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# Evaluation of Allelopathic Potential and Quantification of Momilactone A,B from Rice Hull Extracts and Assessment of Inhibitory Bioactivity on Paddy Field Weeds

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Rice (*Oryza sativa* L.) hull extracts were used in a bioassay to evaluate the allelopathic potential of rice on the germination and growth of barnyard grass (*Echinochloa crus-galli* P. Beauv. var. *oryzicola* Ohwi), to quantify momilactone A and B levels in rice hull germplasm and to assess the inhibitory bioactivity of momilactone A and B as a potential natural source of herbicide for weed control in paddy fields. Four varieties of weeds including *E. crus-galli* P. Beauv. var. *oryzicola* Ohwi, *Monochoria vaginalis* var. *plantaginea*, *Scirpus juncoides*, and *Eleocharis kuroguwai* were tested in the paddy field. Of 99 rice varieties, the top five including Noindari exhibited inhibition effects greater than 50% in average inhibition of dry weight (AIDW). Noindari among them exerted the strongest effect (55.6%). The next five in the ranking exhibited inhibition effects of greater than 40%. Also, 46 varieties had inhibition effects between 20 and 40%, and 29 varieties had inhibition effects greater than 10%. Fourteen varieties had very low inhibitory effects (less than 10%), the lowest of which was Heunbe (4.7%). These varieties showed a mean inhibition of 19.8% for germination rate (GR), 9.9% for germination percentage (GP), 16.6% for leaf dry weight (LDW), 38.9% for straw dry weight (SDW), and 26.8% for root dry weight (RDW). Rice varieties were classified into six categories based on their total momilactones (TMs) (momilactone A + momilactone B). The highest level of momilactone A was found in the Baekna rice variety (34.7 $\mu$g g$^{-1}$), and Baekgwangok contained the highest level of momilactone B (37.8 $\mu$g g$^{-1}$). In allelopathic potential with genetic properties and morphological characteristics, the total inhibition rate (TIR) was 18.3% for Korean rice varieties, 19.0% for middle maturing varieties, 17.8% for colorless hull varieties, 18.3% for awn varieties, and 19.0% for colorless awn varieties. In addition, Korean varieties showed higher TMs (4.5 $\mu$g g$^{-1}$) as compared with varieties that were late maturing (4.4 $\mu$g g$^{-1}$), had colorless hulls (4.1 $\mu$g g$^{-1}$), awns (4.7 $\mu$g g$^{-1}$), and colorless awns (4.8 $\mu$g g$^{-1}$). Momilactone A levels were generally higher than momilactone B levels. Total inhibition rates on barnyard grass correlated with average inhibitions of germination (AIG) ($r^2 = 0.62$***), AIDW ($r^2 = 0.92$***), and were affected by the inhibition rate of GP ($r^2 = 0.57$***). Regions of origin for rice varieties correlated with the AIG ($r^2 = -0.23$***), and maturing time showed a positive correlation with SDW ($r^2 = 0.15$**) and RDW ($r^2 = 0.19$**). Levels of momilactones were also correlated with the region of origin ($r^2 = -0.32$***), maturing time ($r^2 = 0.13$***), and awns (momilactone A, $r^2 = 0.23$***; momilactone B, $r^2 = 0.14$**), suggesting that rice varieties with awns, Korean varieties, and varieties with later maturing times contain higher levels of momilactone. Also, the investigation of the momilactone A and B bioactivity (0, 250, 500, 1000, 2000, and 4000 g a.i./ha) for weed control in paddy fields indicated that momilactones A and B exhibited no toxicity (0 in all concentrations) against rice plants, and the inhibitory bioactivity on weeds with momilactone A was higher than that of momilactone B. When compared with no momilactone control, the highest inhibitory effect (50%) on *E. crus-galli* P. Beauv. var. *oryzicola* Ohwi was shown on the 14th day after the application of momiactone A. The inhibitory effect increased with the concentration of the compound from 250 to 4000 g a.i./ha. Furthermore, momilactone A showed greater suppression than momliactone B toward toward *E. crus-galli* P. Beauv. var. *oryzicola* Ohwi, *M. vaginalis var.* plantaginea, *S. juncoides*, and *E. kuroguwai.* In broad weed species, momilactone A showed the highest inhibitory effect (90% in4000 g a.i./ha) on *S. juncoides*, 14 days after the application as compared with no momilactone control. Further studies on allelopathic effects and momilactones from the germplasm of rice varieties using genetic properties and morphological characteristics may facilitate the development of rice varieties with higher allelopathic potential. Momilactones A and B present in rice hulls may serve as a potential natural herbicide source for weed control in paddy fields reducing the dependence on synthetic herbicides.

**KEYWORDS: Allelopathy; rice; *Oryza sativa* L.; weeds; barnyard grass; bioassay; momilactone A,B; bioactivity; GC/MS**

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## INTRODUCTION

Barnyard grass (*Echinochloa crus-galli*) is one of the most noxious weeds in rice fields, and the yield decreases in rice cropping systems. To control paddy weeds, agricultural systems worldwide currently use three million tons of herbicides per year. In Korea, herbicide use has increased to approximately 0.01 million tons per hectare over the last 30 years (*1*). The increasing use of herbicides has led to environmental problems, tolerant agricultural crops, and human health concerns (*2*). Allelopathic plant properties can be successfully exploited for weed management, and any direct or indirect effects between plants and microorganisms can be controlled through volatilization, leaching, and root exudations during the decomposition of plant residues in the soil (*3*). Many researchers have carried out evaluations of allelopathic effects on various plants including rice, and such effects could be used as a promising method for the biological control of weeds in rice ecosystems (*2*, *4*−*9*). Dilday and colleagues (*4*) screened approximately 12000 rice accessions from U.S. Department of Agriculture/Agricultural Research Service rice germplasm and found allelopathic potential for rice on ducksalad, one of most prevalent aquatic weeds of rice in the United States. Since then, the International Rice Research Institute (IRRI) has been actively studying rice allelopathy in Japan and Korea. Ahn and Chung (*8*) and Chung et al. (*10*) evaluated the allelopathic potential of rice on barnyard grass (*E. crus-galli* Beauv. var. *oryzicola* Ohwi) with extracts of rice leaves, straw, and hulls. They reported higher allelopathic effects in Korean rice varieties, in middle maturing varieties, and in varieties with hull and awn color. In addition, extraction conditions can affect allelopathic potential: Warm extracts exhibited a greater inhibitory effect on barnyard grass germination, seedling growth, weight, and caloric content than hot extracts because temperature can influence the activity and saturation of allelochemicals. Other studies reported that allelopathic effects show significant differences between rice cultivars, different parts of the rice plant, and extract conditions such as temperature, solvent, and time (*4*, *5*, *9*, *11*). Comparing allelochemicals obtained from different extract conditions, we found that extraction time and temperature had the most important influence on allelochemical analysis results (*7*, *8*, *12*).

