

## Functional characterization of a new terpene synthase from *Plectranthus amboinicus*

### ABSTRACT

*Plectranthus amboinicus* (Lour.) Spreng is an aromatic medicinal herb known for its therapeutic and nutritional properties attributed by the presence of monoterpene and sesquiterpene compounds. Up until now, research on terpenoid biosynthesis has focused on a few mint species with economic importance such as thyme and oregano, yet the terpene synthases responsible for monoterpene production in *P. amboinicus* have not been described. Here we report the isolation, heterologous expression and functional characterization of a terpene synthase involved in *P. amboinicus* terpenoid biosynthesis. A putative monoterpene synthase gene (PamTps1) from *P. amboinicus* was isolated with an open reading frame of 1797 bp encoding a predicted protein of 598 amino acids with molecular weight of 69.6 kDa. PamTps1 shares 60–70% amino acid sequence similarity with other known terpene synthases of Lamiaceae. The *in vitro* enzymatic activity of PamTps1 demonstrated the conversion of geranyl pyrophosphate and farnesyl pyrophosphate exclusively into linalool and nerolidol, respectively, and thus PamTps1 was classified as a linalool/nerolidol synthase. *In vivo* activity of PamTps1 in a recombinant *Escherichia coli* strain revealed production of linalool and nerolidol which correlated with its *in vitro* activity. This outcome validated the multi-substrate usage of this enzyme in producing linalool and nerolidol both in *in vivo* and *in vitro* systems. The transcript level of PamTps1 was prominent in the leaf during daytime as compared to the stem. Gas chromatography-mass spectrometry (GC-MS) and quantitative real-time PCR analyses showed that maximal linalool level was released during the daytime and lower at night following a diurnal circadian pattern which correlated with the PamTps1 expression pattern. The PamTps1 cloned herein provides a molecular basis for the terpenoid biosynthesis in this local herb that could be exploited for valuable production using metabolic engineering in both microbial and plant systems.