Review: Rice momilactones, potential allelochemical for weeds suppression

AMY ESTIATI
Research Center for Biotechnology, Indonesian Institute of Sciences. Jl. Raya Bogor Km. 46 Cibinong, Bogor 16911, West Java, Indonesia.
Tel.: +62-87545587, Fax.: +62-21-8754588, email: nugroho_amy@yahoo.com


Abstract. Estiati A. 2019. Rice momilactones, potential allelochemical for weeds suppression. Asian J Agric 3: 6-15. Weeds become one of the important biological constraint declining the productivity and quality of rice. Among the weeds, barnyardgrass is reported as the most destructive weed species. Synthetic herbicides are preferred method to control weeds. However, the excessive and continuous use of synthetic herbicides can have a negative impact on the environment, health and even the emergence of herbicide-tolerant weeds. Therefore, another alternative to overcome weed problems become the concern of scientists. Rice plants have been proven to be able to suppress the growth of weeds nearby by secreting secondary metabolites called allelochemicals. In this article, the achievements of research on rice allelochemicals at laboratory level will be reported. Among rice allelochemicals, momilactones are potential growth inhibitor. The biosynthetic pathway of momilactones and its corresponding genes have been extensively investigated in rice. OsCPS4, OsKSL4, CYP99A2, CYP99A3 and OsMAS are genes that co-regulated in momilactones biosynthetic pathway and production, and they form a gene cluster which is located on chromosome 4. Reverse genetic approach by inserting genes knock-out of OsCPS4 and OsKSL4 into two rice cultivars from Japonica subspecies showed that insertion mutant lines harboring cps4 or ksl4 exhibited a significant loss in inhibition potential due to the lack of momilactones production.

Keywords: Allelochemicals, growth inhibitor, momilactones, rice, weeds

INTRODUCTION

Rice (Oryza sativa L.) become a staple food in many regions in the world especially in Asia, with globally provides approximately 20% of the caloric intake to more than 50% of the world’s population (Khanh et al. 2013; Siddique and Ismail 2013; Amb and Abluwalia 2016). Increasing human population has to be in line with rice production to fulfill food security. However, cultivated rice is always faces problems that come from biotic and abiotic stresses which leads to a decrease in productivity and quality. Weeds, insect-pests, diseases and abiotic stresses are major threats to rice production and climatic changes can worsen the situation (Faroaq et al. 2013). Weeds infestation are reported as the main biological constraint in reducing rice production. The loss of yield due to weeds infestation is greater than the combined yield losses caused by insect pests and diseases (Asaduzzaman et al. 2010; Khang et al. 2016).

In paddy fields, cultivated rice and weeds always grow together although both plants compete for the same needs i.e. water, nutrient, light, space and requirements for photosynthesis (Khanh et al. 2013). Weeds has competitive ability over cultivated rice since weeds grow taller and faster, therefore the presence of interfere weeds is a persistent problem for rice (He et al. 2012).

Over the world, more than 1000 weeds species have been reported in paddy fields (Khanh et al. 2013). However, 12 of them are the important weeds species in Asia, i.e. Cyperus difformis L. (smallflower umbrella), Cyperus iria's L. (rice flatsedge and umbrella sedge), Echinochloa crus-galli (L.) Beauv (barnyardgrass), Echinochloa colona (L.) Link. (jungle rice), Eclipta prostrata (L.) L. (false daisy), Finibrystylis miliacea (L.) Vahl (lesser fimbristylis), Ischaemum rugosum Salish. (wrinkle duck beak), Leptochloa chinensis (L.) Nees (chinese sprangletop), Ludwigia hyssopifolia, Schoenoplectus juncoides, Sphenoclea zeylanica Gaertn (Gosseweed), Oryza sativa L. (weedy rice) (Rice Knowledge Bank). Among 12 weeds, barnyardgrass is reported as the most destructive weed and one of the most serious herbicide-resistant weeds (Tanveer et al. 2012; Zeinali et al. 2013). The presence of barnyardgrass together with rice plants in paddy fields can reduce rice production. When the ratio of rice plants to barnyardgrass in paddy fields is 10:1, this weed can reduce rice biomass and yields by 75% and 50%, respectively (He et al. 2012; Gealy et al. 2013; Yang et al. 2013).

