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ScienceDirect
Phytochemistry 68 (2007) 312–326
PHYTOCHEMISTRY
www.elsevier.com/locate/phytochem

Functional characterization of the rice kaurene synthase-like gene family

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Received 6 August 2006; received in revised form 25 September 2006
Available online 1 December 2006

Abstract

The rice (*Oryza sativa*) genome contains a family of kaurene synthase-like genes (*OsKSL*) presumably involved in diterpenoid biosynthesis. While a number of OsKSL enzymes have been functionally characterized, several have not been previously investigated, and the gene family has not been broadly analyzed. Here we report cloning of several *OsKSL* genes and functional characterization of the encoded enzymes. In particular, we have verified the expected production of *ent*-kaur-16-ene by the gibberellin phytohormone biosynthesis associated OsKS1 and demonstrated that OsKSL3 is a pseudo-gene, while OsKSL5 and OsKSL6 produce *ent*-(iso)kaur-15-ene. Similar to previous reports, we found that our sub-species variant of OsKSL7 produces *ent*-cassa-12,15-diene, OsKSL10 produces *ent*-(sandaraco)pimar-8(14),15-diene, and OsKSL8 largely *syn*-stemar-13-ene, although we also identified *syn*-stemod-12-ene as an alternative product formed in ~20% of the reactions catalyzed by OsKSL8. Along with our previous reports identifying OsKSL4 as a *syn*-pimara-7,15-diene synthase and OsKSL11 as a *syn*-stemod-13(17)-ene synthase, this essentially completes biochemical characterization of the *OsKSL* gene family, enabling broader analyses. For example, because several OsKSL enzymes are involved in phytoalexin biosynthesis and their gene transcription is inducible, promoter analysis was used to identify a pair of specifically conserved motifs that may be involved in transcriptional up-regulation during the rice plant defense response. Also examined is the continuing process of gene evolution in the *OsKSL* gene family, which is particularly interesting in the context of very recently reported data indicating that a *japonica* sub-species variant of OsKSL5 produces *ent*-pimara-8(14),15-diene, rather than the *ent*-(iso)kaur-15-ene produced by the *indica* sub-species variant analyzed here.

Keywords: *Orzya sativa*; Poaceae; Terpene synthase; *ent*-kaurene synthase; *ent*-isokaurene synthase; *ent*-sandaracopimaradiene synthase; *ent*-cassadiene synthase; *syn*-stemarene synthase; *syn*-stemodene synthase; *syn*-pimaradiene synthase; Labdane-related diterpenoids; Phytoalexin; Natural products biosynthesis; Plant defense; Gene family evolution

Abbreviations: AtKS, *Arabidopsis thaliana* kaurene synthase; CPP, copalyl diphosphate; CPS, copalyl diphosphate synthase; cv, cultivar; GGPP, (*E*,*E*,*E*)-geranygeranyl diphosphate; GST, glutathione-*S*-transferase; KO, kaurene oxidase; KOL, kaurene oxidase-like; KS, kaurene synthase; KSL, kaurene synthase-like; ORF, open reading frame; OsCPS, rice (*Oryza sativa*) copalyl diphosphate synthase; OsKS(L), rice (*Oryza sativa*) kaurene synthase(-like); ssp, sub-species.

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doi:10.1016/j.phytochem.2006.10.016

1. Introduction

Rice is an important food crop, and has become a model plant for the cereal plant family with the recent availability of draft genome sequences (Goff et al., 2002; Yu et al., 2002), as well as large numbers of defined full-length cDNAs (Kikuchi et al., 2003). This extensive sequence information has enabled functional genomics based approaches towards elucidating the biosynthetic machinery underlying rice metabolism. One specific area of interest is the production of natural products with antimicrobial activity, which are termed phytoalexins if their biosynthesis is induced by microbial infection and phytoanticipins if their biosynthesis is constitutive (VanEtten et al., 1994), as the production of these small organic compounds is associated with resistance to microbial diseases such as that caused by the agronomically devastating rice blast pathogen *Magneporthe grisea*.

