Isolation, cloning and characterisation of new fragrance-related floral transcripts of Vanda Mimi Palmer

ABSTRACT

A subtracted cDNA library of open flower was constructed using the suppression subtractive hybridization (SSH) technique, to identify fragrance-related transcripts of Vanda Mimi Palmer. In total, 107 transcripts up-regulated during blooming were identified and sequenced. Only 33 clones (4 singletons and 29 contigs) showed similarities to known sequences in the public database. Of these, thirty-two clones were transcripts encoding fragrance-related enzymes including sesquiterpene synthase, (±)-germacrene D synthase, tyrosine decarboxylase and putative acyltransferase. Two fragrance-related transcripts, VMPAAT encoding a putative alcohol acyltransferase and VMPSTS encoding a sesquiterpene synthase, were subjected to full-length cDNA isolation and characterization. The full length cDNA of VMPAAT has a 1343bp open reading frame (ORF) of 448 amino acid residues whereas VMPSTS is predicted to encode a polypeptide of 561 amino acid residues with 1682bp ORF. VMPAAT and VMPSTS show high homologies with plant alcohol acyltransferase and terpene synthase, respectively. Real time RT-PCR indicated that both transcripts were expressed preferentially in floral tissues, with high levels in blooming and full bloom flowers. VMPAAT and VMPSTS transcripts were expressed in a rhythmic pattern. The results presented in this study will be potentially useful in providing additional insights into the fragrance-related pathways of Orchidaceae members, which until today is still limited.

Keyword: Orchid; Expressed sequence tags; Suppression subtractive hybridization