Medical & healthcare, Functional foods, Industrial materials

High-yield bioactive triterpenoid production by heterologous expression of biosynthetic genes in *Nicotiana benthamiana*

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Abstract

Maslinic acid is a natural triterpenoid contained in olive fruit. It has been reported that maslinic acid is very effective in improving locomotive ability of old subjects in a clinical test. Transient protein expression in *Nicotiana benthamiana* leaves via agroinfiltration is a simpler and flexible method for producing high levels of recombinant proteins. In this study, maslinic acid was produced in *N. benthamiana* leaves by transiently introducing five biosynthetic enzyme genes for maslinic acid, yielding 20.7 times higher concentration than that in olive fruit (cv. Kalamata), which have been reported to contain high maslinic acid concentration. This study established a general strategy for rapidly producing substantial quantities of useful triterpenoids.

Background & Results

Triterpenoids are a class of structurally diverse plant specialized (secondary) metabolites commonly derived from 2,3-oxidosqualene (the precursor of sterols, steroids, and triterpenoids). These compounds have been shown to have various health beneficial properties. Maslinic acid contained in olive fruits is very effective in improving locomotive ability of elderly people. Supplement products containing maslinic acid were accepted by the Consumer Affairs Agency as "Foods with Functional Claims" in Japan.

The plant *N. benthamiana* is used as a heterologous expression host for rapid and high-level recombinant protein production by agroinfiltration-based transient transformation. The Tsukuba system is one of the most powerful transient protein expression systems utilizing agroinfiltration, based on the use of a geminiviral replication vector for boosting expression. In this study, we used Tsukuba system to produce maslinic acid by transiently expressing five biosynthetic enzymes of maslinic acid. Using this system, we demonstrate that maslinic acid was produced in *N. benthamiana* leaves, yielding 27.2 ± 3.0 mg/g dw at 7 days after agroinfiltration. This concentration is 20.7 times higher than that in olive fruit (cv. Kalamata), which have been reported to contain high maslinic acid concentration. In conclusion, this study established a framework for optimizing triterpenoids production via agrobacterium-mediated transient expression in *N. benthamiana* using the Tsukuba system.

Significance of the research and Future perspective

The accumulation levels of triterpenoids in raw plant materials are sometimes very low and may also depend on the age of the plant. In this study, we produced substantial amounts of maslinic acid, as an example of a health-beneficial plant-derived triterpenoid, by transiently transformed *N. benthamiana* leaves in a week. This research will contribute to the development of a more efficient platform for synthesizing useful triterpenoids and sustainable use of plant resources.

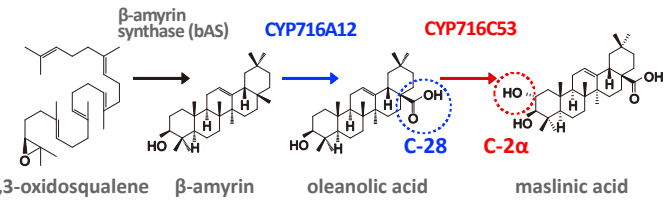


Figure 1 Engineered biosynthetic pathway of maslinic acid constructed in *Nicotiana benthamiana* leaves

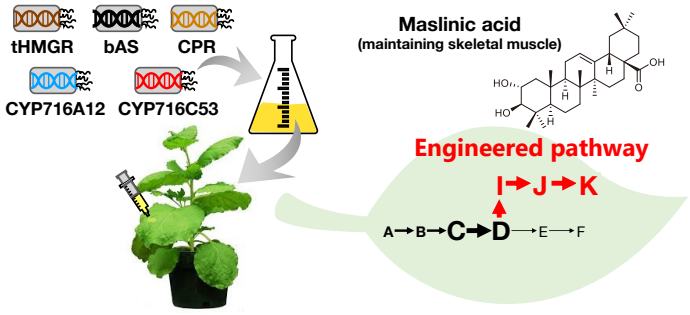
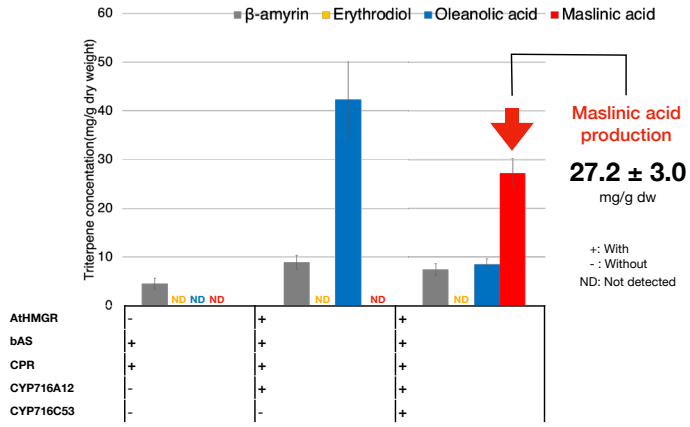


Figure 2 Schematic representation of the agroinfiltration process to construct engineered pathway of maslinic acid in *N. benthamiana* leaves



Supplementary Figure Schematic representation of the agroinfiltration process to construct engineered pathway of maslinic acid in *N. benthamiana* leaves

Patent

Treatise
Romsuk, Jutapat; Yasumoto, Shuhei; Fukushima, Ery Odette et al. High-yield bioactive triterpenoid production by heterologous expression in Nicotiana benthamiana using the Tsukuba system. Frontiers in Plant Science. 2022, 13, 991909. doi: 10.3389/fpls.2022.991909
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