

Isolation and characterization of linalyl UGTs from *Coffea arabica*

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Introduction

Linalool, is one of the monoterpene, is important aroma compound in coffee. Linalool is accumulated as glycoside in coffee plants¹⁾. These glycosides are catalyzed by UDP-glycosyltransferase (UGT) and Glycoside-specific glycosyltransferase (GGT) (Fig. 1).

To reveal the accumulation system of aroma compounds in coffee, we identified and analyzed *UGT* genes from *C. arabica*.

Materials and Methods

Total RNA extraction

From leaves, flower buds and fruits of *C. arabica* L...

In silico screening

Based on the sequence of *UGT85K11*²⁾ from tea. Five candidate genes were selected.

Gene cloning

RT-PCR and subcloning were conducted. Recombinant enzymes were produced.

Recombinant enzymatic assay

The transfer of glucose to the substrate was detected as radioactivity TLC assay. LC-ESI-MS/MS analysis was conducted to identify for UGT85A192 product.

Gene expression analysis

RNA extracts were used for Northern blot analysis.

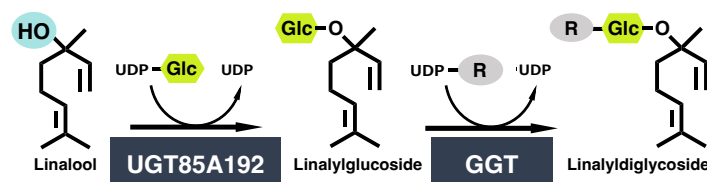
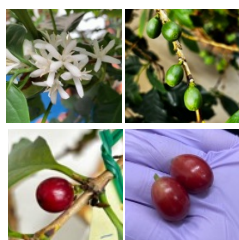


Fig. 1. Glycosylation of linalool

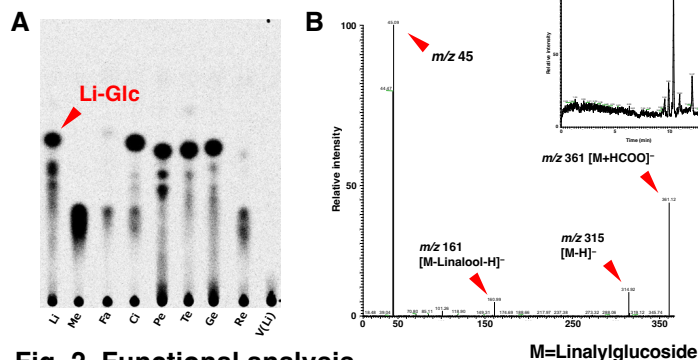


Fig. 2. Functional analysis

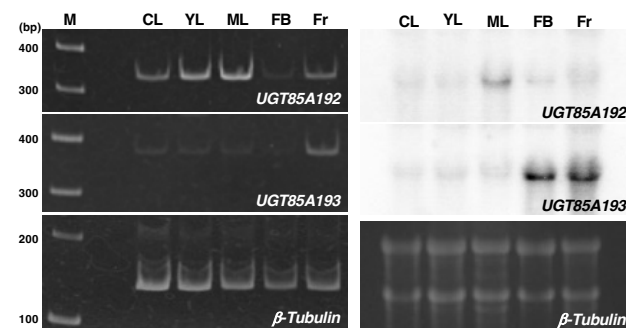


Fig. 3. Gene expression analysis of *CaUGTs*

Results and Discussion

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- UGT85A192 and UGT85A193 could catalyze glycosylation of linalool, citronellol, perillyl alcohol, terpineol, and geraniol. In the TLC assay of UGT85A192, a clear signal of the linalyl glucoside was observed (Fig. 2A).
- The LC-ESI-MS/MS analysis revealed that UGT85A192 showed exhibited signals similar to UGT85A84³⁾ that glycosylates linalool, which are thought to be derived from linalyl glucoside, and were detected (Fig.2B).
- The gene expression analysis results indicated that *UGT85A192* was expressed in the leaves, while *UGT-85A193* was found in the flower buds and fruits of coffee (Fig. 3).

Conclusion

This study suggests that UGT85A192 was initially isolated from coffee and has been identified as a glycosyltransferase, mainly producing linalyl glucoside. Now, to regulate the terpene glycosylation, we are analyzing these mutated enzymes.

These results were published in 2024.

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