

The Role of Momilactones in Rice Allelopathy

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Abstract Large field screening programs and laboratory experiments in many countries have indicated that rice is allelopathic and releases allelochemical(s) into its environment. A number of compounds, such as phenolic acids, fatty acids, phenylalkanoic acids, hydroxamic acids, terpenes, and indoles, have been identified as potential rice allelochemicals. However, the studies reviewed here demonstrate that the labdane-related diterpenoid momilactones are the most important, with momilactone B playing a particularly critical role. Rice plants secrete momilactone B from their roots into the neighboring environments over their entire life cycle at phytotoxic levels, and momilactone B seems to account for the majority of the observed rice allelopathy. In addition, genetic studies have shown that selective removal of the momilactones only from the complex mixture found in rice root exudates significantly reduces allelopathy, demonstrating that these serve as allelochemicals, the importance of which is reflected in the presence of a dedicated momilactone biosynthetic gene cluster in the rice genome.

Keywords Allelopathy · Allelochemical · Ecosystem · Momilactone · Phenolic acid · *Oryza sativa* · Root exudate

Introduction

Allelopathy can be defined as antagonistic chemical interactions among various plant species. Evidence for allelopathy

has accumulated in the literature over many years. In particular, it has been demonstrated that certain plants release natural products, termed allelochemicals, into their immediate environment, with these presumably affecting the growth and development of other nearby plant species (Rice, 1984; Putnam and Tang, 1986; Inderjit, 1996; Bais et al., 2004; Belz, 2007). For example, the roots of sorghum produce the quinone sorgoleone, which is secreted from root hairs (Czarnota et al., 2003; see also Weston et al., 2013, this issue), and whose resulting concentration in the soil is 10–100 μM (Netzley et al., 1988). Sorgoleone has potent allelopathic activity, and inhibits the germination and growth of susceptible weeds at 10 μM (Nimbal et al., 1996). The allelopathic activity of wheat has been correlated with its ability to exude benzoxazinoids, which are constitutively produced as glucoside conjugates in the plant (Belz and Hurle, 2005, see also Schulz et al., 2013, this issue). Thus, allelopathic interactions may play an important role in natural plant ecosystems. However, the evidence for allelochemical mediated plant-plant allelopathy is mostly correlative, and this area has been rather controversial (Field et al., 2006).

Rice has been found to inhibit the growth of several other plant species under field and laboratory conditions (e.g., Dilday et al., 1998; Kim et al., 1999; Olofsdotter et al., 1999; Azmi et al., 2000; Kato-Noguchi and Ino, 2001). These findings indicate that rice is allelopathic, and presumably produces and releases allelochemical(s) into the neighboring environment. The implication is that allelopathy may have played an important ecological role in the evolution of rice.

Given the agricultural importance of rice, its allelopathic activity has been extensively studied, with a number of compounds identified as potential allelochemicals in rice plant extracts, root exudates, and decomposing residues (Rimando and Duke, 2003; Khanh et al., 2007). This review provides a short overview of rice allelopathy and potential allelochemicals, and highlights the importance of momilactones, particularly momilactone B, for this purpose.

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Observations of Rice Allelopathy Under Field Conditions

The first observation of rice allelopathy came from field/paddy examinations in Arkansas, U.S.A. in which about 191 of 5,000 rice accessions inhibited the growth of the aquatic weed ducksalad (*Heteranthera limosa* (SW.) Willd./Vahl) (Dilday et al., 1989). This finding led to a large field screening program. More than 16,000 rice accessions from 99 countries in the germplasm collection of the USDA-ARS have been screened. Of these, 412 rice accessions inhibited the growth of ducksalad, and 145 inhibited the growth of *Ammannia coccinea* Rottb. (Dilday et al., 1994, 1998). In Egypt, 1,000 rice varieties were screened for allelopathy against barnyard grass (*Echinochloa crus-galli* (L.) Beauv.) and *Cyperus difformis* L. under field conditions, and inhibitory activity was found in more than 40 varieties (Hassan et al., 1998). Similar screening programs have been carried out in other countries, and certain rice varieties have been found to inhibit the growth of various plant species (Kim and Shin, 1998; Olofsdotter et al., 1999; Pheng et al., 1999).

Observations of Rice Allelopathy Under Controlled Conditions

Plant-to-plant interference is a complex combination of competition for resources such as light, nutrients, and water, along with allelopathic interactions (Qasem and Hill, 1989; Einhellig, 1996). Therefore, it is important to eliminate the effects of such competition from experimental systems to conclusively demonstrate allelopathy (Fuerst and Putnam, 1983; Leather and Einhellig, 1986; 1988; Inderjit and Olofsdotter, 1998). It also is important to carefully control bioassay conditions, particularly pH and osmolarity of the growth media (Wardle et al., 1992; Haugland and Brandsaeter, 1996; Hu and Jones, 1997). Thus, only well-designed bioassays under controlled environments are able to evaluate plant allelopathy (Leather and Einhellig, 1986; 1988; Inderjit and Olofsdotter, 1998).