Secondary plant metabolites such as terpenoids, steroids, phenols, coumarins, alkaloids, and flavonoids are also involved in allelopathy (*13*). Phenolic compounds are most widely studied with regard to phytotoxicity (*14*). Phenolic compounds, diterpenoid substances, momilactones, long chain hydrocarbons, and fatty acids are expected to play significant functions in rice allelopathic effects. Among allelopathic compounds identified in aqueous extracts of rice (leaves, stems, and hulls), the presence of phenolic compounds was detected, including *p*-hydroxybenzoic, vanillic, *p*-coumaric, and ferulic acids (*12*, *15*−*17*). However, it is improbable that these phenolic compounds demonstrate allelopathic effects of rice because their soil concentrations never reach phytotoxic levels. Chou and Lin (*15*) have identified six allelopathic compounds similar to *p*-hydrobenzoic acid by thin-layer chromatography from decomposing rice straw. Chung et al. (*12*) noted that *p*-coumaric acid, *m*-coumaric acid, ferulic acid, and *p*-hydrobenzoic acid from rice hull extracts at a concentration of $10^{-3}$ M exhibited high inhibitory effects of more than 90% on barnyard grass germination. Furthermore, Song et al. (*17*) reported that long chain fatty acids, phenols, phthalic acids, and benzene derivatives were isolated from root exudates of rice varieties such as Sathi, Jinmi, and Nongan at the yellow ripening stage and that root exudates containing these chemicals significantly inhibited the height, dry weight, and number of barnyard grass tillers.

Several studies indicated that momilactones A and B isolated from rice leaves, straw, and hull extracts and root exudates using liquid chromatography/tandem mass spectrometry and gas chromatography/mass spectroscopy (GC/MS) are associated with rice allelopathy properties (*18*−*24*). Kato et al. (*19*) reported that momilactones A and B isolated from rice husks suppressed the germination of lettuce seeds and the growth of rice roots. Momilactone B showed a stronger inhibition than momilactone A (*18*) by inhibiting root and hypocotyl growth of cress at concentrations greater than 3 $\mu$M and those of lettuce at concentrations greater than 30 $\mu$M (*21*, *22*). Weston and Duke (*25*) reported that momilactones are the most phytotoxic compounds identified in rice hull extracts. Also, Chung et al. (*24*) isolated momilactones A and B from ethyl acetate extract of rice hulls, in which both momilactones exhibited high inhibitory effects against *Lemna paucicostata*, particularly momilactone B, which completely inhibited germination of all three weed species *Leptochloachinensis* L., *Amaranthus retroflexus* L., and *Cyperus difformis* at 20 ppm in culture tubes. Furthermore, the herbicidal activity of one new compound, lanast-7,9(11)-dien-3α,15α-diol-3α-D-glucofuranoside, was found in ethyl acetate extracts of rice hull and exhibited great inhibition against the growth of duckweed (*26*). On the basis of bioassays of seed germination and seedling growth with identified momilactones, these researches suggest that momilactones may have an important role in the rice allelopathy. Also, momilactones A and B have been conducted as useful molecular markers for studying rice allelopathic responses at the molecular level (*21*, *22*, *27*).

Although many studies have attempted to evaluate the allelopathic activity of various rice parts and to analyze allelochemicals, phenolic compounds, and momilactones in rice, little information is available on the correlation between bioassays and one of the potential allelochemicals such as momilactones A and B from rice germplasm hulls. The objectives of this study were to evaluate the allelopathic effects in rice hull extracts, quantify the concentration of momilactones A and B in rice hulls, and further characterize the varieties by genetic properties and morphological characteristics. We also assess the inhibitory bioactivity of momilactones A and B on weeds in the paddy field.

## MATERIALS AND METHODS

Ninety-nine rice (*Oryza sativa* L.) varieties were grown at the Konkuk University farm and harvested in October 2003. The agronomic characteristics such as maturing time, colored and colorless hulls, colored and colorless awns, and awn or awnless varieties are exhibited in **Table 1**. Hulls from harvested plants were separated, then dried at 24 °C for 7 days, and stored at −35 °C until required for bioassays and analysis of momilactones A and B. Analyses of momilactones were performed by GC/MS. Barnyard grass (*E. crus-galli* P. Beauv. var. *oryzicola* Ohwi) seeds were collected in October 2003. Seeds were floated in distilled water ($dH_2O$) to remove contaminants and then stored at −40 °C until bioassay use.

**Preparation and Bioassay of Rice Hull Extracts.** Dried rice hulls were ground with a Wiley mill through a 40 mesh screen, and then, powdered hulls (5 g) were soaked in 100 mL of $dH_2O$ for 24 h at 24 °C in a lighted room. Extract solutions were filtered through four layers of cheesecloth to remove debris, after which representative 5 g samples

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**Table 1.** Evaluation of Allelopathic Potential and Momilactones A and B on Barnyard Grass Using Hull Extracts from 99 Rice Varieties