Synthetic herbicides are believed can control weeds problems effectively, prevent crop yield loss and reduce labor in weeding. However, the use of synthetic herbicide can raise another problems such as high cost for crop production, the use of non-renewable energy resources, the excessive and overuse of herbicides might lead to the evolution of resistance in some paddy weeds to herbicides. Moreover, the negative effects to the environment and human health could not be neglected (Fereira and Reindhart 2010; Bhadoria 2011; Mohammadi 2013). Thus, an alternative method to control weeds problem by...
minimizing the use of synthetic herbicide should be necessary to be considered.

Allelopathy is reported as a self defense mechanism of rice plants against weeds by suppressing weeds growth. Although, in the rice fields, weed-suppressive effect is more complex. It is a combination of competition for resources and the release of allelochemicals from rice varieties (Kato-Noguchi and Ino 2001; Kong et al. 2008). Laboratory bioassay is one of the appropriate methods for conducting preliminary studies on allelopathy because it was conducted under controlled environment that can distinguish allelopathy effect from other competitive interference (Kato-Noguchi and Ino 2001). This paper reviews the published literatures focused on allelopathy potential in rice plants and determine the allelopathy compounds that play an important role in inhibiting weed growth at laboratory level.

ALLELOPATHY, AN ALTERNATIVE TO SYNTHETIC BIOHERBICIDES

Allelopathy is defined as any direct or indirect harmful or beneficial effects on germination, growth and development of one plant on another through the production of chemical compounds called allelochemicals that release into the environment (de Bertoldi et al. 2009; Bhadoria 2011; Bravo et al. 2013; Khanh et al. 2013). Although allelopathy has negative or positive effect, however many ecologist tend to interpret that allelopathy has only negative effect from one plant to the surrounding plants which is considered as interfere plants by inhibiting their growth. This mechanisms occur in an attempt to defend plants themselves (Bhadoria 2011; Bravo et al. 2013). The negative impacts of allelochemicals are harmful to the receiver plants but give a benefit to the donor plants (Bravo et al. 2013).

Many crops including rice have been reported possess allelopathy properties (Dilday et al. 1989; Bhadoria 2011; Bravo et al. 2013; Amb and Ahluwalia 2016). In order to select rice accessions with strong allelopathic, International Rice Research Institute in the Philippines had been developed well-designed bioassays in laboratory level under controlled environments called relay-seedling assay. This method eliminated the effect of resource competition between rice and test plants, thus the data obtained will represent only plant allelopathy (Kato-Noguchi 2004; Kato-Noguchi and Peters 2013). Relay-seedling assay had been routinely used in screening of hundreds rice accessions and resulting in several rice accessions with strong allelopathic potential (Jensen et al. 2001; Olofsdotter et al. 2002).

By using relay-seedling assay, Olofsdotter et al. (2002) evaluated the effect of allelopathy from 50 rice cultivars on root elongation of barnyardgrass (one of the most destructive weed species) as a test plant. Thirty sterilized rice seeds were grown in Petri dish in two parallel rows and covered by Perlite. The distilled water flowed into Petri dish to maintain the moisture of Perlite. The Petri dishes were placed under 1300-3000 lx light density, with a photoperiod of 12 h and temperature between 29-33°C. On day 7, 20 barnyardgrass seeds as test plant were sown in between two rows of 7 -day-old rice seedlings in Petri dish. The root growth of barnyardgrass were measured at 17 day after incubation. The results exhibited that the roots growth of test plant were inhibited by 50 rice cultivars used with different reduction rate between 55%-81%. Data showed that 50 rice cultivars have different abilities in suppressing root growth of barnyardgrass, thus the activity of allelopathy is cultivars-specific. Based on these data, the 50 rice cultivars can be categorized as allelopathic cultivars and non-allelopathic cultivars (Olofsdotter et al. 2002). Microscope studies showed that secondary root growth of barnyardgrass were inhibited with the presence of allelopathic rice seedlings, meanwhile no significance reduction were exhibited when they were grown together with non-allelopathic rice (Figure 1).

Figure 1. The secondary root growth of barnyardgrass when they grown together with rice seedlings of (a) non-allelopathic rice (Aus 196) and (b) allelopathic rice (IR64) in Petri dish. Secondary root growth of barnyardgrass were inhibited by the presence of allelopathic rice, IR64 (Source: Olofsdotter et al. 2002)
Since cultivated rice has defence mechanisms toward weed species through the production of allelochemicals, this allelopathic compounds are considered as plant-produced herbicides (Colquhoun 2006). And, since rice allelopathy is environmentally friendly and secreted from rice itself, utilizing allelopathy to suppress weeds growth surrounding cultivated rice as a promising approach for biological control of weeds in sustainable agriculture practice (Fang et al. 2013; Khanh et al. 2013).

