

Production of coumaroylserotonin and feruloylserotonin in Transgenic Rice Expressing Pepper Hydroxycinnamoyl-CoA:Serotonin *N*-(Hydroxycinnamoyl)transferase

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Objectives

Transgenic rice plants expressing a pepper THT gene were generated by transforming wild-type rice (*Oryza sativa* cv. Nakdong) with the binary vector pGA1611:THT (Fig. 1A).

Materials and Methods

1. Materials: Plant - rice (*Oryza sativa*).
2. Methods: *Agrobacterium* mediated transformation was utilized to generate transgenic rice plants. Scutellum derived rice calli (*Oryza sativa* cv Nakdong) were co-cultured with *Agrobacterium tumefaciens* LBA4404 harboring pGA1611:THT binary vector.

Results and Discussion

Transgenic rice plants were engineered to express pepper hydroxycinnamoyl-CoA:tyramine *N*-(hydroxycinnamoyl) transferase (THT), a key enzyme in the synthesis of *N*-(hydroxycinnamoyl)-amines, from a constitutive maize ubiquitin promoter. The transgenic rice plants require foliar application of amines to support synthesis of *N*-hydroxycinnamoylamines, suggestive of limiting amine substrates in rice cells. In addition, when T2 homozygous transgenic rice plants were grown in the presence of amines or phenolic acids, two novel compounds were identified in the leaves of the transgenic plants. These compounds eluted earlier than *p*-coumaroyltyramine and feruloyltyramine during HPLC chromatography and were identified as *p*-coumaroylserotonin and feruloylserotonin by LC/MS and other methods. To test whether the unpredicted production of serotonin derivatives is associated with the pepper THT, the substrate specificity of the pepper THT was analyzed again. Purified recombinant pepper THT exhibited a serotonin *N*-hydroxycinnamoyltransferase (SHT) activity, synthesized *p*-coumaroylserotonin and feruloylserotonin *in vitro*, and demonstrated a low *K_m* for serotonin. SHT activity was inhibited by 10 to 50 mM tyramine. Transgenic rice plants were capable of producing large quantities of these serotonin derivatives (48 g to 424 g per gram fresh tissue). This is the first report of SHT activity and the first demonstration that serotonin derivatives can be produced *in vivo* in transgenic rice plants that express SHT.