Such a laboratory based whole-plant bioassay, termed “relay-seedling”, has been developed for rice at the International Rice Research Institute in the Philippines (Navarez and Olofsdotter et al., 1996). This bioassay eliminates the effects of resource competition between rice and test plants in the experimental system, and provides a means to evaluate rice allelopathy. Use of this relay-seedling bioassay led to the identification of several rice varieties that possess strong allelopathy. In addition, 111 rice varieties were tested for their growth inhibitory activity under laboratory and field conditions, but the results were inconsistent (Olofsdotter et al., 1999).

Screening for allelopathic rice varieties also has been undertaken in several other laboratories. These studies show that there is a marked difference in activity among rice varieties, with about 3 - 4 % of tested varieties exhibiting

strong allelopathy (Fujii, 1992; Hassan et al., 1998; Kim et al., 1999; Olofsdotter et al., 1999; Azmi et al., 2000).

The allelopathic potential of rice seedlings from eight cultivars was determined in Petri dishes under controlled laboratory conditions (Kato-Noguchi and Ino, 2001). Three species, alfalfa (*Medicago sativa* L.), cress (*Lepidium sativum* L.), and lettuce (*Lactuca sativa* L.) were chosen as test plants for these bioassays due to their known sensitivity to allelopathic inhibition. Following the methods of Weidenhamer et al. (1987), phosphate buffer (pH 6.0) was chosen as the growth media. This buffer did not affect the germination and growth of cress, lettuce, or alfalfa as well as rice, and prevented any significant pH changes during the bioassay. In addition, the differences in osmotic potential of the growth media in all dishes did not affect the germination and growth of these plant species.

In that study, all eight rice cultivars inhibited the growth of roots and shoots, leading to reduced fresh weight, of all three test plant species. However, cv. Koshihikari exhibited the greatest growth inhibition, with more than 60% reduction of growth (Fig. 1; Kato-Noguchi and Ino, 2001). These bioassays were carried out at the early seedling stage where nutrients generally are supplied from initial seed reserves

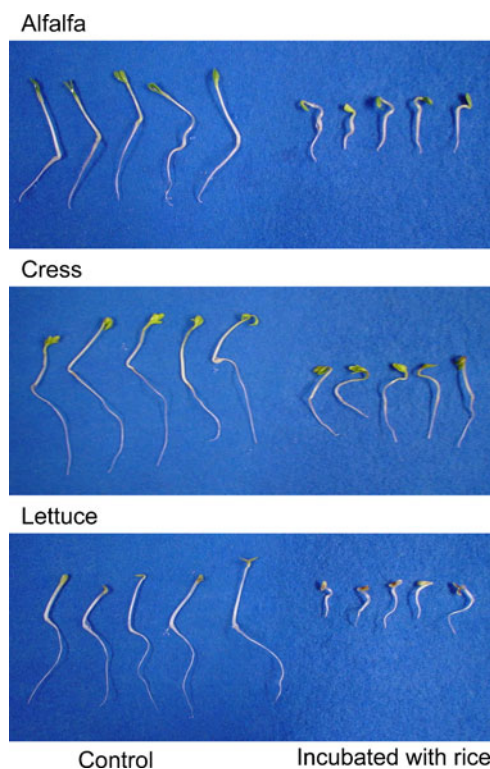


Fig. 1 Effects of rice (cv. Koshihikari) on the growth of the hypocotyls and roots of alfalfa, cress and lettuce. Alfalfa, cress and lettuce seeds were allowed to germinate and grow with 7-day-old rice seedlings at 25 °C and 12-h photoperiod. After 3 days, hypocotyl and root length of their seedlings were determined. Control bioassays did not contain rice seedlings. Reprinted with permission from Kato-Noguchi (2004). Copyright 2004 Elsevier

(Fuerst and Putnam, 1983), to enable growth without nutrient addition or the need for light, thus removing any complicating effects from resource competition. Therefore, the observed growth inhibition is consistent with the release of allelochemicals by rice.

Candidate Rice Allelochemicals

A number of secondary metabolites, phenolic acids, phenylalkanoic acids, hydroxamic acids, fatty acids, terpenes, and indoles, have been suggested as putative allelochemicals from rice plants extracts and decomposing rice residues, as summarized by Rimando and Duke (2003) and Khan et al. (2007). However, it was not yet clear whether these compounds are released from living rice plants, and truly function as allelochemicals.