**RICE MOMILACTONE INVOLVEMENT IN ALLELOPATHY EFFECT**

Most allelochemicals are classified as secondary metabolites, which are not required in primary metabolic processes (growth, development and reproduction) of the allelopathic organisms (Ghahari and Miransari 2009; Belel and Rahimata 2012). Many secondary compounds such as phenolic acids, hydroxamic acids, fatty acids, terpenes and indoles were identified both in rice root exudates and rice residues as potential allelochemicals (Kato-Noguchi et al. 2010; Kato-Noguchi 2011; Kato-Noguchi 2012). However, although many secondary compounds have been reported as potential allelochemicals, only compounds that released from the plants into environment and has the ability to inhibit the growth of neighboring plants that are considered as allelochemicals (Kato-Noguchi 2008; Kato-Noguchi et al. 2010).

Phenolic acid such as p-coumaric acid, p-hydroxybenzoic acid, ferulic acid and vanillic acid are found in aqueous extracts of rice straws, roots and residues and are often mentioned as the most important among potential allelochemicals (Kato-Noguchi 2004; Hui et al. 2010; Siddique and Ismail 2013). However, their growth inhibition was relatively weak (Kato-Noguchi and Ino 2005a).

In order to investigate which secondary compounds actually possess allelopathic property in rice plants, eight Japanica type cultivars from Kinuhikari, Hinohikari, Nipponbare, Sasamikihiki, Yukihihikari, Norin 8, Kamenoo, and Koshihikari as donor plants were investigated on three sensitive plants to allelochemicals i.e. alfalfa (Medicago sativa L), cress (Lepidium sativum L.) and Lettuce (Lactuca sativa L.) as receiver plants/test plants. In this experiment, the effects of resource competition between donor and test plants such as water, nutrients and light were eliminated. Sterilized sixth day-rice seedlings were put on filter paper in Petri dish, moistened with phosphate buffer. Each of three day old test plants seedlings as describe above were then grown together with rice seedlings in Petri dish at 25°C and 12 h photoperiod. The control experiment was conducted by growing each of test plant without rice seedlings. All experiments were replicated seven times in complete randomized block designs. On three days after incubation, the lengths of roots and shoots and fresh biomass of three test plants were measured. The results showed that all of eight rice cultivars could inhibited the growth of the roots, shoots and fresh mass of three test plants with different inhibition indexes, where the greatest inhibition index were reported from Koshihikari cultivar with the precentage of inhibition indexes on alfalfa, cress and lettuce as 60.6, 63.2 and 71.9, respectively (Kato-Noguchi and Ino 2001; Kato-Noguchi 2004; Kato-Noguchi and Peters 2013) (Tabel 1; Figure 2).

In order to find an allelochemical in rice root exudates which possess the greatest allelopathic potential, rice seedlings of Koshihikari cultivar were hydroponically grown for 14 days. The culture solution was then purified by chromatographic fractionations and finally the putative compound with the inhibitory effect was isolated and the chemical structure of the putative inhibitor was determined by spectral data as momilactone B (3,20-epoxy-3α-hydroxy-synpimarin-7,15-dien-19,6β-olide, C20H26O4) (Kato-Noguchi 2004; Kato-Noguchi and Ino 2005b).