| | | | | | | inhibition (%) | | | | | | $\mu g\ g^{-1}$ | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | varieties characteristics | | | | | germination | | dry weight | | | | momilactones | | |
| varieties | O[a] | M[b] | H[c] | A[d] | C[e] | GR | GP | LDW | SDW | RDW | AIDW | MA[f] | MB[g] | TM[h] |
| 143 (PI 274471) | 1 | 1 | 2 | 1 | 1 | 17.0 | 10.8 | 2.7 | 16.0 | 27.1 | 15.4 | 11.0 | nd | 11.0 |
| AC 1423 | 2 | 2 | 1 | 1 | 2 | 16.3 | 11.5 | 52.7 | 53.6 | 48.8 | 51.7 | nd | 3.5 | 3.5 |
| Agudo | 1 | 2 | 2 | 1 | 1 | 10.2 | 14.2 | 10.0 | 37.3 | 43.6 | 30.3 | 5.8 | nd | 5.8 |
| Arongbyeo | 1 | 2 | 2 | 1 | 1 | 17.7 | 8.1 | 8.7 | 25.0 | 10.2 | 14.6 | 1.2 | nd | 1.2 |
| B1293b-pn-24 | 2 | 3 | 2 | 2 | 2 | 9.5 | 5.4 | 9.0 | 48.0 | 36.0 | 31.7 | 3.2 | 2.2 | 5.4 |
| Badolbyeo | 1 | 2 | 2 | 1 | 2 | 33.3 | 9.5 | 15.8 | 52.9 | 54.4 | 41.0 | 18.2 | 3.9 | 22.1 |
| Baekchalbyeo | 1 | 3 | 2 | 1 | 2 | 27.9 | 15.5 | 15.7 | 38.8 | 33.8 | 29.4 | 14.5 | 3.8 | 18.3 |
| Baekgwangok | 1 | 3 | 2 | 1 | 1 | 23.1 | 13.5 | 0 | 55.8 | 52.5 | 36.1 | 16.8 | 37.8 | 54.6 |
| Baekhaedal | 1 | 3 | 2 | 1 | 1 | 19.7 | 12.2 | 28.0 | 59.1 | 63.9 | 50.5 | 1.7 | 3.5 | 5.2 |
| Baekjicheongbyeo | 1 | 3 | 2 | 1 | 2 | 12.3 | 14.9 | 40.0 | 60.5 | 57.1 | 52.5 | 5.2 | 3.9 | 9.1 |
| Baekjo | 1 | 2 | 2 | 2 | 2 | 25.9 | 16.2 | 36.1 | 63.7 | 56.1 | 52.0 | 8.9 | 2.8 | 11.7 |
| Baekkyeongjo | 1 | 2 | 2 | 1 | 2 | 31.3 | 14.9 | 28.7 | 53.0 | 52.0 | 44.5 | 20.2 | 2.7 | 22.9 |
| Baekmangjo | 1 | 1 | 2 | 2 | 2 | 29.9 | 18.9 | 24.7 | 35.2 | 0 | 20.0 | 14.5 | 3.2 | 17.7 |
| Baekna | 1 | 2 | 2 | 1 | 2 | 22.5 | 6.1 | 7.3 | 57.8 | 46.0 | 37.0 | 34.7 | 3.1 | 37.8 |
| Bakkye | 1 | 3 | 2 | 2 | 2 | 23.8 | 9.5 | 20.4 | 41.2 | 36.8 | 32.8 | 8.4 | 2.1 | 10.5 |
| Bandalbyeo | 1 | 1 | 1 | 1 | 2 | 21.8 | 7.4 | 19.7 | 43.4 | 33.9 | 32.3 | nd | nd | 0 |
| Baramdungkuri | 1 | 3 | 1 | 2 | 2 | 7.5 | 11.5 | 2.2 | 33.9 | 14.9 | 16.7 | 6.4 | nd | 6.4 |
| Basmati | 2 | 2 | 2 | 1 | 2 | 17.0 | 10.1 | 1.0 | 32.8 | 28.3 | 20.7 | nd | nd | 0 |
| Boribyeo | 1 | 3 | 2 | 2 | 2 | 26.5 | 18.2 | 38.0 | 60.4 | 43.3 | 47.2 | nd | nd | 0 |
| Buldo | 1 | 3 | 1 | 1 | 1 | 29.9 | 9.5 | 26.7 | 40.3 | 22.4 | 29.8 | 4.4 | 3.2 | 7.6 |
| Chanarak | 1 | 1 | 2 | 2 | 2 | 18.4 | 5.4 | 25.4 | 48.2 | 58.4 | 44.0 | 4.4 | 2.7 | 7.1 |
| Cheonggunbyeo | 1 | 1 | 1 | 1 | 1 | 18.4 | 4.7 | 30.7 | 9.1 | 0 | 13.3 | nd | 5.1 | 5.1 |
| Cheongsando | 1 | 1 | 1 | 1 | 1 | 14.3 | 4.1 | 24.9 | 1.7 | 12.3 | 17.4 | nd | nd | 0 |
| Che-shau-nan-bir | 2 | 2 | 2 | 2 | 2 | 24.5 | 13.5 | 19.4 | 39.0 | 28.7 | 29.0 | nd | 2.1 | 2.1 |
| Chindadchiki | 1 | 1 | 1 | 1 | 1 | 17.7 | 11.5 | 15.2 | 25.0 | 0 | 13.4 | 5.1 | 3.4 | 8.5 |
| Cica4 | 2 | 3 | 2 | 2 | 2 | 6.8 | 5.4 | 2.8 | 34.0 | 7.5 | 14.8 | 3.2 | 4.2 | 7.4 |
| Cuba 65-V-58 | 2 | 3 | 2 | 2 | 2 | 1.4 | 0.0 | 11.4 | 8.0 | 1.7 | 7.0 | nd | nd | 0 |
| Dabaegjo | 1 | 3 | 2 | 1 | 2 | 20.4 | 6.8 | 0 | 45.2 | 39.2 | 28.1 | 4.6 | 9.3 | 13.9 |
| Daegoldo | 1 | 1 | 2 | 1 | 1 | 25.9 | 8.1 | 5.7 | 40.2 | 0 | 22.9 | nd | nd | 0 |
| Damagung | 1 | 2 | 1 | 1 | 2 | 20.4 | 7.4 | 4.0 | 31.9 | 33.9 | 23.3 | nd | 3.5 | 3.5 |
| Danganeuibangju | 1 | 1 | 2 | 2 | 2 | 10.9 | 12.2 | 0 | 35.6 | 0 | 11.9 | 15.9 | 2.5 | 18.4 |
| Deokjeokjodo | 1 | 1 | 2 | 1 | 1 | 11.6 | 1.4 | 15.5 | 0 | 0 | 5.2 | 1.9 | 3.2 | 5.1 |
| Dong o byeo | 1 | 2 | 1 | 1 | 1 | 9.5 | 6.8 | 11.7 | 34.7 | 24.2 | 23.5 | 8.2 | 6.2 | 14.4 |
| Dongsanjo | 1 | 1 | 2 | 1 | 2 | 10.2 | 6.1 | 14.2 | 28.3 | 18.1 | 20.2 | 3.8 | 6.1 | 5.9 |
| Donduni kunluz | 2 | 3 | 1 | 2 | 2 | 21.1 | 13.5 | 16.4 | 36.0 | 21.2 | 24.5 | nd | nd | 0 |
| Donna | 1 | 3 | 2 | 1 | 1 | 25.2 | 8.1 | 9.0 | 0 | 0 | 26.1 | 5.1 | nd | 5.1 |
| Duchungjong | 1 | 2 | 1 | 1 | 1 | 26.5 | 5.4 | 6.0 | 32.1 | 31.0 | 28.5 | 2.1 | 3.3 | 5.4 |
| Dorae | 1 | 2 | 1 | 1 | 2 | 23.8 | 6.8 | 0 | 38.2 | 41.2 | 21.0 | 1.5 | 3.6 | 5.1 |