Even though most plant tissues contain potential allelochemicals, only those released into the environment can inhibit the germination and growth of neighboring plant species and act as allelochemicals in natural ecosystems (Putnam and Tang, 1986; Bais et al., 2004). For this reason, it has been postulated that natural products found in roots exudates are more likely to act as allelochemicals than those simply identified in plant tissues (Perez and Ormeno-Nunez, 1991; Neimeyer and Perez, 1995). Moreover, Wu et al. (2001) have found that there is no significant correlation between the level of growth inhibitory substances in plants and their levels in root exudates, further emphasizing the need to couple measurement of phytotoxicity with relevant localization – i.e., secretion to the environment.

Phenolic Acids Do not Function in Rice Allelopathy

Phenolic acids often are mentioned as putative allelochemicals and are the most commonly investigated compounds among potential allelochemicals as they have been found in a wide range of plants and soils (e.g., Hartley and Whitehead, 1985; Inderjit, 1996; Dalton, 1999). Phenolic acids also have been found in rice field/paddy soils, decomposing rice residues, and rice roots exudates (Kuwatsuka and Shindo, 1973; Chou and Lin, 1976; Chou and Chiou, 1979; Mattice et al., 1998; Kim and Kim, 2000; Seal et al., 2004a).

However, the levels of phenolic acids found in rice soil are not sufficient to cause phytotoxic effects, leading to doubts about their relevance to rice allelopathy (Tanaka et al., 1990). In support of this view, phenolic acids usually are present in rice soils at concentrations less than 5 mg/kg soil, which is below the threshold for phytotoxic effects, and additionally, allelopathic rice cultivars do not release significantly greater amount of phenolic acids than non-allelopathic rice cultivars (Olofsdotter et al., 2002).

To investigate the possibility that synergistic action of phenolic acids might influence rice allelopathy, the five major phenolic acids in rice root exudates, 4-hydroxybenzoic acid, vanillic acid, syringic acid, *p*-coumaric acid, and caffeic acid, were mixed, and the phytotoxicity of this mixture against arrowhead (*Sagittaria monotevidensis* Cham. & Schldtl.) was determined. However, the concentration required for 50% growth inhibition of the mixture of these five phenolic acids was 502 μ M, much higher than the level actually found in rice root exudates (Seal et al., 2004b). Collectively, these studies indicate that the phenolic acids in rice root exudates are not the allelochemicals responsible for rice allelopathy.

Discovery of Momilactones in Rice Root Exudates

As described above, rice cv. Koshihikari exhibits significant allelopathic activity. About 5,000 rice cv. Koshihikari seedlings were hydroponically grown for 14 days in order to isolate the exuded chemicals. Bioactivity guided fractionation led to purification of 2.1 mg of a phytotoxic compound (Kato-Noguchi et al., 2002; Kato-Noguchi and Ino, 2003a). The chemical structure of this putative allelochemical was determined from spectral data to be momilactone B (3,20-epoxy-3 α -hydroxy-*syn*-pimara-7,15-dien-19,6 β -olide, C₂₀H₂₆O₄). Secretion of momilactone B was later confirmed for other rice cultivars as well (Kong et al., 2004). In addition, another potential allelochemical, momilactone A (3-oxo-*syn*-pimara-7,15-dien-19,6 β -olide, C₂₀H₂₆O₃) also was found in the root exudates of rice cv. Koshihikari (Kato-Noguchi et al., 2008b). Momilactones A and B further were found to be secreted by many other rice cultivars (Chung et al., 2006; Mennan et al., 2012).

Momilactones A and B were first isolated from rice husks as plant growth inhibitors (Kato et al., 1973; Takahashi et al., 1976). However, both were later (re)isolated as inducible antibiotics, which are termed phytoalexins (VanEtten et al., 1994), against the fungal rice blast disease pathogen *Magnaporthe oryzae* in rice leaves and straw (Cartwright et al., 1977, 1981). Thereafter, the function of momilactone A as a phytoalexin was extensively studied, and several lines of evidence suggest that momilactone A plays a role in rice defense against fungal pathogens (e.g., Tamogami and Kodama, 2000; Jung et al., 2005; Okada et al., 2007; Hasegawa et al., 2010). By contrast, much less has been reported regarding momilactone B function as a phytoalexin.

Two independent laboratories recently isolated momilactones A and B from the moss *Hypnum plumaeforme* Wils as allelochemicals (Kobayashi et al., 2007; Nozaki et al., 2007; Kato-Noguchi and Kobayashi, 2009). Given that these are the only two species known that produce these natural products, and the obvious phylogenetic distance between rice and *H. plumaeforme*, it seems likely that their common ability to produce momilactones results from convergent evolution.

