

In response to environmental stressors such as blast fungal infections, rice produces phytoalexins, an antimicrobial diterpenoid compound. Together with momilactones, phytocassanes are among the major diterpenoid phytoalexins. The biosynthetic genes of diterpenoid phytoalexin are organized on the chromosome in functional gene clusters, comprising diterpene cyclase, dehydrogenase, and cytochrome P450 monooxygenase genes. Their functions have been studied extensively using *in vitro* enzyme assay systems. Specifically, P450 genes (*CYP71Z6*, *Z7*; *CYP76M5*, *M6*, *M7*, *M8*) on rice chromosome 2 have multifunctional activities associated with *ent*-copalyl diphosphate-related diterpene hydrocarbons, but the *in planta* contribution of these genes to diterpenoid phytoalexin production remains unknown. Here, we characterized *cyp71z7* T-DNA mutant and *CYP76M7/M8* RNAi lines to find that potential phytoalexin intermediates accumulated in these P450-suppressed rice plants. The results suggested that *in planta*, *CYP71Z7* is responsible for C2-hydroxylation of phytocassanes and that *CYP76M7/M8* is involved in C11 α -hydroxylation of 3-hydroxy-cassadiene. Based on these results, we proposed potential routes of phytocassane biosynthesis *in planta*.

C2-hydroxylation and C11-hydroxylation are active *in planta* catalyzed by *CYP71Z7* and *CYP76M7/M8* leading to phytocassanes. New intermediates are found in this study.