Studies on the biosynthetic enzymes of abscisic acid toward its fermentation production

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Research aims

Abscisic acid (ABA, **1**) is a sesquiterpene phytohormone that regulates physiological functions in plants as well as in a wide range of organisms from bacteria to human cells. For example, **1** mediates adaptive responses toward abiotic stresses such as drought stress and salt stress in plants, as well as temperature-induced oxygene consumption and water filtration in sponges. In a previous study, we successfully elucidated the biosynthetic pathway of **1** by through the heterologous expression of four ABA biosynthetic genes (*bcABA1-4*) in *Aspergillus oryzae* (**Fig 1**). [1, 2] Efficient production of **1** (68 mg/kg) in the transformant revealed that cyclization of farnesyl diphosphate (FPP) by the action of BcABA3 can be a bottleneck in the metabolic pathway, strongly suggesting that improved catalytic activity of BcABA3 would enable ABA production with a more efficient yield. Considering this hypothesis, in this study, we performed 1) re-analysis of the metabolic profile in the transformant and 2) a kinetics study of BcABA3.

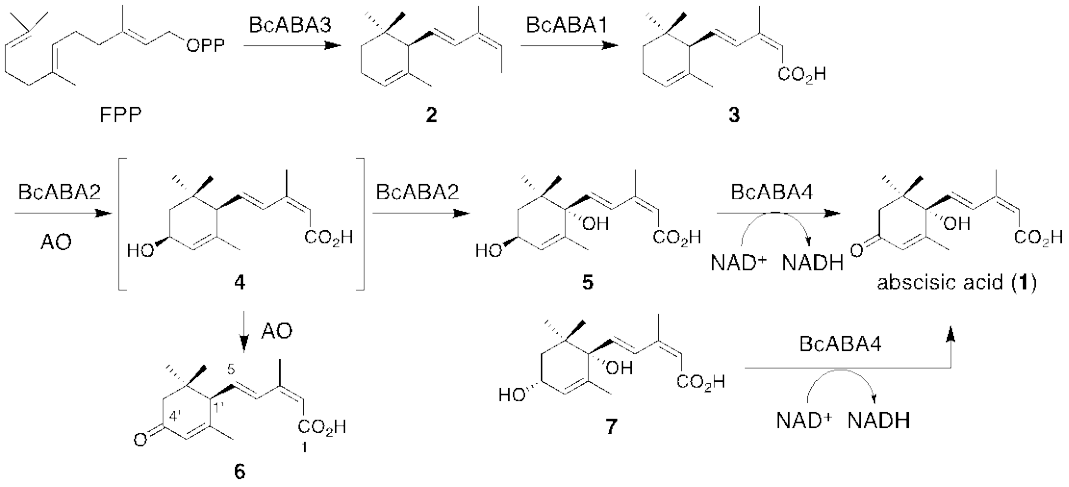

Figure 1. Proposed biosynthetic pathway of ABA in fungi

Methods

1) Re-examination of the metabolic profile in the transformant

The previously decribed transformant harboring four *bcABA* genes was constructed using traditional methods via non-homologous end joining. In this case, the *bcABA* genes were integrated into random positions in the chromosome, necessitating the search for

positive clones producing ABA. Further, the production yield as well as the metabolic profiles of each positive clone were different.

To obtain a reliable result especially focusing on the metabolic profile in the transformant, I decided to re-construct the transformant using an established hot spot knock-in method. In this method, the introduced genes are highly expressed in the transformant because they locate at recently identified hot spot loci, which guarantees their efficient expression. The metabolites produced by the new transformant were analyzed by UPLC-MS.

2) Kinetic study of BcABA3

BcABA3 catalyzes the cyclization of FPP to afford a cyclization product **2** via two neutral intermediates, β-farnesene and allofarnesene (**Figure 2**). To avoid complicated discussion, we quantified **2** to determine the optimal pH, temperature, metal ion requirement, and kinetic parameters of BcABA3.

Results

1) Re-examination of the metabolic profile in the transformant

Applying the established method, we constructed the expected transformant with 70% efficiency. Metabolite analysis revealed that the transformant only produced 228 mg/kg of ABA. The fact that no intermediates such as **2–5** were observed in the metabolites indicated that, as expected, cyclization is a bottleneck in the ABA biosynthetic pathway.

2) Kinetic study of BcABA3

The highest BcABA3 activity with FPP was observed with 50 mM HEPES buffer, pH 8.0, at 30°C. The Mg^{2+} cation (10 mM) was effective for BcABA3 activity. These requirements are almost the same as those of the traditional sesquiterpene cyclase. The kinetic parameters are as follows: Km = 3.2 mM and kcat = 6.7×10^{-2} s^{-1}.

Conclusion

In this study, we constructed an ABA-producing transformant by harnessing the four *bcABA* genes in *A. oryzae*. Efficient production of ABA revealed that BcABA3 catalyzed cyclization is a limiting step in ABA biosynthesis. Kinetic study of BcABA3 revealed the Km and kcat values under the optimal conditions. Thus, fine-tuning of the BcABA3 function based on these kinetic studies will allow efficient production of ABA in *A. oryzae*.

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References

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2） Takino, J., Kozaki, T., Ozaki, T., Liu, C., Minami, A., and Oikawa, H. (2019) Elucidation of biosynthetic pathway of a plant hormone abscisic acid in phytopathogenic fungi. *Biosci. Biotechnol. Biochem.* **83:** 1642-1649.