Abstract

Allene oxide synthase (AOS) and hydroperoxide lyase (HPL) mediate the formation of precursors of jasmonates and carbon-six volatiles, respectively. AOS and HPL utilize fatty acid hydroperoxides and belong to the plant cytochrome P450 74 (CYP74) family that mediates plant defense against herbivores, pathogens, or abiotic stresses. Although members of the CYP74 family have been reported in mosses and other species, the evolution and function of multiple CYP74 genes in plants remain elusive. Here, we show that the liverwort *Marchantia polymorpha* belongs to a basal group in the evolution of land plants; has two closely related proteins (59 % identity), MpAOS1 and MpAOS2, that are similar to moss PpAOS1 (49 and 47 % identity, respectively); and exhibits AOS activity but not HPL activity. We also found that the green microalgae *Klebsormidium flaccidum*, consist of multicellular and non-branching filaments, contains an enzyme, KfAOS, that is similar to PpAOS1 (37 % identity), and converts 13-hydroperoxide of linolenic acid to 12-oxo-phytodienoic acid in a coupled reaction with allene oxide cyclase. Phylogenetic analysis showed two evolutionarily distinct clusters. One cluster comprised AOS and HPL from charophytic algae, liverworts, and mosses, including MpAOSs and KfAOS. The other cluster was formed by angiosperm CYP74. Our results suggest that plant CYP74 enzymes with AOS, HPL, and divinyl ether synthase activities have arisen multiple times and in the two different clades, which occurred prior to the divergence of the flowering plant lineage.