

A bifunctional geranyl and geranylgeranyl diphosphate synthase is involved in terpene oleoresin formation in *Picea abies*.

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Abstract

The conifer *Picea abies* (Norway spruce) defends itself against herbivores and pathogens with a terpenoid-based oleoresin composed chiefly of monoterpenes (C(10)) and diterpenes (C(20)). An important group of enzymes in oleoresin biosynthesis are the short-chain isoprenyl diphosphate synthases that produce geranyl diphosphate (C(10)), farnesyl diphosphate (C(15)), and geranylgeranyl diphosphate (C(20)) as precursors of different terpenoid classes. We isolated a gene from *P. abies* via a homology-based polymerase chain reaction approach that encodes a short-chain isoprenyl diphosphate synthase making an unusual mixture of two products, geranyl diphosphate (C(10)) and geranylgeranyl diphosphate (C(20)). This bifunctionality was confirmed by expression in both prokaryotic (*Escherichia coli*) and eukaryotic (*P. abies* embryogenic tissue) hosts. Thus, this isoprenyl diphosphate synthase, designated PaIDS1, could contribute to the biosynthesis of both major terpene types in *P. abies* oleoresin. In saplings, PaIDS1 transcript was restricted to wood and bark, and transcript level increased dramatically after methyl jasmonate

treatment, which induces the formation of new (traumatic) resin ducts. Polyclonal antibodies localized the PaIDS1 protein to the epithelial cells surrounding the traumatic resin ducts. PaIDS1 has a close phylogenetic relationship to single-product conifer geranyl diphosphate and geranylgeranyl diphosphate synthases. Its catalytic properties and reaction mechanism resemble those of conifer geranylgeranyl diphosphate synthases, except that significant quantities of the intermediate geranyl diphosphate are released. Using site-directed mutagenesis and chimeras of PaIDS1 with single-product geranyl diphosphate and geranylgeranyl diphosphate synthases, specific amino acid residues were identified that alter the relative composition of geranyl to geranylgeranyl diphosphate.

Involved units

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