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Metabolic engineering of transgenic spearmint (*Mentha spicata*) through limonene synthase gene for useful terpenoids production

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Objectives

Mint plants belong to the Labiatae family are widely cultivated for their essential oil. This valuable product is mainly composed of monoterpenes and is largely used in the production of food, cosmetics and pharmaceuticals. Metabolic engineering can lead to increased yields of theses compounds and spearmint could be considered as a model plant because monoterpene pathways are particularly well known in the plant. Using a reliable regeneration system previously set up with spearmint. It would be interesting to extend transformation model system to other plant species. Therefore, we have obtained, transgenic *M. spicata* by *Agrobacterium tumefaciens* mediated limonene synthase gene for useful terpenoids production.

Materials and Methods

1. Materials: Plant- Spearmint was provided by Agricultural farm of GyeongSang National University
Bacteria strain- *Agrobacterium tumefaciens* GV3101
Expression vector: pBIlimo (pBI121+Limonene synthase gene in *citrus limo),* Accession n.: AF514287.
2. Methods: Regeneration system: MS medium including with BA 1.0 ppm
Confirmation of limonene synthase gene: putative transgenic plants by a CTAB based method
PCR analysis and Southern blotting
Yield of essential oil (%): Volume of essential oil (ml) / Weight of oven-dry sample (g)
GC-MS analysis: HP 5890 Series (60 mm × 0.25 mm × 0.25 ㎛) GC, HP-1 column

Results and Discussion

We made transgenic 1, 3, 4 lines through pBIlimo expression vector with *Agrobacterium tumefaciens.* After a co-cultivation of 1 day, the regeneration of plants occurred on the condition of MS medium with BA 1.0 ppm and kanamycin 200 ppm, cefotaxime 250 ppm. Concerning the plant transformation of *M. spicata* gives evidence that a method to transform with limonene synthase gene coding for enzymes involved in the monoterpene metabolism in this plant is now available. In the three transgenic lines analyzed by PCR, a 1.8 Kb fragment was amplified corresponding to the region between the limonene synthase gene primers. For Southern blot hybridization, a limonene synthase detection system was set up. Concerning the plant transformation of *M. spicata* gives evidence that a method to transform with limonene synthase gene coding for enzymes. This data was involved in the monoterpene metabolism by characterization of GC-MS after extraction of essential oil in the plants. So if we wish to understand the regulatory mechanisms of monoterpenes through the analysis of transgenic spearmint plants, we need to transform *M. spicata* belonging to different pathways of monoterpenes, such as other species that respectively accumulate carvone and menthol.

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