

SHORT COMMUNICATION

New perspective of the bHLH-MYB complex in jasmonate-regulated plant fertility in arabidopsis

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ABSTRACT

Jasmonates (JAs) are a class of plant hormones, essential in plant development and defense. JA induces the interaction of the JA receptor Coronatine Insensitive 1 with jasmonate ZIM-domain (JAZ) proteins, and promotes subsequent JAZs degradation, leading to the release of downstream factors and activation of diverse plant development and defense processes. We recently revealed that the IIIe bHLH transcription factors MYC2, MYC3, MYC4 and MYC5 interact with the MYB transcription factors MYB21 and MYB24 to form the bHLH-MYB complex, and JAZs repress the bHLH-MYB complex to regulate JA-mediated stamen development. Here, we further discuss the different properties of the components of the bHLH-MYB complex in expression pattern and stamen regulation.

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The phytohormone jasmonates (JAs) are oxylipin signaling molecules, participating in the control of various aspects of the plant life, such as plant fertility,^{1,2,3} anthocyanin accumulation,^{4,5} root growth,^{6,7,8} fruit ripening⁹ and leaf senescence.^{10,11} They also act as defense signals to mediate plant responses against abiotic and biotic stresses.^{12,13,14,15} Upon exogenous or endogenous JA induction, the JA receptor protein Coronatine Insensitive 1 interacts with and triggers the degradation of the jasmonate ZIM-domain (JAZ) proteins, which interact with and repress diverse downstream transcription factors to inhibit multiple aspects of JA responses.¹⁶⁻²²

The R2R3-MYB transcription factors MYB21 and MYB24 are essential for floral organ development, including petal expansion, stamen development and gynoecium growth, and secondary metabolism in flower.²³⁻²⁶ We previously showed that JAZ proteins interact with MYB21 and MYB24 to regulate JA-mediated stamen development, including filament elongation, anther dehiscence and pollen maturation.²⁴ Recently we reported that the IIIe bHLH transcription factors MYC2, MYC3, MYC4 and MYC5, which are also targets of JAZs,^{6,7,8,27} function redundantly in promoting stamen development and plant fertility, and regulate JA-mediated stamen development and fertility by forming the bHLH-MYB transcription complex with MYB21 and MYB24.²⁷ Here, we focus on investigating the variant characters of the MYB and bHLH factors in the bHLH-MYB complex.

Microarray expression data and histochemical staining analysis have shown that the MYB components (MYB21 and MYB24) of the bHLH-MYB complex are expressed in sepals, petals, stamens and carpels,^{26,28} supporting their roles in the regulation of floral organ development and secondary metabolism (e.g. sesquiterpene synthesis).²³⁻²⁶ Here, we performed quantitative real-time PCR assays to analyze the expression of the bHLH components of the bHLH-MYB complex in the sepal, petal, stamen and carpel of flower. Expressions of MYC2, MYC3, MYC4 and MYC5 were all detected in stamens (Fig. 1), supporting their role in stamen development and plant fertility.^{25,26,27} Furthermore, we found that MYC2 and MYC3 exhibited high expression level in all four floral organs (Fig. 1A, 1B and 1D), while MYC4 and MYC5 were preferentially expressed in sepals or carpels respectively (Fig. 1C). The expressions of the MYC genes in floral organs indicate that they might also control the development of other flower organs and floral secondary metabolism, which remain to be elucidated. MYC2 has been shown to regulate sesquiterpene synthesis in flower.²⁹

Mutation, inhibition and overexpression of MYB21 and MYB24 obviously inhibit stamen development, demonstrating that proper stamen development requires the appropriate expression level of MYB21 and MYB24.²⁴ Our recent study demonstrated that the malfunction of MYC2, MYC3, MYC4, and MYC5 delays stamen development and reduces fertility.²⁷ Now, we generated MYC2, MYC3, MYC4, and