| Eumseon | 1 | 1 | 2 | 2 | 2 | 32.7 | 16.2 | 21.4 | 35.9 | 0.5 | 19.3 | 6.0 | nd | 6.0 |
| Eunjo | 1 | 1 | 1 | 2 | 2 | 25.9 | 10.1 | 0 | 40.3 | 13.6 | 19.0 | 4.1 | nd | 4.1 |
| F3-220 | 1 | 1 | 2 | 1 | 2 | 35.4 | 7.4 | 0 | 0 | 0 | 0 | 9.9 | 22.0 | 31.9 |
| Gangcheongdo | 1 | 2 | 2 | 2 | 2 | 13.6 | 7.4 | 0 | 0 | 0 | 0 | 3.5 | 3.6 | 7.1 |
| Geumchangdo | 1 | 1 | 2 | 1 | 2 | 25.2 | 10.8 | 14.5 | 18.2 | 2.9 | 11.9 | 16.3 | 2.8 | 19.1 |
| Geumjeomdo | 1 | 2 | 2 | 1 | 2 | 20.4 | 4.4 | 0 | 0 | 0 | 0 | 2.5 | nd | 2.5 |
| Gin shun | 2 | 1 | 2 | 2 | 2 | 3.4 | 3.0 | 17.6 | 6.7 | 0 | 8.1 | 1.8 | 2.7 | 4.5 |
| Gpno 12856 | 2 | 1 | 2 | 2 | 2 | 19.1 | 9.5 | 20.1 | 25.0 | 0 | 15.0 | 1.6 | nd | 1.6 |
| Gpno 3005 | 2 | 1 | 2 | 1 | 2 | 10.2 | 8.1 | 4.7 | 35.6 | 24.8 | 21.7 | nd | 2.8 | 2.8 |
| Guando | 1 | 2 | 1 | 1 | 1 | 3.5 | 2.7 | 10.4 | 37.2 | 32.1 | 26.6 | 17.4 | nd | 17.4 |
| Hamburebyeo | 1 | 2 | 1 | 1 | 2 | 37.4 | 15.5 | 8.6 | 22.6 | 0 | 10.4 | 5.2 | 2.8 | 8.0 |
| Heugbal | 1 | 1 | 1 | 1 | 2 | 11.6 | 13.5 | 0 | 27.1 | 40.0 | 22.4 | 8.7 | 7.6 | 16.3 |
| Heugsaekdo | 1 | 1 | 2 | 1 | 1 | 10.9 | 2.7 | 0 | 25.6 | 3.1 | 9.6 | 5.2 | nd | 5.2 |
| Heunbe | 1 | 2 | 2 | 2 | 2 | 20.4 | 8.8 | 3.5 | 9.6 | 1.2 | 4.7 | 4.5 | nd | 4.5 |
| Hochokjindo | 1 | 3 | 2 | 1 | 2 | 22.5 | 10.8 | 0 | 15.1 | 0 | 5.0 | 4.1 | 4.7 | 8.8 |
| Hongdodo | 1 | 2 | 2 | 1 | 1 | 28.6 | 12.2 | 22.1 | 26.7 | 33.8 | 27.6 | 8.4 | 4.7 | 13.1 |
| Huado | 1 | 3 | 2 | 1 | 1 | 23.8 | 12.8 | 5.5 | 48.7 | 35.2 | 29.8 | 8.9 | nd | 8.9 |
| Hwangjo | 1 | 3 | 2 | 1 | 1 | 21.8 | 6.1 | 0 | 37.2 | 17.5 | 18.2 | 7.9 | 3.8 | 11.7 |
| Hwangju | 1 | 1 | 2 | 2 | 2 | 24.5 | 10.1 | 0 | 62.7 | 33.2 | 32.0 | nd | nd | 0 |
| Hwangtodo | 1 | 3 | 2 | 1 | 2 | 16.3 | 8.1 | 3.8 | 40.6 | 44.1 | 29.5 | 7.9 | nd | 7.9 |
| IRI 268 (Nongkwang) | 1 | 3 | 2 | 1 | 1 | 31.3 | 12.2 | 25.8 | 35.2 | 37.5 | 32.9 | 8.3 | 2.7 | 11.0 |
| IR 1044-56 | 2 | 3 | 2 | 1 | 2 | 17.0 | 10.8 | 15.9 | 34.6 | 49.1 | 33.2 | 1.1 | nd | 1.1 |
| IR 329-19-5-2-2 | 2 | 2 | 2 | 2 | 2 | 13.6 | 14.2 | 23.6 | 30.6 | 19.8 | 24.7 | 3.2 | 4.2 | 7.4 |
| IR 644-1-63-1-1 | 2 | 3 | 2 | 2 | 2 | 3.4 | 6.8 | 11.1 | 37.3 | 16.2 | 21.5 | 2.8 | 4.0 | 6.8 |
| IR 781-497-2-3 | 2 | 3 | 1 | 2 | 2 | 11.6 | 8.1 | 5.8 | 20.5 | 2.2 | 9.5 | 5.0 | 6.5 | 11.5 |
| IRI293 (Palgeum) | 1 | 3 | 2 | 2 | 2 | 41.5 | 12.8 | 8.1 | 31.2 | 0 | 13.1 | nd | nd | 0 |
| IRI301 (Mangyung) | 1 | 3 | 2 | 1 | 2 | 20.4 | 14.9 | 0 | 26.6 | 27.4 | 18.0 | 8.0 | 3.0 | 11.0 |
| Jaeraejongna | 1 | 3 | 2 | 1 | 2 | 20.4 | 12.8 | 2.2 | 23.3 | 1.6 | 9.0 | nd | 2.4 | 2.4 |
| Jangjo | 1 | 1 | 2 | 1 | 1 | 16.3 | 14.2 | 9.3 | 33.4 | 0 | 14.2 | nd | nd | 0 |
| Jangsamdo | 1 | 3 | 2 | 2 | 2 | 31.3 | 10.8 | 4.6 | 17.5 | 2.2 | 8.1 | nd | nd | 0 |
| Jangwang | 1 | 3 | 2 | 1 | 2 | 13.6 | 14.2 | 15.7 | 15.9 | 7.5 | 13.0 | nd | 3.7 | 3.7 |
| Jeokmosaek | 1 | 1 | 2 | 2 | 2 | 27.9 | 16.2 | 28.1 | 18.2 | 12.6 | 19.6 | nd | nd | 0 |
| Jeona | 1 | 1 | 2 | 2 | 2 | 37.4 | 12.8 | 12.6 | 25.4 | 0 | 12.6 | 5.3 | 4.3 | 9.6 |
| Jeongdaldo | 1 | 3 | 1 | 1 | 1 | 8.8 | 9.5 | 43.4 | 45.5 | 43.9 | 44.3 | nd | nd | 0 |
| Jeongjo | 1 | 3 | 2 | 2 | 2 | 24.5 | 6.8 | 0 | 0 | 0 | 0 | nd | nd | 0 |
| Jinhwa | 1 | 3 | 2 | 1 | 1 | 11.6 | 4.1 | 16.9 | 44.5 | 0 | 20.5 | 18.9 | 3.5 | 22.4 |
| Kasarwala mundara | 2 | 2 | 2 | 1 | 2 | 18.4 | 16.2 | 3.5 | 40.8 | 40.5 | 28.3 | 2.8 | nd | 2.8 |
| Kingmen toumen chiumu | 2 | 1 | 2 | 1 | 2 | 21.8 | 14.9 | 18.3 | 39.1 | 43.3 | 33.6 | 1.3 | 6.1 | 7.4 |
| Mamoriak | 2 | 1 | 2 | 1 | 2 | 15.7 | 4.7 | 11.5 | 27.2 | 29.7 | 22.8 | nd | 5.3 | 5.3 |
| Mon-z-wuan | 2 | 1 | 2 | 2 | 2 | 17.7 | 6.1 | 4.9 | 26.7 | 25.5 | 19.0 | nd | nd | 0 |
| Namseon 1 | 1 | 3 | 2 | 1 | 2 | 25.2 | 18.2 | 18.2 | 43.2 | 52.6 | 37.4 | nd | 9.3 | 9.3 |
| Namkangbaekjo | 1 | 1 | 2 | 1 | 2 | 25.2 | 6.1 | 0 | 22.9 | 39.1 | 20.7 | 1.6 | nd | 1.6 |
| Noindari | 1 | 2 | 1 | 1 | 2 | 14.3 | 6.1 | 0 | 78.5 | 88.2 | 55.6 | 5.4 | nd | 5.4 |

