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Molecules of Interest

Uncovering the complex metabolic network underlying diterpenoid phytoalexin biosynthesis in rice and other cereal crop plants

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Abstract

Rice (*Oryza sativa*) is a staple food crop and serves as a model cereal crop plant for scientific study. Phytochemical investigations of the agronomically devastating rice blast disease have identified a number of rice phytoalexins exhibiting significant direct anti-fungal activity against the causative agent, *Magneporthe grisea*. Current evidence strongly indicates that these phytoalexins, largely a family of labdane-related diterpenoids, are important as general antibiotics, and that similar phytoalexins are produced more broadly throughout the cereal crop family. From the extensive sequence information available for rice it has been possible to functionally identify the genes for the enzymes catalyzing the two consecutive cyclization reactions that initiate biosynthesis of these labdane-related diterpenoid phytoalexins. This has led to several insights into the underlying evolution of diterpene biosynthesis throughout the cereal crop family. The hydrocarbon olefins resulting from cyclization must be further elaborated to form bioactive natural products and, because not much is currently known, necessarily speculative biosynthetic pathways for these processes are presented. Given the significant antibiotic activity of the labdane-related diterpenoid phytoalexins from rice, and the presence of similar secondary metabolism throughout the cereal crop plant family, study of this type of biosynthesis will continue to be an area of active investigation.

Keywords: *Oryza sativa*; Poaceae; Diterpenoids; Labdane-related diterpenoids; Phytoalexins; Biosynthesis; Terpene synthases

1. Introduction

Rice is an important source of food, providing 20% of the total direct human caloric intake world-wide, as well as being the predominant staple food for many developing countries (FAO, 2004). Furthermore, rice also has become a model plant for the widespread grass plant family (Poaceae), which covers ~20% of the earth's land mass surface area. In particular, the relatively small size of the rice genome (~430 Mb), ease of transformation, and extensive genetic resources, along with agronomic importance, led to its selection for both public and private genomic sequencing (Goff et al., 2002; Yu et al., 2002). This has stimulated the additional development of a host of related resources, such as the availability of large numbers of defined full-length cDNAs (Kikuchi et al., 2003) and various types of mutant rice lines (Hirochika et al., 2004). Thus, rice is not only important for its own sake, but also as a model system for study of all the cereal crop plants, which altogether provide approximately half of the global total direct human caloric intake (FAO, 2005), and further provides critical sources of forage and processed feed for many agronomically important domesticated animals.

Among the many factors contributing to plant fitness and, specifically, disease resistance, seems to be the deployment of antimicrobial small molecules. These are termed phytoalexins if their biosynthesis is induced by microbial

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Abbreviations: CPP, copalyl diphosphate; CPS, copalyl diphosphate synthase; CPSL, copalyl diphosphate synthase-like; P450, cytochrome P450; GGPP, geranylgeranyl diphosphate; KO, kaurene oxidase; KOL, kaurene oxidase-like; KS, kaurene synthase; KSL, kaurene synthase-like; MeJA, methyl jasmonate; MYA, million years ago.

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infection, and phytoanticipins if preformed (VanEtten et al., 1994). Not surprisingly, there are a number of reports in the literature detailing the isolation and identification of rice phytoalexins. These studies have generally focused on finding compounds that exhibit antibiotic activity against *Magneporthe grisea* (originally known as *Pyricularia oryzae*), a filamentous ascomycete fungus that is the causal agent of rice blast disease. This devastating disease has been estimated to cause the loss of 10–30% of the total rice harvest, and *M. grisea* also has been observed to infect wheat, barley, and millet crops, producing a similar blast disease and loss of grain production (Talbot, 2003). Accordingly, there is considerable interest in elucidating any means by which *M. grisea* infections might be contained, which includes early work demonstrating the generalized production of phytoalexins by rice plants in response to *M. grisea* (Uehara, 1958). Of particular relevance here is identification of the corresponding natural products, along with elucidation of the associated biosynthetic pathways.

