

Accepted Manuscript

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PII: S0981-9428(15)30057-7

DOI: [10.1016/j.plaphy.2015.07.011](https://doi.org/10.1016/j.plaphy.2015.07.011)

Reference: PLAPHY 4229

To appear in: *Plant Physiology and Biochemistry*

Received Date: 12 June 2015

Revised Date: 6 July 2015

Accepted Date: 15 July 2015

Please cite this article as: X. Zhan, S.S. Bach, N.L. Hansen, C. Lunde, H.T. Simonsen, Additional diterpenes from *Physcomitrella patens* synthesized by copalyl diphosphate/kaurene synthase (*PpCPS/KS*), *Plant Physiology et Biochemistry* (2015), doi: 10.1016/j.plaphy.2015.07.011.

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Additional diterpenes from *Physcomitrella patens* synthesized by copalyl diphosphate/kaurene synthase (*PpCPS/KS*)

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Abstract

The bifunctional diterpene synthase, copalyl diphosphate/kaurene synthase from the moss *Physcomitrella patens* (*PpCPS/KS*), catalyzes the formation of at least four diterpenes, including *ent*-beyerene, *ent*-sandaracopimaradiene, *ent*-kaur-16-ene, and 16-hydroxy-*ent*-kaurene. The *in planta* enzymatic activity has been confirmed through generation of a targeted *PpCPS/KS* knock-out mutant in *P. patens* via homologous recombination, transient expression of *PpCPS/KS* in *N. benthamiana*, and expression in *E. coli*. GC-MS analysis of the knock-out mutant shows that it lacks the diterpenoids, supporting that all are products of *PpCPS/KS* as observed in *N. benthamiana*. These results provide additional knowledge of the mechanism of this bifunctional diterpene synthase, and are in line with proposed reaction mechanisms in kaurene biosynthesis.

Keywords: diterpene, copalyl diphosphate/kaurene synthase, *Physcomitrella patens*, *ent*-beyerene, *ent*-sandaracopimaradiene.

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