Allelopathic Activity of the Momilactones

Momilactones A and B inhibit the growth of barnyard grass (*Echinochloa crus-galli*) and *Echinochloa colonum*, which are the most noxious weeds in rice fields (Rao et al., 2007; Kong, 2008), at concentrations greater than 1 and 10 μM , respectively (Kato-Noguchi et al., 2008a). The concentrations required for 50% growth inhibition (defined as I_{50}) of barnyard grass roots and shoots, respectively, are 28.7 and 46.4 μM for momilactone A, and 6.1 and 6.3 μM for momilactone B. The I_{50} values for *E. colonum* roots and shoots, respectively, are 65.4 and 240 μM for momilactone A, and 5.0 and 12.5 μM for momilactone B. Comparing I_{50} values, the inhibitory activities of momilactone B on the root and shoot growth of barnyard grass were 4.7- to 19-fold greater than those of momilactone A. Accordingly, momilactone B exhibits significantly greater growth inhibitory activity than momilactone A. This has been verified in other bioassay systems (Takahashi et al., 1976; Kato et al., 1977; Chung et al., 2005; Fukuta et al., 2007; Toyomasu et al., 2008).

The Momilactones Are Not Toxic to Rice

Momilactones A and B only inhibit root and shoot growth of rice seedlings at concentrations greater than 100 and 300 μM , respectively. These inhibitory activities are only 1 - 2% and 0.6 - 2% of the effect of the momilactones on the root and shoot growth of barnyard grass and *E. colonum* (Kato-Noguchi et al., 2008a). Thus, the ability of momilactones A and B to suppress the growth of rice seedlings is much less than their effect on barnyard grass and *E. colonum*, with no visible damage to rice seedlings exerted by momilactones A and B at levels that are cytotoxic to these other plant species (Kato-Noguchi et al., 2008a). Considering the reduced inhibitory activity, and amount of momilactones A and B secreted into the rice rhizosphere described below, it seems intuitive that these natural products exert negligible effects on rice plants themselves. The basis for rice resistance is currently unknown, but presumably it involves either efflux (e.g., via the same transport mechanism responsible for momilactone secretion), insensitivity of the molecular target (which is currently unknown), and/or degradation.

Rice selectively Secretes Momilactone B

Rice secretes momilactones A and B into its rhizosphere throughout its entire life cycle (Kato-Noguchi et al., 2003b; 2008a). The secretion level increases until flowering initiates, and decreases thereafter. The secretion rates for momilactones A and B, respectively, at day 80 (around flowering initiation) are 1.1 and 2.3 $\mu\text{g plant}^{-1} \text{day}^{-1}$, which

are 55- and 58-fold greater than they are at day 30. Interestingly, the endogenous concentrations of momilactone A and B, respectively, in rice are 4.5 and 3 $\mu\text{g/g}$ in the straw (Lee et al., 1999), 4.9 and 2.9 $\mu\text{g/g}$ in the seed husks (Chung et al., 2006), and 140 and 95 $\mu\text{g/g}$ in whole plants, where levels also increase until the initiation of flowering (Kato-Noguchi et al., 2008a). Hence, momilactone A is present at 1.5 to 1.7-fold higher levels than momilactone B in rice plants themselves. By contrast, momilactone B is secreted at higher rate than is momilactone A, which suggests that momilactone B is preferentially secreted into the rhizosphere relative to momilactone A. Plants secrete a wide variety of compounds from root cells by plasmalemma-derived exudation, endoplasmic-derived exudation, and proton-pumping mechanisms (Hawes et al., 2000; Bais et al., 2004; Bardi and Vivanco, 2009). However, the mechanism underlying secretion of the momilactones from rice roots is unknown.

Momilactones Contribution to Rice Allelopathy

When 8 different cultivars of 7-d-old rice seedlings were grown for 4 days with 4-d-old barnyard grass seedlings at 25°C with a 12-h photoperiod in medium consisting only of 1 mM MES buffer (pH 6.0), all cultivars inhibited the growth of barnyard grass, but to varying degrees. Notably, the resulting concentrations of momilactones A and B in the bioassay medium varied from 0.21 - 1.45 μM and 0.66 - 3.84 μM , respectively (Kato-Noguchi et al., 2010). Based on the growth inhibitory activity (defined above) and concentrations found in the bioassay medium, momilactone A appears to only account for 1.0 - 4.9% of the observed growth inhibition of barnyard grass by rice seedlings. By contrast, momilactone B then accounts for 58.8 - 81.9% of the observed inhibition. Thus, the momilactone B secreted from rice seedlings appears to be the major contributor to the allelopathic activity of rice seedlings against at least barnyard grass, while momilactone A contributes relatively little. Moreover, the cultivar-specific concentration of momilactone B in the medium is significantly ($P < 0.01$) correlated with the extent of growth inhibition of barnyard grass by the 8 tested rice cultivars (Kato-Noguchi et al., 2010). Thus, the variation in allelopathic activity observed among different rice cultivars may primarily reflect differences in their level of momilactone B secretion, which seems to be the major allelochemical in rice.