**Table 1.** Continued

| varieties | varieties characteristics | | | | | inhibition (%) | | | | | | $\mu g\ g^{-1}$ | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | germination | | dry weight | | | | momilactones | | |
| | O[a] | M[b] | H[c] | A[d] | C[e] | GR | GP | LDW | SDW | RDW | AIDW | MA[f] | MB[g] | TM[h] |
| Noindo | 1 | 3 | 2 | 2 | 2 | 17.0 | 4.7 | 0.8 | 60.0 | 59.0 | 39.9 | 2.1 | 2.6 | 4.7 |
| Oegukbyeo | 1 | 3 | 2 | 1 | 1 | 21.5 | 19.6 | 15.1 | 50.4 | 36.8 | 34.1 | nd | 3.2 | 3.2 |
| Olbyeo | 1 | 1 | 2 | 1 | 2 | 15.7 | 19.6 | 9.1 | 26.7 | 22.6 | 19.4 | 1.2 | nd | 1.2 |
| Patbyeo | 1 | 1 | 2 | 2 | 2 | 23.8 | 6.1 | 15.2 | 23.8 | 9.3 | 16.1 | nd | nd | 0 |
| Philippine 2 | 2 | 1 | 2 | 2 | 2 | 12.3 | 6.1 | 14.6 | 41.1 | 45.9 | 33.8 | nd | nd | 0 |
| Pyeongbuk 4 | 1 | 2 | 2 | 1 | 1 | 16.3 | 2.0 | 7.5 | 20.1 | 0 | 9.2 | 7.4 | 3.0 | 10.4 |
| Pyeongyang | 1 | 3 | 2 | 1 | 2 | 32.0 | 21.6 | 5.3 | 43.3 | 34.5 | 27.7 | 21.9 | nd | 21.9 |
| Red khosha cerma | 2 | 1 | 2 | 2 | 2 | 11.6 | 4.7 | 14.2 | 21.5 | 5.8 | 13.8 | nd | nd | 0 |
| San chiao tswen | 2 | 1 | 2 | 2 | 2 | 21.1 | 10.1 | 6.2 | 24.7 | 17.1 | 16.0 | 1.2 | 5.7 | 6.9 |
| Sancheongdo | 1 | 2 | 2 | 1 | 1 | 26.5 | 12.8 | 37.0 | 51.5 | 0 | 29.5 | 8.0 | 6.0 | 14.0 |
| Sangpung | 1 | 2 | 1 | 1 | 2 | 15.7 | 11.5 | 3.2 | 38.8 | 48.5 | 30.2 | nd | 3.4 | 3.4 |
| Sanjo | 1 | 2 | 2 | 1 | 2 | 27.2 | 6.8 | 20.3 | 48.4 | 18.6 | 29.1 | nd | nd | 0 |
| Seogandodo | 1 | 2 | 2 | 2 | 2 | 18.4 | 7.4 | 12.0 | 30.3 | 2.8 | 15.1 | nd | nd | 0 |
| Seungsiljo | 1 | 3 | 2 | 2 | 2 | 13.6 | 7.4 | 17.6 | 28.6 | 13.4 | 19.9 | nd | 3.0 | 3.0 |
| Sinbaegseog | 1 | 3 | 2 | 1 | 1 | 12.9 | 8.8 | 13.3 | 21.0 | 8.6 | 14.3 | 9.3 | 3.1 | 12.4 |
| Taichung native 1 | 2 | 2 | 2 | 2 | 2 | 14.3 | 4.7 | 0 | 42.2 | 28.7 | 23.7 | 2.3 | 6.4 | 8.7 |
| Tsai yuan chon | 2 | 2 | 2 | 2 | 2 | 21.8 | 14.2 | 18.0 | 28.9 | 27.3 | 24.7 | nd | 6.6 | 6.6 |
| Woo-co-chin-yu | 2 | 2 | 2 | 2 | 2 | 21.8 | 10.8 | 7.5 | 26.6 | 23.4 | 19.2 | 2.5 | nd | 2.5 |
| $LSD_{0.05}$ | | | | | | 11.5 | 8.0 | 22.5 | 31.3 | 46.3 | 11.7 | 2.0 | 1.5 | 1.3 |

