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[P005] Poster

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[P03-447] Impact of the *HISE1* mutation on the heterologous triterpenoids production in Arabidopsis

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[Purpose]
The mevalonate pathway provides precursors for terpenes and sterols, with HMG-CoA reductase (HMGR) serving as a key enzyme. In the leaves of the *Arabidopsis thaliana* high sterol ester 1 mutant (*hise1*), which shows abnormal sterol biosynthesis, HMGR levels are over 100 times higher than in the wild type. These results indicate that HIGHSTEROL ESTER 1 (HISE1) negatively regulates HMGR protein levels and helps maintain sterol balance. The mevalonate pathway is involved not only in sterol biosynthesis but also in producing valuable triterpenoids with potential pharmaceutical uses. Therefore, introducing HISE1 mutations is likely to increase triterpenoid production. In this study, we examined whether *hise1* mutants can serve as effective hosts for heterologous triterpenoid synthesis.

[Method]
As a model of heterologous terpenoid biosynthesis, a part of the pathway for glycyrrhizin, a sweet triterpenoid saponin found in licorice roots, was used. The genes encoding β-amyrin synthase (bAS) and β-amyrin 11-oxidase (CYP88D6) were introduced and expressed under the CaMV35S promoter in both wild-type and *hise1* mutant Arabidopsis. The levels of intermediates, β-amyrin and 11-oxo-β-amyrin, in leaves were quantified using GC-MS. Additionally, the levels of phytosterols (campesterol, stigmasterol, β-sitosterol, and cycloartenol) were also analyzed.

[Results]
Two T-DNA insertion mutants (*hise1-2* and *hise1-3*) were used as the *hise1* Arabidopsis lines. As a result, both mutant backgrounds accumulated nearly eight times more β-amyrin than the wild-type background. Additionally, the mutant backgrounds showed a five-fold increase in 11-oxo-β-amyrin accumulation. Stigmasterol and cycloartenol were not detected in any lines. Campesterol and β-sitosterol levels were similar between the wild-type and *hise1-3* backgrounds. However, the *hise1-2* background strains accumulated significantly higher levels of these two phytosterols compared to the wild-type.

[Consideration]
These findings indicate that *hise1* mutants have potential as hosts for terpene production. However, further analysis of the expression levels of the introduced genes, *bAS* and *CYP88D6*, will be necessary to better demonstrate how the HISE1 mutation contributes to the overproduction of intermediates.

[Conclusion]
hise1 mutants may serve as a useful platform for boosting heterologous triterpenoid production. This method could also be applied to other plant species, such as *Nicotiana benthamiana*, as a platform for producing heterologous triterpenoids through transient

expression of pathway enzymes via agroinfiltration.

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