**Table 1.** Inhibition index of rice seedlings on alfalfa, cress and lettuce seedlings (Kato-Noguchi and Ino 2001)

<table>
<thead>
<tr>
<th>Rice cultivars</th>
<th>Inhibition index (%)</th>
<th>Alfalfa</th>
<th>Cress</th>
<th>Lettuce</th>
</tr>
</thead>
<tbody>
<tr>
<td>Norin 8</td>
<td>43.9</td>
<td>53.7</td>
<td>51.4</td>
<td></td>
</tr>
<tr>
<td>Kamenoo</td>
<td>22.3</td>
<td>46.6</td>
<td>53.2</td>
<td></td>
</tr>
<tr>
<td>Nipponbare</td>
<td>16.3</td>
<td>31.4</td>
<td>30.5</td>
<td></td>
</tr>
<tr>
<td>Sasamikihiki</td>
<td>29.6</td>
<td>48.2</td>
<td>51.5</td>
<td></td>
</tr>
<tr>
<td>Kinuhikari</td>
<td>22.6</td>
<td>49.2</td>
<td>43.9</td>
<td></td>
</tr>
<tr>
<td>Koshihikari</td>
<td>60.6</td>
<td>63.2</td>
<td>71.9</td>
<td></td>
</tr>
<tr>
<td>Hinohikari</td>
<td>7.4</td>
<td>33.5</td>
<td>51.1</td>
<td></td>
</tr>
<tr>
<td>Yukihihikari</td>
<td>27.1</td>
<td>30.4</td>
<td>39.9</td>
<td></td>
</tr>
</tbody>
</table>

Note: * The inhibition indexes were calculated on average of inhibition rate of root length, shoot length and fresh mass. Inhibition rate of root length, shoot length and fresh mass was scored so that those of control plants were 100%.

**Figure 2.** The growth of hypocotyls and roots of test plants i.e. (A) alfalfa; (B) cress and (C) lettuce when grown with and without rice seedlings cv. Koshihikari. The growth of test plants without rice seedlings/control (left); the growth of test plants with the presence of rice seedings/incubated with rice (right) (Kato-Noguchi 2004; Kato-Noguchi and Peters 2013)
In addition, another potential allelochemicals that was found in rice exudates of *Koshihikari* cultivar had been identified as momilactone A. However, the ability of momilactone A to suppress the growth of neighboring plants is weaker compared to momilactone B (Kato-Noguchi and Ino 2005a).

**RICE MOMILACTONES POTENTIAL FOR WEEDS SUPPRESSION**

To study the effectiveness of momilactone A and B against weed species, experiment using four weeds species had been conducted by Kato-Noguchi and Ota (2013). Four weeds species, i.e. *timothy* (*Phleum pratense* L.), *barnyardgrass* (*Echinochloa crus-galli* (L.) Beauv), *jungle rice* (*Echinochloa colona* L. Link) and *crabgrass* (*Digitaria sanguinalis* L.) were subjected to bioassay as test plants. Momilactone A and B were isolated from husks of rice cv. *Koshihikari* and dissolved in methanol and added to two sheets of filter paper in a Petri dish and allow to evaporate. The filter paper in Petri dish was moistened with 3 ml of 1 mM MES buffer. The final concentrations of momilactone A and B were 0.01, 0.03, 0.1, 0.3, 1.3, 10, 30, 100, 300, 1000, 3000 and 10000 µM. Sterilized ten germinated of each test plant seedlings were put on filter paper in Petri dish containing momilactones solution with different concentration as above. The length of shoots and roots of test plants seedlings were measured after 48 h of incubation in the darkness at 25°C. For control experiment, sterilized ten germinated seeds of each test plant was placed on filter paper in Petri dish containing MES buffer without momilactones filtrate. Percentage of inhibition was calculated following the formula: [(control plant length - plant length incubated with momilactone A or B)/control plant length] × 100. The results showed that momilactone A and B can inhibited the growth of shoots and roots of all test plants. The concentrations of momilactone A and B required for 50% growth inhibition (*I*$_{50}$) for shoots and roots of all test plants are presented in Table 2.

Table 2 showed that the *I*$_{50}$ values of momilactone A on four weeds species were 66.7-79.5 µM and 145-275 µM for roots and shoots, respectively, meanwhile, the *I*$_{50}$ values of momilactone B were 5.6-9.5 µM and 6.3-12.4 µM for roots and shoots, respectively. By comparing the *I*$_{50}$ values of momilactone A and B on roots and shoots of four weeds cultivars, it showed that inhibitory activity of momilactone B on the roots of *timothy, barnyardgrass, jungle rice* and *crabgrass* were 13.7, 13.6, 9.26, and 10.4 fold higher than that of momilactone A. And, the inhibitory activity of momilactone B on the shoots of *timothy, barnyardgrass, jungle rice* and *crabgrass* were 19.9, 23.0, 20.5, and 22.2 fold higher than that of momilactone A. Therefore, it could be suggested that momilactone B plays a more important role in weeds growth inhibition than momilactone A (Kato-Noguchi and Ota 2013; Kato-Noguchi and Ino, 2005b).