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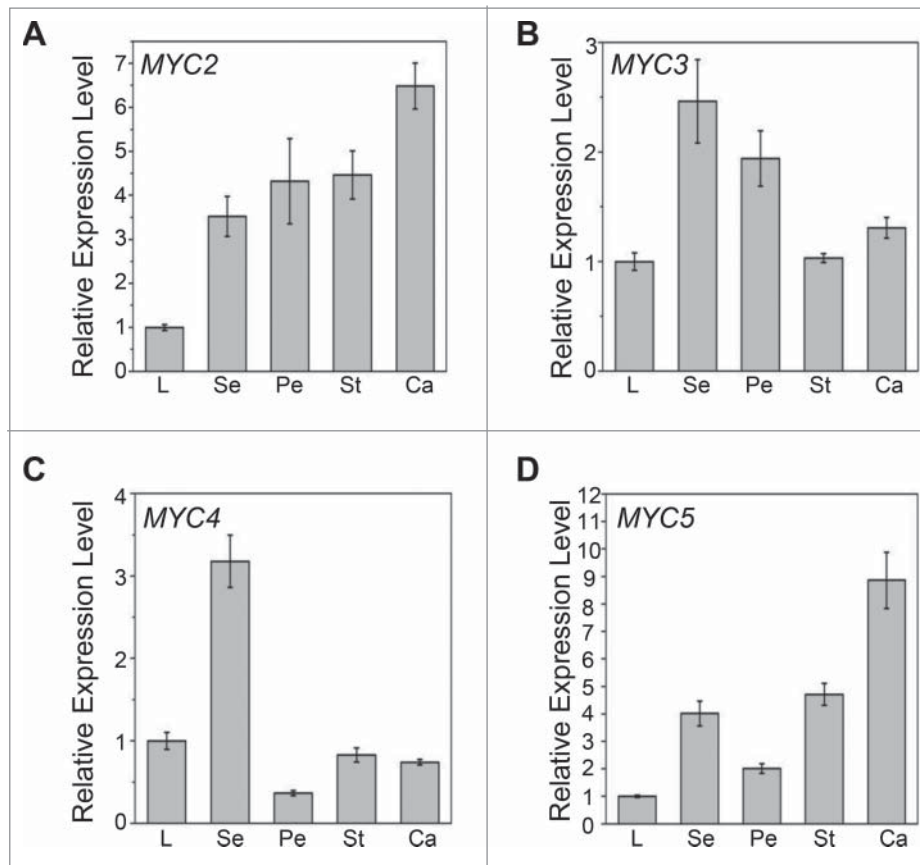


Figure 1. Expression Levels of *MYC2*, *MYC3*, *MYC4* and *MYC5* in Floral Organs. (A–D) Quantitative real-time PCR analyses of *MYC2* (A), *MYC3* (B), *MYC4* (C), and *MYC5* (D) in leaves and floral organs of 8-week-old Col-0 wild type using *ACT1N8* as the internal control. L, leaf; Se, sepal; Pe, petal; St, stamen; Ca, carpel. Each value is the mean (\pm SE) of 3 biological replicates.

MYC5 overexpressing transgenic plants to investigate whether overexpression of these bHLH factors represses stamen development. Among all the transgenic lines of every gene (101, 68, 71, and 87 overexpressing transgenic lines respectively for *MYC2*, *MYC3*, *MYC4*, and *MYC5*), none of them showed altered stamen development or plant fertility (2 representative overexpression lines for each gene are shown in Fig. 2), suggesting that overexpression of *MYC2*, *MYC3*, *MYC4*, or *MYC5* does not affect stamen development, which is distinct from that of *MYB21* and *MYB24*.

JA regulates the expression of the bHLH and MYB components of the bHLH-MYB complex.^{7,25,26} The transcript levels of *MYB21*, *MYB24*, and *MYC2* increased rapidly and significantly to about 20-, 30-, and 16-fold in response to JA treatment,²⁵ whereas the expression of *MYC3* and *MYC4* were only mildly (2 fold) induced by JA.⁷ Here, we found that the expression of *MYC5* cannot be elevated by JA in wild type (Fig. 3), suggesting *MYC5* and the other components of the bHLH-MYB complex exhibit different JA-induced expression patterns.

The MYB factors (*MYB21* and *MYB24*) and bHLH factors (*MYC2*, *MYC3*, *MYC4* and *MYC5*) form the

bHLH-MYB complex to control JA-mediated stamen development.^{24,27} Here we noticed that studies have revealed differences existing among these factors: 1) *MYC2* and *MYC3* exhibit high expression levels in all four floral organs, while *MYC4* and *MYC5* preferentially express in sepals or carpels, respectively; 2) Overexpression of MYB factors inhibits stamen development, while overexpression of these bHLH factors cannot influence stamen development; 3) The expression of *MYB21*, *MYB24*, *MYC2*, *MYC3* and *MYC4* can be either significantly or mildly induced by JA, whereas *MYC5* cannot. So far, *MYB21* and *MYB24* have been reported to mainly function in the flower, such as in floral organ development and floral sesquiterpene biosynthesis, while *MYC2*, *MYC3*, and *MYC4* regulate diverse plant responses, including root growth, leaf senescence, secondary metabolic processes, and plant defense responses, as well as stamen development. As these MYB and bHLH factors share common functions and exhibit differences at the same time, it will be interesting to investigate what other plant responses the bHLH-MYB complex regulates besides stamen development, and what other plant responses the bHLH and MYB factors separately control, and how.