Extensive phytochemical investigation has demonstrated that rice produces a number of phytoalexins in response to *M. grisea* infection (Peters, 2006). Intriguingly, the rice phytoalexins, with the exception of the flavonoid sakuranetin, all fall into the large family of labdane-related diterpenoid natural products characterized by minimally containing a labdane type bicyclic core structure. Thus, along with the ubiquitous gibberellin phytohormone, rice produces more than 10 other labdane-related diterpenoids as phytoalexins. These are momilactones A & B (Cartwright et al., 1977, 1981), oryzalexins A–F (Akatsuka et al., 1985; Kato et al., 1993, 1994; Sekido et al., 1986), oryzalexin S (Kodama et al., 1992), and phytocassanes A–E (Koga et al., 1997; Koga et al., 1995). In addition, momilactone B is constitutively secreted from rice roots and acts as an allelochemical in suppressing germination in nearby seeds (Kato-Noguchi and Ino, 2003; Kato-Noguchi et al., 2002). The identified rice labdane-related diterpenoid natural products fall into five structurally related groups (Fig. 1), with the gibberellins being elaborated from *ent*-kaurene, oryzalexins A–F from *ent*-sandaracopimaradiene, phytocassanes A–E from *ent*-cassadiene, oryzalexin S from *syn*-stemodene, and momilactones A and B from *syn*-pimaradiene (Mohan et al., 1996; Wickham and West, 1992; Yajima et al., 2004).

Labdane-related diterpenoids share an unusual biogenetic origin, as their biosynthesis is uniquely initiated by a consecutive pair of terpene synthase catalyzed reactions. In the first reaction, the characteristic bicyclic core structure is formed by class II labdane-related diterpene cyclases, which catalyze protonation-initiated cyclization of the universal diterpenoid precursor (*E*,*E*,*E*)-geranylgeranyl diphosphate (GGPP, **1**) to produce a specific stereoisomer of labdadienyl/copalyl diphosphate (e.g. **2** and **3**) or rearranged derivative structure such as clerodanyl diphosphate (MacMillan and Beale, 1999). The resulting cyclized diphosphate compounds can then be further cyclized and/or rearranged by more typical class I terpene synthases, which initiate catalysis via ionization of the allylic pyrophosphate group (Davis and Croteau, 2000). Notably, class I labdane-related diterpene synthases exhibit stereospecificity, e.g. all of the identified copalyl diphosphate (CPP) specific enzymes only react with single stereoisomers of CPP (Cho et al., 2004; Nemoto et al., 2004; Otomo et al., 2004a; Peters et al., 2000; Wilderman et al., 2004).

Prototypical plant terpene synthases are similar in size and consist of two structurally defined domains that have been simply termed the N- and C-terminal domains because these are associated with the corresponding region of their polypeptide sequence (Starks et al., 1997; Whittington et al., 2002). While both class II and class I labdane-related diterpene synthases are phylogenetically related to

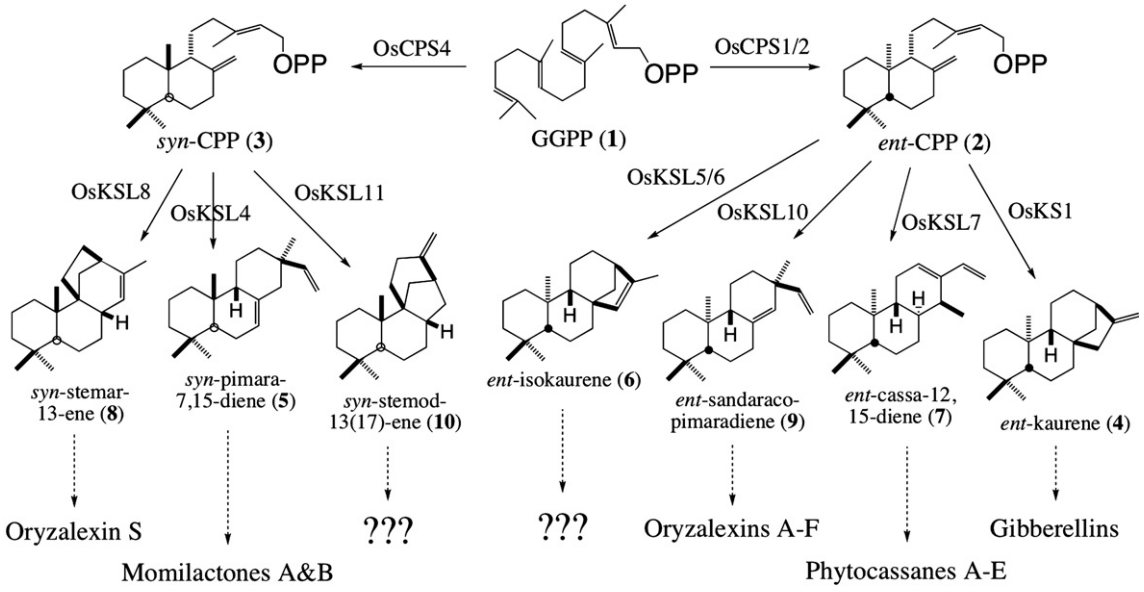

Fig. 1. Known labdane-related diterpene cyclization reactions in rice. The corresponding cyclases are indicated, along with their products and, where known, the derived natural products (dashed arrows indicate multiple biosynthetic steps).