Momilactones A and B contribute 61.4 - 86.6% of the observed growth inhibition of barnyard grass by rice seedlings. The remaining 14.4 - 38.6% of activity presumably is caused by other putative rice allelochemicals, such as 3-isopropyl-5-acetoxycyclohexene-2-one-1 and 5,7,4'-trihydroxy-3'5'-dimethoxy-flavone (Kong et al., 2004). In any case, given that rice

secretes increasing amounts of momilactones A and B up to the initiation of flowering (Kato-Noguchi et al., 2008a), it also is possible that rice allelopathic activity increases over this timeframe.

Barnyard-grass Induces Rice Allelopathy and Momilactone Secretion

Rice allelopathic activity is increased in the presence of barnyard grass seedlings and barnyard grass root exudates (Zhao et al., 2005; Kong et al., 2006; Kato-Noguchi, 2011). This increase is not due to nutrient competition between the two plant species (Kato-Noguchi, 2011). Levels of momilactone B, both endogenous concentration in rice seedlings and secretion rate, also were increased by the presence of barnyard grass seedlings and barnyard grass root exudates. Presumably rice detects components of the barnyard grass root exudates, which trigger increased production and secretion of momilactone B (Kato-Noguchi, 2011). Similarly, production of the sorghum allelochemical, sorgoleone, also is induced by root extracts of the agriculturally relevant weed velvetleaf (Dayan, 2006). Accordingly, allelopathy potentially can act as an inducible defense mechanism mediated by recognition of root exudate components specific to other plant species found in the relevant ecosystem (or agricultural setting - e.g., barnyard grass in rice paddies).

Stress-induced Rice Allelopathy and Momilactone Production

Jasmonic acid, a plant defense signaling hormone (MacKintosh et al., 1994; Rakwal et al., 2001; Glazebrook, 2005; Halim et al., 2006), was found to increase rice allelopathic activity (Bi et al., 2007). Jasmonic acid and cantharidin, a protein serine/threonine phosphatase inhibitor that has been shown to mimic elicitor action in plants and activate defense responses (MacKintosh et al., 1994; Rakwal et al., 2001), increased the endogenous concentrations, as well as secretion rates, of momilactones A and B (Fig. 2; Kato-Noguchi et al., 2007). As momilactones A and B exhibit antifungal activity, and have been suggested to act as phytoalexins (Yamada et al., 1993; Dillon et al., 1997), their induction by jasmonic acid and cantharidin may be associated with plant microbial defense. Indeed, infection with the fungal blast pathogen *M. oryzae* also induces the production of momilactones, with greater accumulation of momilactone A than B (Hasegawa et al., 2010).

However, in addition to pathogen infection, it has long been appreciated that UV-irradiation also induces the production and increases the endogenous concentrations of momilactones A and B in rice (Cartwright et al., 1981; Kodama et al., 1988a). Moreover, the production of momilactones A and

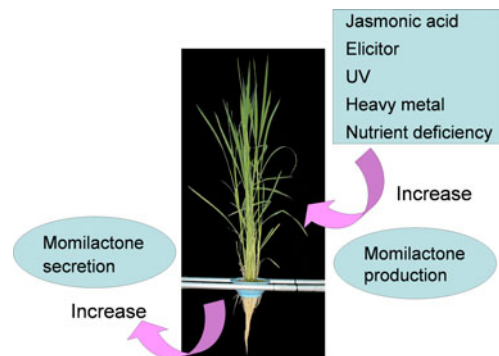


Fig. 2 Various stresses and elicitation increase the production and secretion of momilactones by rice

B can be induced by the application of heavy metal ions (Kodama et al., 1988b). Thus, the induction of momilactone production may be part of a more general stress, rather than specific microbial defense response. Accordingly, although UV-irradiation also elevates the rate at which momilactone B is secreted by rice into its rhizosphere (Kato-Noguchi et al., 2007), such induction may similarly reflect a more general, rather than specific defense response. Nevertheless, it also was reported that nutrient deficiency, which is often caused by competition with other plants, increases the production and secretion of momilactones by rice (Kato-Noguchi, 2011). Given that momilactones A and B possess strong allelopathic activities (e.g., Chung et al., 2005; Kato-Noguchi et al., 2008a, b; 2010), such increased secretion may provide a competitive advantage for rice by inhibiting the growth of competing plant species.