2. Identified rice phytoalexins

The first identified rice phytoalexins were the 9,10-*syn*-pimarane diterpenoids momilactones A and B (Fig. 1). These compounds were originally isolated and identified as plant growth inhibitors from rice seed husks (Kato et al., 1973). Later work has demonstrated that at least momilactone B acts as an allelochemical, inhibiting seed germination of other plant species (Kato-Noguchi et al., 2002), as well as being constitutively secreted from rice roots (Kato-Noguchi and Ino, 2003). Nevertheless, work by Cartwright and co-workers, as first communicated to Nature (Cartwright et al., 1977) and later detailed in Phytochemistry (Cartwright et al., 1981), demonstrated that momilactones A and B are also phytoalexins. In particular, the momilactones exhibit antifungal activity against *M. grisea* and only appear in rice leaves after infection. In their initial report Cartwright et al. (1977) also reported that biosynthesis of these two phytoalexins was ‘primed’ by treatment with the systemic antifungal WL 28325 (2,2-dichloro-3,3-dimethyl cyclopropane carboxylic acid), which does not induce production of momilactones directly but rather increases the amounts produced following infection. Indeed, the authors suggest that this ‘priming’ of phytoalexin biosynthesis is the mode of action accounting for dichlorocyclopropane-induced blast resistance. In both reports Cartwright et al. also demonstrated that momilactone biosynthesis could be induced by UV-irradiation, an observation that was later reported to extend to a wider range of rice phytoalexins (Kodama et al., 1988), and has proven useful in a number of the following studies.

Many of the known rice phytoalexins were identified by Tadami Akatsuka, Osamu Kodama, and co-workers. Their efforts were initiated by the isolation of a group of four compounds from *M. grisea* infected rice leaves, wherein the first, oryzalexin A, was suggested to be a pimarane diterpenoid in their initial report (Akatsuka et al., 1983). Further spectroscopic analysis demonstrated that oryzalexin A was configured with *enantio*-stereochemistry (Fig. 2) and, thus, was an *ent*-pimarane diterpenoid (Kono et al., 1984). Oryzalexins B and C were also identified as *ent*-pimarane diterpenoids, and found in *M. grisea* infected, but not healthy rice leaves (Akatsuka et al., 1985; Kono et al., 1985); likewise for oryzalexin D (Sekido et al., 1986). Accordingly, all four oryzalexins have been classi-

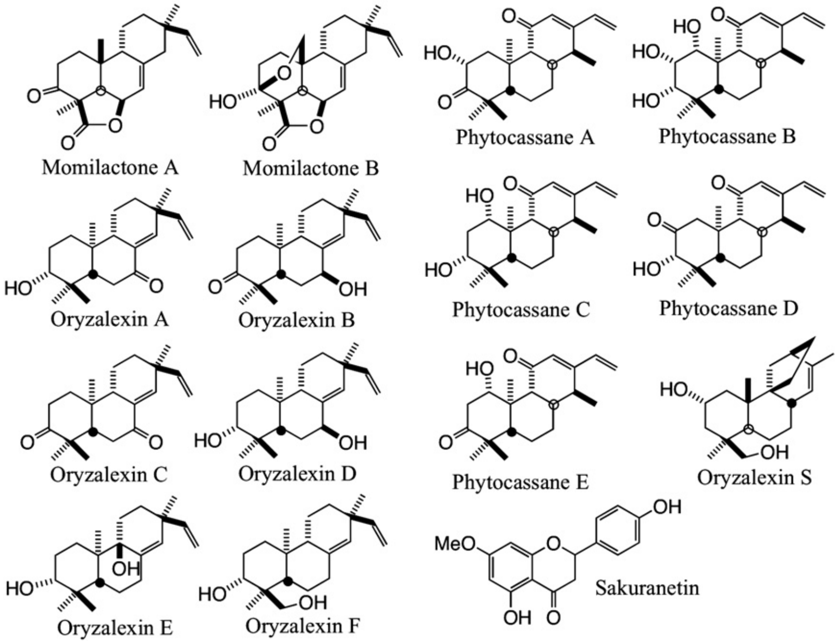

Fig. 1. Known rice phytoalexins.