[a] O, origin (1, Korean; 2, foreign). [b] M, maturing time (1, early maturing; 2, middle maturing; and 3, late maturing). [c] H, color of hull (1, colored hull; 2, colorless hull). [d] A, awn (1, awn; 2, awnless). [e] C, color of awn (1, colored awn; 2, colorless awn). [f] MA, momilactone A. [g] MB, momilactone B. [h] TM, total momilactones.

were centrifuged at 3000 rpm for 4 h. The supernatant was filtered through one layer of Whatman no. 42 filter paper. To prevent microorganism growth, the solutions were then filtered through a 0.2 mm Nalgene filter (Becton Dickinson Labware, Lincoln Park, NJ).

Before the bioassay, barnyard grass seeds were surface sterilized in a 1:10 (v/v) dilution of commercial hypochlorite bleach for 10 min and rinsed several times with $dH_2O$. Seeds were placed on moistened paper towels for 2 h, and then, 50 barnyard grass seeds were placed on filter paper in a sterilized 9 cm Petri dish. The rice hull extract solution (10 mL) was added to each Petri dish, and $dH_2O$ was used as a control. All Petri dishes were placed in a lit growth chamber at 24 °C. The germination rate (GR) and germination percentage (GP) were determined after 3 and 14 days, respectively. Seedlings were separated into leaf dry weight (LDW), straw dry weight (SDW), and root dry weight (RDW) and oven-dried at 65 °C for 4 days. The percentage of inhibition was calculated using the following equation:

$$\text{inhibition percentage} = [(\text{control} - \text{aqueous extracts})/\text{control}] \times 100$$

**Preparation for the Analysis of Momilactones A and B.** Dried ground rice hulls (30 g) were extracted in 100% methyl alcohol (150 mL) in a shaking bath at 25 °C for 24 h according to the method of Chung et al. (*24*). The extracts were filtered through Whatman no. 42 filter paper. The filtrates were concentrated to a volume of 3 mL using a rotary vacuum evaporator at a temperature of less than 35 °C and then freeze-dried at −40 °C. Finally, dried filtrate samples were redissolved in 150 mL of ethyl acetate (EtOAc) and $dH_2O$ at a dilution of 1:1 (v/v), concentrated to a volume of 2 mL, and used for the quantification of momilactones A and B from rice hulls. Momilactone A and B standards isolated from rice hulls and identified by Chung et al. (*24*) were used for calibration curves. The purity (>98%) of each standard was determined by GC/MS and obtained by plotting standard concentrations (at five concentrations: 1, 25, 50, 75, and 100 ppm). High linearity ($r^2 > 0.99$) was obtained for each curve and identified by retention time or by cochromatography with authentic standards. Concentrations were calculated by comparing peak areas of samples with those of the standards.

**Quantification of Momilactones A and B.** GC/MS analysis was performed using a new method developed in this study to identify momilactones A and B separated from rice hulls. A Hewlett-Packard G 1800B GCD MS system interfaced with a Hewlett-Packard 5890 series II gas chromatograph was used for the separation and identification of rice hull extracts. A capillary column (DB-5; J & W Scientific Inc., Folsom, CA; length, 30 m, i.d., 0.25 mm, 0.25 $\mu$m) was connected directly to the ionizer without the jet separator normally used for packed columns. The helium gas carrier flow rate was 0.8 mL $min^{-1}$, the sample injection volume was 1 $\mu$L, the injector temperature was 290 °C, and the program for column temperature was as follows: (i) isocratic for 5 min at 200 °C; (ii) gradient for 36 min (2.5 °C $min^{-1}$) from 200 to 290 °C; and (iii) isocratic for 20 min at 290 °C. The conditions for the mass spectrometer were as follows: (i) mass range, 45−450 *m*/*z*; (ii) analysis time, 61 min; (iii) ion mode, EI; (iv) scan time (5 $\mu$scans), 1 s; (vi) background mass, 48 *m*/*z*; and (vii) peak threshold, one count.

**Inhibitory Bioactivity Study in the Field.** This experiment was conducted in 2004 to assess the inhibitory bioactivity of momilactones A and B as potential natural herbicide sources in the Experimental Farm, KonKuk University. Momilactones A and B identified from the rice hulls showed a high activity in the Lemna assay and GACT (*24*). The paddy field soil was composed of sandy clay loam at pH 6.9 and 61% sand, 27% silt, 12% clay, and 3.2% organic matter at 8.6me CEC (cation exchange capacity). The paddy field had not had herbicidal or other special treatment in the past 5 years. The soil was divided into 5 × 9 $m^2$ plots. A common Korean rice species, Ilpumbyeo (*O. sativa* L. var. *indica*), was grown in seedling boxes for 40 days and transplanted by hand. There were 15 cm of space between each plant on a row, and each row was 30 cm apart (30 cm × 15 cm). Momilactones A and B were dissolved in acetone and Tween-20; the final concentration was 50% acetone and 0.2% Tween-20. The diluted compounds were applied to the paddy field, which was under 3 cm of water. The control had no momilactones and no herbicides treatment. Also, to compare momilactones A and B with commercial synthetic herbicides, cyhalofop-B, carfentrazone, and pyrazosulfuron were applied. Fertilizers (N, $P_2O_5$, and $K_2O$ at the rate of 7.5, 12.6, and 9.0 g $m^{-2}$, respectively) were applied to the field 1 day before saturating the soil with water. All other pesticide treatments were applied according to the standard methods of rice cultivation in Korea. The experimental field had six treatments with three replications each in the completely randomized block design. Treatments were applied 7 and 14 days after planting. The inhibitory bioactivity of weeds in species *E. crus-galli* P. Beauv. var. *oryzicola* Ohwi, *Monochoria vaginalis* var. *plantaginea*, *Scirpus juncoides*, and *Eleocharis kuroguwai*, and the toxicity on rice were simultaneously investigated 30 days after planting. The inhibitory bioactivity effect of momilactones A and B was rated visually on a scale of 0−100 index (no visible inhibition to weed death, respectively). Rice toxicity was also rated visually on a scale of 0−9 index (no visible injury to total rice death; necrosis).

