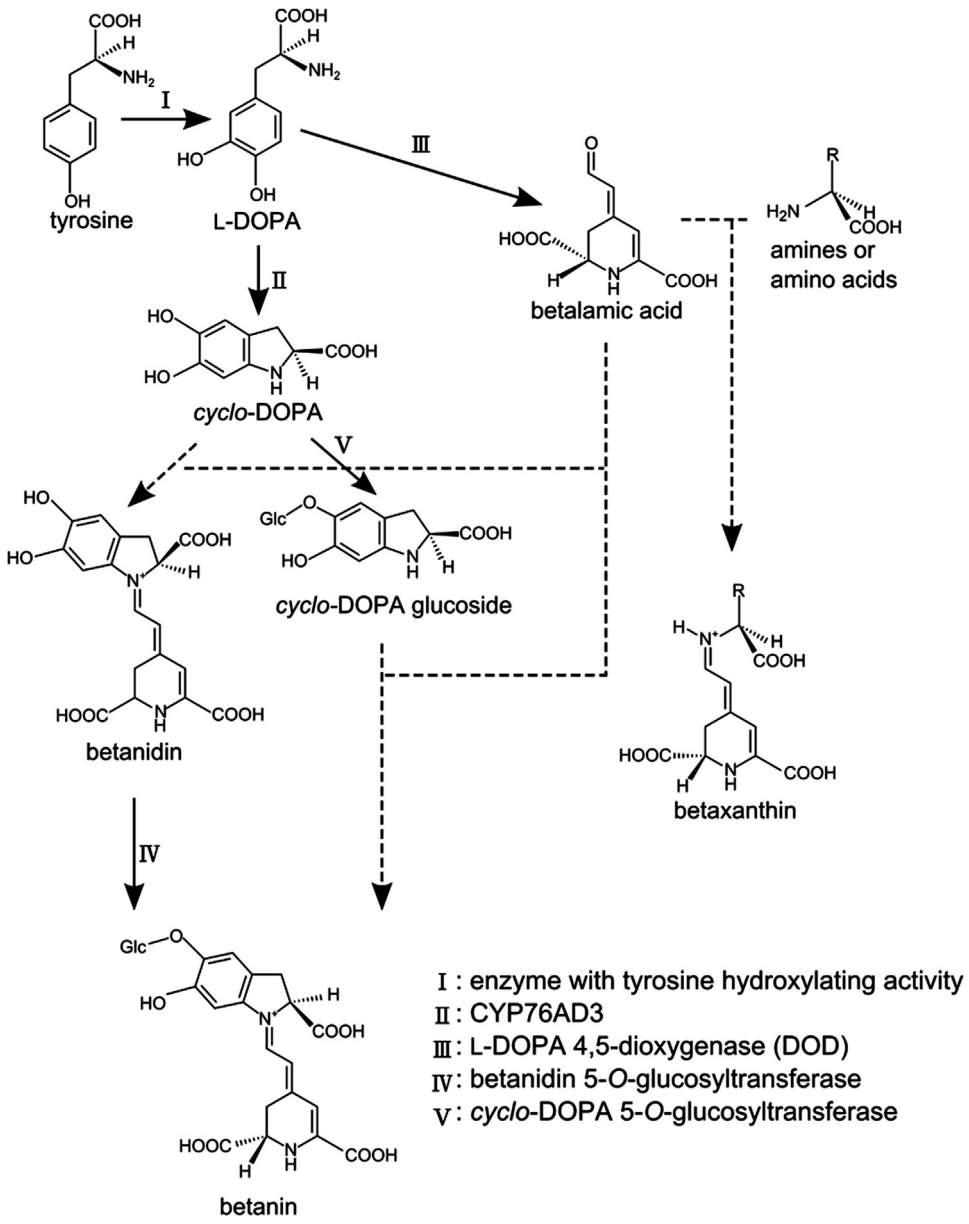

Fig. 1. Schematic outline of the likely biosynthetic pathways for betalains. Reactions indicated by solid lines have been suggested to be mediated by enzymes, those indicated by dotted lines are expected to occur spontaneously *in vivo*.

of mutable genes. Transposable elements, especially mobile class II DNA transposable elements, have been found to disrupt genes encoding enzymes, transcription regulatory factors and transporters involved in anthocyanin synthesis in several plant species (e.g., Iida et al., 2004). To date, however, transposable element mediated gene mutation has not been reported for betalain synthesis. Variegated flowers occur commonly in four o'clock (Fig. 2A), but the mechanism that generates this phenotype has not been elucidated. One possibility might be transposon-mediated mutation of genes for betalain synthesis similar to that described for anthocyanins.

Here, we isolated the *CYP76AD3* gene from four o'clock plants and compared its genomic structure in red and yellow perianth phenotypes ('R' and 'Y', respectively) to that in a variegated perianth phenotype ('R/Y') to determine whether there was any identifiable genetic alteration associated with the generation of variegated phenotypes.

Materials and methods

Plant materials

Four o'clock (*Mirabilis jalapa* L.) plants bearing red or yellow flowers (Fig. 2A), abbreviated as 'R' and 'Y' plant lines in this report, were collected in the yard of Tokyo University of Agriculture and Technology (Koganei, Tokyo, Japan); a plant bearing variegated flowers with red sectors or dots on a yellow background, abbreviated as the 'R/Y' line, was purchased from a local flower shop (Fig. 2A). Perianths were collected from 'R' plants and assigned to one of the five developmental stages: stage 1, perianths that were 7 or 8 d before flower opening, usually a greenish color without any indication of red, and less than 10 mm in length; stage 2, perianths that were 5 or 6 d before flower opening, with a clear red coloration of the top, and 10–15 mm in length; stage 3, perianths 3 or 4 d before flower opening, with a length of 15–20 mm and