In addition, experiment using lettuce and Chinese cabbage has proven that the concentration of momilactone B at 10 µM drastically could inhibited the growth of hypocotyls of lettuce and Chinese cabbage seedlings, meanwhile the application of momilactone A at 100 µM exhibited only slightly inhibited both of test plants (Toyomasu et al. 2008). This data explained that momilactone B can suppress the growth of test plants with a concentration at 10$^{-1}$ of momilactone A. In other words, momilactone B only requires low concentration to produce a high inhibition ability against surrounding plants (Trezz et al. 2016). Extensively studied on momilactone A reported that the function of momilactone A more refer to defense mechanisms against fungal pathogen which is termed as phytoalexin (Kato-Noguchi and Peters 2013; Atawong et al. 2002). Thus, momilactones have dual functions i.e. 1) as germination and seedling growth inhibitor (allelochemicals) and 2) as antimicrobial natural products (phytoalexins) (Wang et al. 2011; Toyomasu et al. 2008).

**RICE MOMILACTONES POTENTIALLY CAN INHIBITED THE GROWTH OF BARNYARDGRASS**

Most of allelopathy studies were focused on one of the most noxious weed in rice field i.e. *barnyardgrass* (Khan et al. 2013; Kato-Noguchi and Peters 2013; Ma et al. 2014). To understand the correlation between allelopathic activity and momilactones secretion of eight rice cultivars, bioassay on *barnyardgrass* using method called donor-receiver bioassay had been conducted by Kato Noguchi (2010a). In this research, the interfere effects such as nutrients, light and pH were eliminated during the period of bioassay, thus the inhibitor effect of rice toward *barnyardgrass* was solely due to an allelopathy effect.

Sterilized seeds of eight rice cultivars i.e. *Kinuhikari*, *Hinohikari*, *Nipponbare*, *Sasanishiki*, *Yukihikari*, *Norin 8*, *Kameno*, and *Koshihikari* were germinated on a moist filter paper in Petri dish and incubated in growth chamber at 25°C and 12 h photoperiod. Four days after incubation, seedlings of each of eight rice cultivars with uniform roots and shoots length were transferred in Petri dish containing two sheets of filter paper moistened with MES buffer and allow to grow for another three days. In the meantime, sterilized seeds of *barnyardgrass* were grown on a moist filter paper in Petri dish at 25°C in the darkness condition for three days. Further, *barnyardgrass* seedlings with uniform roots and shoots length were then moved into Petri dish that already contains 7-day-old rice seedlings and they

<table>
<thead>
<tr>
<th>Cultivars</th>
<th>Momilactone A</th>
<th>Momilactone B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Root</td>
<td>Shoot</td>
</tr>
<tr>
<td><em>Timothy</em></td>
<td>76.5±6.3</td>
<td>157±12</td>
</tr>
<tr>
<td><em>Barnyardgrass</em></td>
<td>91.2±7.2</td>
<td>145±11</td>
</tr>
<tr>
<td><em>Jungle rice</em></td>
<td>66.7±5.4</td>
<td>238±21</td>
</tr>
<tr>
<td><em>Crabgrass</em></td>
<td>98.5±7.3</td>
<td>275±19</td>
</tr>
</tbody>
</table>
Rice momilactone B play an important role in inhibiting the growth of barnyardgrass and their secretion was induced by the presence of weeds

Rice momilactone B was reported to play an important role in weeds growth inhibition than momilactone A. To study the presence effect of barnyardgrass on the concentration of secreted momilactone B from rice seedlings and its inhibitor activity as well, bioassay where rice seedlings were incubated with (mixed-incubation) and without (mono-incubation) barnyardgrass seedlings had been conducted by Kato Noguchi (2011b). Fifty sterilized seeds of rice cv. Koshihikari and 50 barnyardgrass seeds were grown separately on moist filter paper in a growth chamber at 25°C and 12 h photoperiod for 10 days. Ten-day-old uniform rice and barnyardgrass seedlings were then transferred onto a holed plate of polystyrene foam that was floated on Hoagland medium with pH 6.0 in plastic container at 25°C and 12 h photoperiod. After 10 days of incubation, rice seedlings were harvested for barnyardgrass bioassay and momilactone B was determined.