Biosynthesis of the Momilactones

Momilactones are members of the labdane-related diterpenoids, a large superfamily of ~7,000 natural products defined by an initial pair of cyclization reactions (Peters, 2010). In particular, bicyclization of the general diterpenoid precursor (*E,E,E*)-geranylgeranyl diphosphate (GGDP), which proceeds via an eponymous labdaenyl carbocation intermediate that is most often directly deprotonated to produce copalyl/labdadienyl diphosphate (CPP) in reactions catalyzed by class II diterpene cyclases then termed CPP synthases (CPS). The resulting bicycles are further cyclized and/or rearranged, generally to a polycyclic olefin, by class I diterpene synthases termed kaurene synthase-like (KSL) because of their derivation from the presumably ancestral *ent*-kaurene synthases required in all plants for gibberellin phytohormone biosynthesis. The CPS involved in rice momilactone biosynthesis, which produces an unusual (9*S*,10*R*) stereoisomer termed *syn*-CPP, has been identified (Xu et al., 2004; Otomo et al., 2004b), as has the subsequently acting *syn*-pimara-7,15-diene producing class I

diterpene synthase (Otomo et al., 2004a; Wilderman et al., 2004). While there was some initial confusion over enzyme nomenclature, these are now termed OsCPS4 and OsKSL4, respectively (Peters, 2006; Toyomasu, 2008).

Strikingly, the genes encoding OsCPS4 and OsKSL4 are found relatively close together in the rice genome (Sakamoto et al., 2004), with biochemical characterization demonstrating that these together form a functional pairing that defines a biosynthetic gene cluster (Wilderman et al., 2004). This gene cluster includes cytochromes P450 (CYP) and short chain alcohol dehydrogenases (SDR) as well, all of which are co-regulated with OsCPS4 and OsKSL4 (Fig. 3) - i.e., the level of these gene transcripts all are increased by the same stimuli that induce momilactone production (Okada et al., 2007). Moreover, simultaneous RNAi knockdown of the two closely related CYP found in this gene cluster (CYP99A2 & 3) led to decreased momilactone accumulation, demonstrating that one or both of these also function in momilactone biosynthesis (Shimura et al., 2007). It has since been demonstrated that CYP99A3 converts *syn*-pimaradiene to the *syn*-pimaradien-19-oic acid required for formation of the eponymous 19,6 β -lactone ring of the momilactones (Wang et al., 2011). In addition, at least one of the two SDR from this gene cluster

is a momilactone A synthase (OsMAS), catalyzing oxidation of the penultimate intermediate 3 β -hydroxy-*syn*-pimaradiene-19,6 β -olide (Shimura et al., 2007), an enzymatic activity previously demonstrated in rice cell-free assays (Atawong et al. 2002).

Genetic Evidence Demonstrates a Role for the Momilactones in Rice Allelopathy

While the extensive phytochemical investigations discussed above have provided strong evidence that the rice momilactones do act as allelochemicals, rice nevertheless secretes a number of phytotoxic compounds from its roots (Fig. 4). Particularly given such chemical complexity, assignment of allelochemical activity is most directly testable by selective removal of the compounds of interest via genetic manipulation, but no such experiments have been previously reported for any allelochemical. Because the OsCPS4 and OsKSL4 required for production of the *syn*-pimaradiene that represents the committed intermediate in momilactone biosynthesis have been identified (Fig. 3), it was possible to envision a reverse genetic approach to defining the physiological role of these natural products. Such an approach was enabled by the extensive mutant collections available for rice (Jiang and Ramachandran, 2010), from which it was possible to obtain insertion gene knockouts for OsCPS4 and