**Statistical Analysis.** All experiments were conducted twice using a randomized design with six replications. Analysis of variance was performed for all data using a general linear model procedure (*28*). Data from two experiments were pooled, and mean values were

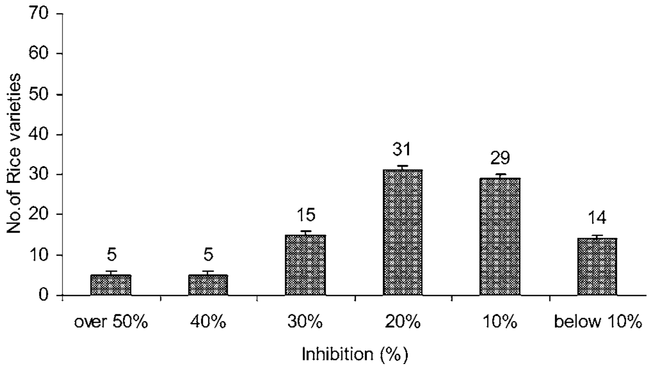


**Figure 1.** Distribution of rice varieties with allelopathic potential.

separated according to least significant differences (LSD) at the 0.05 probability level.

## RESULTS AND DISCUSSION

**Comparison of Allelopathic Potential and Quantification of Momilactones in Rice Germplasm.** Rice varieties were divided into three groups according to germination, dry weight, and momilactone level. Of the hull extracts from 99 rice varieties, five inhibited the germination and growth of barnyard grass by more than 50%. Noindari exerted the greatest inhibition of dry weight (AIDW) (55.6%), followed by Baekjicheongbyeo (52.5%). The varieties ranked six to 10 had inhibition effects greater than 40%. Fifteen varieties had inhibition effects in excess of 30%, 31 in excess of 20%, and 29 in excess of 10%, and 14 had weak effects (less than 10%), the lowest of which was Heunbe (4.7%). Mean inhibitory effects of the 99 varieties of rice were 27.5% for dry weight and 14.8% for germination (**Table 1** and **Figure 1**).

Allelopathic effects of rice hulls on the germination of barnyard grass were examined using soluble rice hull extracts. The average inhibition of GR was 19.8%, with Iri 293 (Palgeum) exhibiting the highest inhibition rate (41.5%). Cuba 65-V-58 had the lowest inhibition rate (1.4%). Eight rice varieties showed GR inhibitions of over 30%, of which the varieties with the greatest inhibition rates were IRI 293 (Palgeum) at 41.5% and Hamburebyeo 3 at 37.4%. The average GP inhibition rate was 9.9%, and only Pyeongyang showed a GP inhibition rate of over 20%. Fifty-one varieties had GP inhibition rates below 10%, with Deokjeokjodo displaying the lowest (1.4%). Overall, the inhibition rate of GR was higher than GP (**Table 1**).

The average inhibition rate of dry weight of the various plant parts analyzed occurred in the following order: SDW (38.9%) > RDW (26.8%) > LDW (16.6%). The inhibition of LDW was greatest in AC 1423 (52.7%) and lowest in Noindo (0.8%). Two rice varieties, Jeongdaldo and Baekjicheongbyeo, showed inhibition rates of 40%. However, 44 varieties showed LDW inhibition rates of less than 10%, of which 17 varieties, including Taichung native 1, showed no LDW inhibition rate. The Noindari variety showed the highest SDW inhibition (78.5%), and 10 varieties inhibited SDW to below 10%. In particular, the LDW, SDW, and RDW rates of the four varieties F3-220, Gangcheongdo, Geumjeomdo, and Jeongjo were zero. Noindari also had the highest RDW inhibition rate (88.2%). Eight varieties exhibited rates greater than 50%. Sixteen varieties did not exert any RDW inhibition (**Table 1**).

The allelopathic effect is influenced by various biotic or abiotic factors, and many methods exist for evaluating allelopathic potential in rice varieties. The results of this study support previous conclusions that allelopathic properties vary between rice varieties (*4*, *5*). Chung et al. (*2*) reported significant variability of allelopathic effects in rice cultivars, of which rice hull extracts contained more water soluble substances toxic to barnyard grass than leaf or stem extracts. Furthermore, Ahn and Chung (*8*) found that a warm water extraction method (24 °C) is more effective than hot water extraction (80 °C). They proposed that the low pH of the warm water extract partially contributes to the inhibitory effect. Chung et al. (*10*) and Jung et al. (*9*) examined the allelopathic potential of rice varieties identical to those used in the present study and obtained similar results. However, it was thought that the results of previous trials and those of the current study would not be compatible because the previous experiments were carried out using different weeds, cropping years, and body extracts or residues. Theoretically, about 14580 kg of rice hulls per hectare would be required to produce an inhibitory effect on weeds. The use of rice hulls to control paddy weeds may be possible because farmers in Korea usually leave a large amount of rice hulls in the field. In addition, rice extracts are slightly acidic, which may inhibit the GR, GP, and TDW of barnyard grass (*2*, *10*, *29*).