<table>
<thead>
<tr>
<th>Rice cultivars</th>
<th>Concentration (µmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Momilactone A</td>
</tr>
<tr>
<td>Kinuhikari</td>
<td>0.21a</td>
</tr>
<tr>
<td>Hinohikari</td>
<td>0.27b</td>
</tr>
<tr>
<td>Nipponbare</td>
<td>0.29b</td>
</tr>
<tr>
<td>Sasamishiki</td>
<td>0.34bc</td>
</tr>
<tr>
<td>Yukihikari</td>
<td>0.40c</td>
</tr>
<tr>
<td>Norin 8</td>
<td>1.03d</td>
</tr>
<tr>
<td>Kamenoo</td>
<td>1.17d</td>
</tr>
<tr>
<td>Koshihikari</td>
<td>1.45f</td>
</tr>
</tbody>
</table>

Barnyardgrass bioassay was conducted by growing five-day-old uniform barnyardgrass in Petri dish containing rice extracts which is homogenized from 10 g fresh weight of rice seedlings. The control experiment did not contain rice extracts. The length of roots and shoots of barnyardgrass were measured 48 h after incubation in the darkness at 25°C. Inhibitor activity (%) was calculated by the formula: [ (control plant length-plant length treated with rice extract) /control plant length ] x 100. To determine momilactone B, 10 g fresh weight of rice seedlings were homogenized with aqueous methanol and homogenate was filtered through filter paper No.2. This procedure was repeated two times to have an aqueous residue and further loaded into a coloumn of synthetic polystyrene adsorbent, purified and finally momilactone B was calculated following method described by Kato-Noguchi et al. (2002).

Bioassay where rice seedlings were incubated without (mono-incubation) barnyardgrass seedlings was conducted in the container. Barnyardgrass root exudates was resulted by growing barnyardgrass seedlings onto a holed plate of polystyrene foam for 10 days. Then, the medium in the
container was collected and filtered and the filtrate was
loaded onto a column of synthetic polystyrene adsorbent,
eluted with methanol, evaporated and dissolved with
methanol again and added to a filter paper in incubation
container. Next step, 1L of Hoagland nutrient solution were
poured into incubation container with different final
concentration of barnyardgrass root exudates in the
medium i.e. 0, 10, 30, 100, 200 and 300 mg/L and the
uniform rice seedlings were transferred onto a holed plate
of polystyrene foam and floated in the container containing
different concentration of barnyardgrass root exudates as
explained above for 10 days. At 10 days after incubation,
the rice seedlings were then harvested and used for
barnyardgrass bioassay and momilactone B was also
quantified. Barnyardgrass bioassay and determination of
momilactone B in rice seedlings were performed by the
same methods as above.

The results from mixed- and mono-incubation showed
that the extracts resulted from mono-incubated rice could
inhibited the roots and shoots of barnyardgrass as 15% and
12%, respectively. Meanwhile, the extracts from mixed-
incubated rice could inhibited 79% and 75% of roots and
shoots growth of barnyardgrass, respectively. Thus,
mixed-incubation induced 5.3-fold increase in roots
inhibition and 6.3-fold increase in shoots inhibition
comparing with mono-incubated rice. In line with
allelopathy activity, the concentration of momilactone B
was also higher in mixed-incubated rice than in mono-
incubated rice. In high allelopathic rice varieties, the concentration of momilactone B per day
can reach 2-3 µg (Soltys et al. 2013).

In rice plants, momilactone B was released into the
environment during their life cycle and the released level
was increasing until flowering initiation and afterwards
was decreased (Kato-Noguchi 2008; Kato-Noguchi et al.
2013). The concentration level of momilactone B at day 80
(around flowering) was 58-fold higher than at day 30
(Figure 8) and the level of momilactone B in the shoots
was 3.8-fold greater than in the roots (Kato-Noguchi et al.
2011a; Kato-Noguchi and Ino 2005b). In high allelopathic
rice varieties, the concentration of momilactone B can reach 2-3 µg (Soltys et al. 2013).
Biosynthesis of momilactones and identified genes responsible for momilactones production

The biosynthetic pathway of momilactones and its corresponding genes have been extensively investigated in rice (Okada et al. 2016). Genetic evidence is provided indicating that they form a gene cluster for momilactones production, located on chromosome 4 which includes copalyl diphosphate synthase 4 (OsCPS4), kaurene synthase-like 4 (OsKSL4), two cytochromes P450 (CYP), genes (CYP99A2 and CYP99A3), and a short chain alcohol dehydrogenase (SDR) (Kato-Noguchi and Peters. 2013; Miyamoto et al. 2014; Wang et al. 2011) (Figure 4). CYPs genes and a putative dehydrogenase gene (AK103462/OsMAS) are involved in the downstream steps of momilactones biosynthesis (Shimura et al. 2007; Xu et al. 2012; Miyamoto et al. 2014).