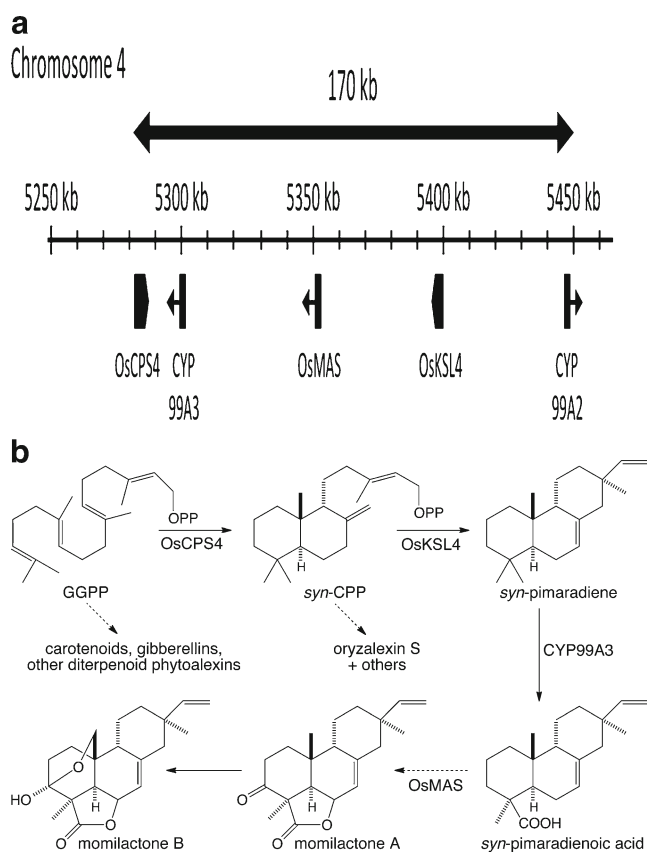


Fig. 3 Rice momilactones. **a** Biosynthetic gene cluster. **b** Biosynthetic pathway. Reprinted with permission from Xu et al. (2012). Copyright 2011 The Authors

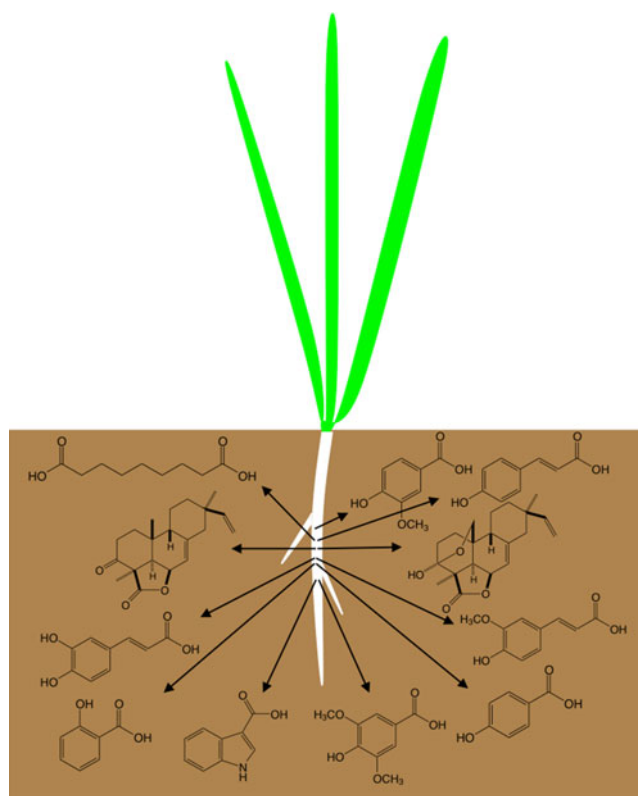


Fig. 4 Phytotoxic compounds secreted by rice roots

OsKSL4. It should be noted that these were obtained from different projects (Zhang et al., 2006 and Jeon et al., 2000, respectively), and are then derived from distinct parental/wild-type cultivars (Zhonghua 11 and Hwayoung, respectively), although both are from the *japonica* subspecies. Given these different genetic backgrounds, the *cps4* and *ksl4* mutants were then compared to their respective parental lines to determine the effect of removing all *syn*-CPP derived labdane-related diterpenoids (*cps4*) or more selectively only the momilactones (*ksl4*) from the rice natural product arsenal (Xu et al., 2012).

Although the momilactones have been extensively studied as phytoalexins against the agriculturally devastating fungal blast pathogen (e.g., Tamogami and Kodama, 2000; Jung et al., 2005; Okada et al., 2007; Hasegawa et al., 2010), with similar activity attributed to the oryzalexin S also derived from *syn*-CPP (Kodama et al., 1992), the rice *cps4* mutant was no more susceptible to infection by *M. oryzae* than plants from its parental/wild-type line. By contrast, *cps4* rice exhibited much less allelopathic activity against not only the sensitive plant species lettuce, but also the agriculturally relevant noxious weed barnyard grass. Similar results were observed with the *ksl4* mutant upon comparison to its parental/wild-type line as well (Fig. 5), demonstrating that this loss of allelopathic activity can be attributed to the specific loss of the momilactones from the

complex composition of rice root exudates, which then verifies the momilactones as bona fide allelochemicals (Xu et al., 2012).