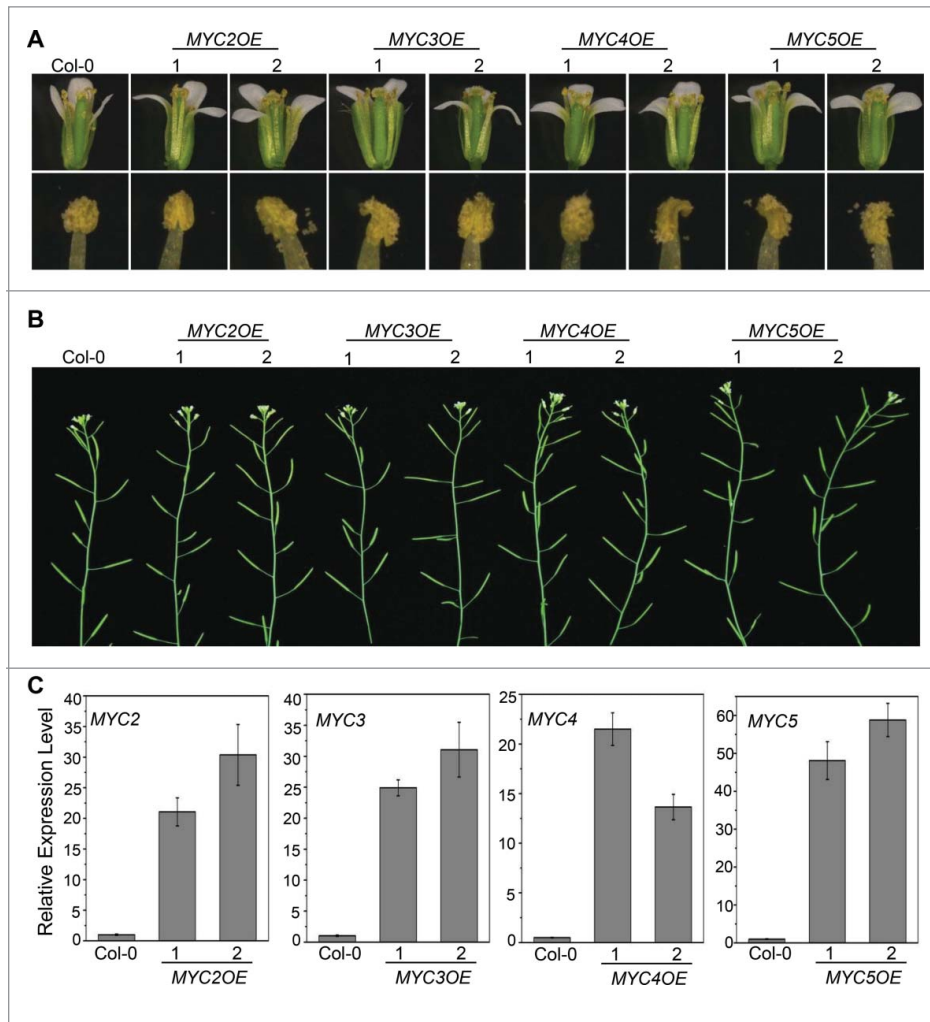


Figure 2. Overexpression of *MYC2*, *MYC3*, *MYC4* and *MYC5* Cannot Affect Plant Fertility. (A) Comparison of flowers (top) and anther dehiscence (bottom) at floral stage 13 from Col-0 wild type, two transgenic lines respectively for *MYC2* (*MYC2OE1* and *MYC2OE2*), *MYC3* (*MYC3OE1* and *MYC3OE2*), *MYC4* (*MYC4OE1* and *MYC4OE2*), and *MYC5* (*MYC5OE1* and *MYC5OE2*). (B) The main inflorescences of the indicated genotypes. (C) Quantitative real-time PCR analyses of *MYC2*, *MYC3*, *MYC4* or *MYC5* in 3-week-old plants of the indicated genotypes using *ACTIN8* as the internal control. Each value is the mean (\pm SE) of three biological replicates.

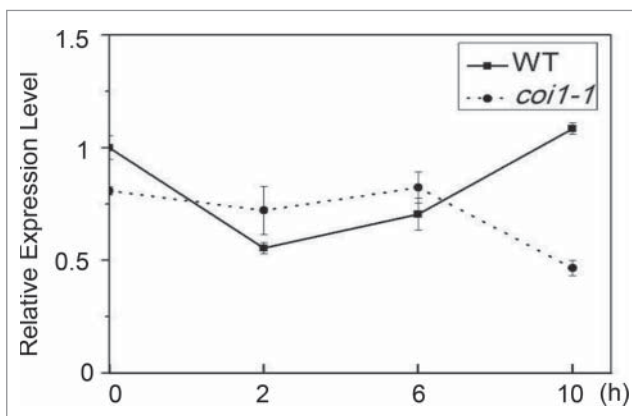


Figure 3. JA Treatment Cannot Induce *MYC5* Expression in *Arabidopsis*. Quantitative real-time PCR analysis of *MYC5* expression level using *ACTIN8* as the internal control. After JA treatment, *MYC5* expression was not significantly raised in WT compared to that in *coi1-1*, a JA receptor mutant. Each value is the mean (\pm SE) of three biological replicates.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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