Momilactone biosynthesis is initiated by dual cyclization reactions (Figure 5). The initial cyclization of common diterpenoid precursor (E,E,E)-geranylgeranyl diphtophosphate (GGPP) to syn-copalyl diphtophosphate (syn-CPP) is catalyzed by copalyl diphtophosphate synthase OsCPS4 (Xu et al. 2012; Okada et al. 2016). Further cyclization of syn-CPP to (9β-H)-pimarane-7,15-diene is catalyzed by (9β-H)-pimarane-7,15-diene synthase OsKSL4. Cytochromes P450 are involved in the downstream oxidation of the (9β-H)-pimarane-7,15-diene. The involvements of one of cytochromes P450, CYP99A3, was found to catalyze consecutive oxidations of the C10 methyl group of the momilactone precursor, (9β-H)-pimara-7,15-diene to form sequentially (9β-H)-pimara-7,15-dien -19-ol, (9β-H)-pimara-7,15 dien -19-al, and (9β-H)-pimara-7,15 dien -19-oic acid. Then, the (9β-H)-pimara -7,15 - dien 19- oic acid give rise to 3 β -hydroxy - (9β-H)-pimara-7,15 dien-19,6 β -olide and finally, the 3 β -hydroxy - (9β-H)-pimara-7,15 dien-19,6 β -olide is converted to momilactone A by AK103462 (OsMAS) (Okada et al. 2009; Zhao et al. 2018). To produce momilactone B, it is proposed to form from momilactone A through C20-hydroxylation and hemiketal ring closure (Wang et al. 2011; Zhao et al. 2018). In this biosynthetic pathway, CYP99A2 as one of cytochromes P450 is involved in a lesser extent comparing with CYP99A3 (Zhao et al. 2018).

The involvements and the important role of OsCPS4 and OsKSL4 in momilactones biosynthetic pathway and momilactones production, had been proven by selective removal those gene using gene knock-out experiments (Xu et al. 2012). Reverse genetic approach by insertion genes knock-out of OsCPS4 and OsKSL4 in Zhonghua 11 and Hwayoung rice cultivars had been conducted (Xu et al. 2012). Bioassays to determine the effect of removing cps4 or ksl4 on plant suppression was conducted by growing homozygous insertional mutant lines either harboring cps4 or ksl4 together with lettuce (the sensitive plant species) or barnyardgrass (noxious weed) in Petri dishes without any supplied nutrients to avoid any confounding. The control experiment was conducted by growing barnyardgrass or lettuce together with wild type rice seedlings. Parameters observed in this experiment was the length of roots and hypocotyls after 6 or 12 days after incubation for lettuce and barnyardgrass, respectively.

The results showed that homozygous insertional mutant lines harboring either cps4 or ksl4 exhibited the decreasing in allelopathy potential on both lettuce and barnyardgrass as shown in Figure 6 and 7. The length of roots and hypocotyls of lettuce and barnyardgrass were longer when they were grown together with mutant rice lines seedlings comparing with their wild type rice. In other words, the wild type has allelopathy effect greater than mutant lines. It is suggested that in mutant rice lines, they lose their ability to suppress the growth of neighboring plants due to the loss of OsCPS4 or OsKSL4 gene expression which directly influence momilactones production (Xu et al. 2012; Kato-Noguchi and Peters. 2013; Kato-Noguchi and Ota 2013). Thus, it is proved that momilactones have a direct role for allelopathy ability. However, phenotypically, those mutant lines exhibited normal growth through all stages of development (Xu et al. 2012).

In addition, it was reported that the OsTGAP1 is an elicitor-inducible rice basic leucine zipper (bZIP) transcription factor which is play an important role for momilactones biosynthesis and regulates the expression of all five genes in the cluster (Figure 4). Knock-out mutant line of OsTGAP1 showed no expression of OsCPS4, OsKSL4, CYP99A2, CYP99A3 and OsMAS and the absence of momilactones production. Therefore, OsTGAP1 is proven as a key regulator for elicitor-inducible production of momilactones and to coordinate the transcription of all five genes essential for momilactones production (Okada 2009; Zhao et al. 2018).