In addition to demonstrating that the momilactones are allelochemicals, certain observations with the *ksl4* mutant also have implications regarding the presence of a momilactone biosynthetic gene cluster in the rice genome. Although the presence of gene clusters for the biosynthesis of certain natural products has been noted as an emerging theme in plants (Osborn, 2010), it remains relatively uncommon, and it is unclear what drives the assembly of such clusters. While there were no obvious ill effects with the *cps4* mutant, it was notable that the *ksl4* mutant exhibited a significantly (~2-fold) reduced germination rate relative to its parental/wild-type strain. Accordingly, while their allelopathic activity provides positive selection for momilactone production, there also is a negative effect from incomplete inheritance of the necessary biosynthetic machinery (i.e., OsCPS4 in the absence of OsKSL4, as in the *ksl4* mutant). As recently highlighted by Takos and Rook (2012), such balancing selection for alternative sets of alleles leads to closer linkage of the relevant genes, as first postulated by R.A. Fisher (1930), and such suppression of recombination can be accomplished by physical proximity, providing a driving force for assembly of gene clusters such as that observed here for momilactone biosynthesis.

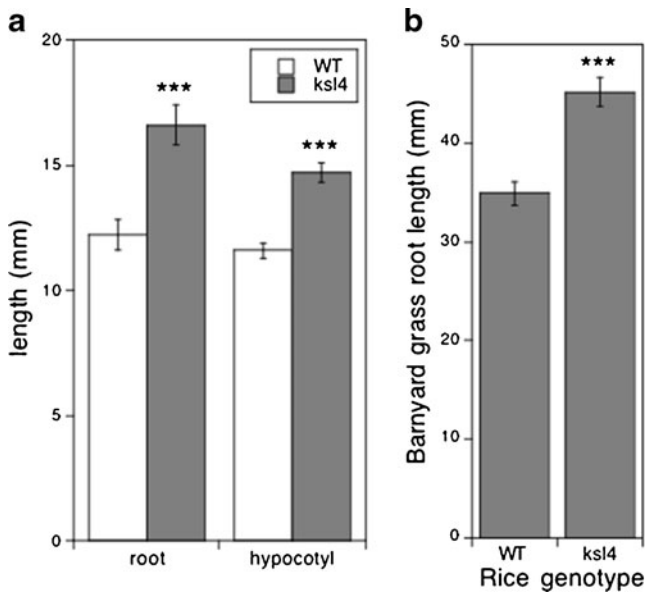


Fig. 5 Effect of OsKSL4 knock-out mutant (*ksl4*) on allelopathy (histograms depict mean length, with error bars showing the associated standard error). All comparison between parental wild-type (*WT*) and *ksl4* exhibit *P*-values < 0.001 (as indicated by ***). **a** Comparison of allelopathic effect of *WT* vs. *ksl4* rice seedlings on growth of lettuce, both roots and hypocotyls. **b** Comparison of allelopathic effect of *WT* vs. *ksl4* rice seedlings on root length of germinated barnyard grass seedlings. Reprinted with permission from Xu et al. (2012). Copyright 2011 The Authors

Future Directions

The mechanism and molecular target of the allelochemical activity of the momilactones remains unknown, as does the basis for the self-resistance of rice. These questions then provide the basis for further basic science investigations. In addition, the ability of allelochemicals to serve as endogenous natural herbicides offers significant benefits when applied to important staple crop plants such as rice. Thus, identification of momilactone B as the major allelochemical provides a molecular marker for breeding and/or engineering efforts directed at increasing rice allelopathy. However, it seems likely that the other rice labdane-related diterpenoids may also serve important roles, as suggested by the presence of another such biosynthetic gene cluster in the rice genome (Swaminathan et al., 2009). Accordingly, such efforts should be approached with some caution to avoid deleterious effects that might arise from suppression of the production of these alternative natural products. For example, simply redirecting metabolic flux towards only momilactone biosynthesis might lead to increased susceptibility to microbial disease if the other such rice labdane-related diterpenoids serve as effective antibiotics against any such pathogens.

Conclusions

Rice has been shown to possess allelopathic activity and release allelochemical(s) based on field and laboratory experiments in many countries (Dilday et al., 1998; Kim et al., 1999; Olofsdotter et al., 1999; Azmi et al., 2000; Kato-Noguchi and Ino, 2001). While a large number of compounds, including phenylalkanoic acids, hydroxamic acids, fatty acids, terpenes, and indoles, have been identified as putative rice allelochemicals (Rimando and Duke, 2003; Khan et al., 2007), the studies reviewed here, including recent genetic evidence, demonstrate an important role for the momilactones in rice allelopathy, particularly momilactone B, which seems to serve as the major allelochemical in rice. Indeed, the allelopathic activity of the momilactones presumably has played an important ecological role in rice evolution, the relevance of which seems to be reflected in the presence of a dedicated biosynthetic gene cluster in the rice genome. Perhaps more importantly, identification of momilactone B as the major allelochemical in rice provides a molecular marker for breeding and/or engineering efforts directed at increasing allelopathy of this critical staple food crop.

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