

“Enhancement of Flavonoid production through Genetic Modification in *Boesenbergia rotunda* cell suspension culture”

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Boesenbergia rotunda (*B. rotunda*), a medicinal ginger, is rich in bioactive flavonoids, including prenylated flavonoids and cyclohexenyl chalcone derivatives (CCDs), such as panduratin A. These flavonoids have been reported to exhibit beneficial effects on human health, such as antioxidant, antibacterial, antifungal, anti-inflammatory, antitumor, anti-tuberculosis and anti-dengue properties. Despite the potential of these compounds, the limited availability in nature continues to be a significant challenge. One of the key enzymes involved in flavonoid production is prenyltransferases (PTs). However, only a few flavonoid-related prenyltransferase genes have been identified so far. In this study, a 1,176 bp full-length cDNA of prenyltransferase (*BrPT2*) was isolated from *B. rotunda*. The isolated *BrPT2* was characterized before introduced into *B. rotunda* cell suspension cultures via *Agrobacterium*-mediated transformation. The deduced protein sequence of PT2 shared the highest gene sequence homology with the predicted homogentisate phytyltransferase 2 chloroplastic isoform X1 from *Musa acuminata* subsp. *Malaccensis*. The *BrPT2*-expressing *B. rotunda* cells were then fed with a substrate, pinostrobin chalcone, and their products were analyzed by a high performance liquid chromatography. About 0.3-fold increase of the potentially panduratin A was detected in *BrPT2*-expressing cells compared to the untransformed cells, suggesting the involvement of *BrPT2* in catalyzing panduratin A.

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