Figure 4. Rice momilactones biosynthetic gene cluster (Xu et al. 2012)
Figure 5. Biosynthesis pathway of momilactone A and B (Zhao et al. 2018)

Figure 6. Effect of OsKSL4 and OsCPS4 knock-out mutants rice (ksl and cps4) on allelopathy potential against lettuce (A and C) and barnyardgrass (B and D). Comparison the length of roots and hypocotyls seedlings of lettuce and barnyardgrass when they were grown together with mutant rice versus their wild type rice seedlings (Xu et al. 2012)
Discussion

One of the most important factors that play a role in declining yield and the quality of rice is weeds. In paddy fields, cultivated rice and weeds always grow together for the same needs. Among weeds species, *barnyardgrass* was reported as the most destructive weed and the most serious herbicide-resistant weed (Zhang et al. 2018). Farmers always control weeds by using synthetic herbicide. However, the overuse and continuous use of synthetic herbicide can lead to the emergence of herbicide-resistant weeds and moreover give the negative effects to the environment and human health. Therefore, an alternative method to control weeds problem by minimizing the use of synthetic herbicide should be necessary to be considered.

Rice, actually has self defense mechanisms by secreting phytotoxic compounds into the environment that can suppress the germination and growth of weeds nearby. This kind of self defense is called allelopathy (Heidarzade et al. 2012; Zhang et al. 2018). Allelopathy is important to prevent rice plants from interfere weeds. Allelopathy is defined as any direct or indirect harmful effects of one plant on another through the production of chemical compounds that releases into the environment called allelochemicals. There are many secondary compounds are potential allelochemicals. The researchers are then interested in studying which allelochemicals as the most responsible compounds for inhibiting weed growth. This article summarized the published literatures to determine the important compounds of rice allelochemicals that play an important role in suppressing weed growth. All experiments has been conducted in laboratories to eliminate other resource competitive interference. Therefore, the data obtained from the experiments solely reflects the allelopathic effect.

Although rice has the abilities in suppressing root growth of *barnyardgrass*, however, its abilities is varies among cultivars. Thus, the activity of allelopathy is cultivars-specific. From microscopic studies, rice cv. *IR64* with potential allelopathic can inhibited the growth of secondary roots of *barnyardgrass* but not with non-allelopathic rice plant cv. Aus 196 (Olofsdotter et al. 2002). Study in more detail explained that among allelochemicals, momilactone A and B are suggested as the most important compounds with allelopathic potential although momilactone B is play a more important role in weeds growth inhibition than momilactone A (Kato-Noguchi and Ota 2013; Kato-Noguchi and Ino 2005a). Momilactone A function is reported more as defense mechanisms against fungal pathogen called phytoalexin.

The genes involved in momilactones biosynthetic pathway and directly affected momilactones production, form a gene cluster consisted of *OsCPS4, OsKSL4, CYP99A2, CYP99A3* and *OsMAS* located on chromosome 4 and all of these five genes are coordinately regulated by *OsTGA1*. In order to prove the involvement of *OsCPS4* and *OsKSL4* genes in momilactones biosynthetic pathway and production, a reverse genetic approach by using gene knock-out of both genes has been conducted by Xu et al. (2012). The homozygous *cps4* or *ksl4* knock-out lines exhibited a significant loss in suppressing the growth of lettuce and *barnyardgrass*. These data become a direct evidence that the loss of allelopathic ability in mutant lines to suppress the neighboring plants is due to the loss of *OsCPS4* and *OsKSL4* genes expression as well as momilactones production (Xu et al. 2012). The same results were obtained in knock-out mutant line of *tgap1* which exhibited no expression of *OsCPS4*, *OsKSL4*, *CYP99A2*, *CYP99A3* and *OsMAS* and no momilactones production. Thus, it could be concluded that momilactones is play an important role in allelopathy activity.

In the future, momilactones as natural plant growth suppressants which is environmental friendly, could be contributed as an alternative to the use of synthetic herbicides in weed management strategy. However, bioassay in greenhouse that imitate the situation in the field and continued with field studies to demonstrate the efficacy of momilactones still need to be carried out.

REFERENCES


Bravo HR, Copaja SV, Lamborot M. 2013. Phytotoxicity of phenolic acids from cereals. DOI: 10.5772/55942