**Table 2.** Distribution of Rice Varieties with TMs

| TMs ($\mu$g g$^{-1}$) | varieties |
|---|---|
| above 50 | Baekgwangok |
| 30–40 | Baekna, F3-220 |
| 20–29 | Baekkyeongjo, Jinhwa, Badolbyeo, Pyeongyang |
| 10–19 | Geumchangdo, Danganeuibangju, Baekchalbyeo, Baekmangjo, Guando, Heugbal, Dongobyeo, Sancheongdo, Dabaegjo, Hongdodo, Sinbvaegseog, Baekjo, Hwangjo, IR 781-497-2-3, IRI 268 (Nongkwang), IRI 301 (Mangyung), 143 (PI 274471), Bakkye, Pyongbuk 4 |
| 1–9 | Jeona, Namseon 1, Baekjicheongbyeo, Huado, Hochokjindo, Taichung Native 1, Chindadchiki, Hamburerbyeo, Hwangtodo, Buldo, Cica, IR 329-19-5-2-2, Kingmen toumen chiumu, Chanrak, Gangcheongdo, San chiao tsween, IR 644-1-63-1-1, Tsai yuan chon, Baramdungkuri, Eunseon, Dongsanjo, Agudo, Duchungjong, Noindari, B1293b-pn24, Mamoriak, Heugsaekdo, Cheonggunbyeo, Donna, Baekhaedai, Dorae, Deokjeokjodo, Noindo, Gin shun, Heunbe, Eunjo, Seungsiljo, Jangwang, AC 1423, Damagung, Sangpung, Oegukbyeo, Kasarwala mundara, Gpno 3005, Woo-co-chin-yu, Geumjeomdo, Jaeraejongna, Che-shau-nan-bir, Gpno 12856, Namkangbaekjo, Olbyeo, Arongbyeo, IR 1044-56 |
| not detected | Basmati, Cuba 65-v-58, Donduni kunluz, Mon-z-wuan, Phillippine 2, Red khosha cerma, Bandalbyeo, Boribyeo, Sanjo, Seogandodo, IRI 293 (Palgeum), Jangsamdo, Jangjo, Jeongdaldo, Jeongjo, Cheongsando, Patbyeo, Jeokmosaek, Hwangju, Daegoldo |

The average of momilactones in the rice varieties analyzed was 4.9 $\mu$g g$^{-1}$ for momilactone A and 2.9 $\mu$g g$^{-1}$ for momilactone B (**Table 1**). Momilactone A was detected in 66 rice varieties, and momilactone B was found in 58 rice varieties. Baekna had the highest level of momilactone A (34.7 $\mu$g g$^{-1}$), and Baekgwangok had the highest level of momilactone B (37.8 $\mu$g g$^{-1}$). Momilactone A was detected at over 10 $\mu$g g$^{-1}$ in 12 rice varieties including AC1423 (PI 274471), whereas only F3-220 and Baekgwangok had momilactone B levels of over 20 $\mu$g g$^{-1}$. The details of concentrations of total momilactones (TMs) in rice hulls varieties are shown in **Table 1**. Rice varieties were classified into six categories based on their TMs (**Table 2**). Baekgwangok was in the first class (total concentrations > 50 $\mu$g g$^{-1}$). The second class (30−40 $\mu$g g$^{-1}$) was composed of Baekna and F3-220. The third class (20−29 $\mu$g g$^{-1}$) was composed of four varieties including Badolbyeo. The fourth

**Table 3.** Evaluation of Allelopathic Effect of Rice Hull Extracts from Different Regions on Barnyard Grass and TMs in Rice Hulls

| | inhibition (%) | | | $\mu$g g$^{-1}$ |
|---|---|---|---|---|
| rice varieties | AIG[a] | AIDW[b] | TIR[c] | TMs |
| Korean (75)[d] | 15.8 | 20.8 | 18.3 | 4.5 |
| foreign (24) | 12.1 | 19.2 | 15.7 | 2.1 |
| $LSD_{0.05}$ | 1.8 | 3.9 | 2.4 | 1.1 |

[a] (GR + GP)/2. [b] (LDW + SDW + RDW)/3. [c] (AIG + AIDW)/2. [d] Number of varieties.

class (10−19 $\mu$g g$^{-1}$) was composed of 19 varieties including Baekchalbyeo. The fifth class (1−19 $\mu$g g$^{-1}$) was composed of 53 varieties including AC 1423. The sixth class (not detected) was composed of 20 varieties including Bandalbyeo.

The momilactones of rice hulls increase when the plant is subjected to biotic or abiotic stress, indicating that momilactones play an important role in plant defense systems against pathogens. Most allelochemicals, including momilactones, are released during early developmental plant stages when there is competition with neighboring plants for resources such as light, water, and nutrients (*22*, *25*). In the present study, we quantified momilactones from 99 rice hull germplasms and found positive but weak correlations with the inhibition of GR ($r^2 = 0.12$*) and SDW ($r^2 = 0.16$**). Furthermore, the TM level correlated better with momilactone A ($r^2 = 0.84$***) than with momilactone B ($r^2 = 0.71$***). In addition, the total inhibition rate (TIR) of barnyard grass correlated with the average inhibition of germination (AIG) ($r^2 = 0.62$***), the AIDW ($r^2 = 0.92$***), and the inhibition of GP ($r^2 = 0.57$***). This study suggests that rice variety with high momilactones may provide one of the most importance gene resources for breeding rice variety with high allelopathic hulls.

**Evaluation of Allelopathic Potential with Genetic Properties in Rice Germplasm.** Korean rice varieties showed a significantly higher TIR (18.3%) than foreign rice varieties (15.7%). The AIG (15.8%) and AIDW (20.8%) of Korean rice varieties had a slightly greater allelopathic effect on barnyard grass growth than that of foreign rice varieties (12.1 and 19.2%, respectively). Furthermore, Korean rice varieties had markedly higher inhibitory effects on GR than on GP, and a greater inhibition of SDW as compared with LDW and RDW (although the values were not significant). TMs were higher in Korean rice varieties (4.5 $\mu$g g$^{-1}$) as compared with foreign rice varieties (2.1 $\mu$g g$^{-1}$), with momilactone A levels higher than those of momilactone B. Some variations in TM and momilactone A were statistically significant between regions of origin, although there was no significant difference between momilactone B levels. It is obvious that the difference in allelopathic effects in rice may be correlated with momilactones (**Table 3** and **Figures 2a** and **3a**).

According to the different maturing times of rice varieties, the TIR occurred in the following order: middle maturing (19.0%) > late maturing (17.8%) > early maturing(16.4%). There was a slightly greater inhibition of GR than of GP, and the AIG was not significant for the three different maturing times. Conversely, AIDW showed significant inhibitory effects, while both SDW and RDW were significantly inhibited as compared with the inhibitory effects on LDW. Late-maturing varieties had higher TM levels (4.4 $\mu$g g$^{-1}$) as compared with early- and middle-maturing varieties. Middle-maturing varieties had a momilactone A level of 6.1 $\mu$g g$^{-1}$, and late-maturing varieties had a momilactone B level of 3.6 $\mu$g g$^{-1}$. There were significant differences in momilactone A levels for the three

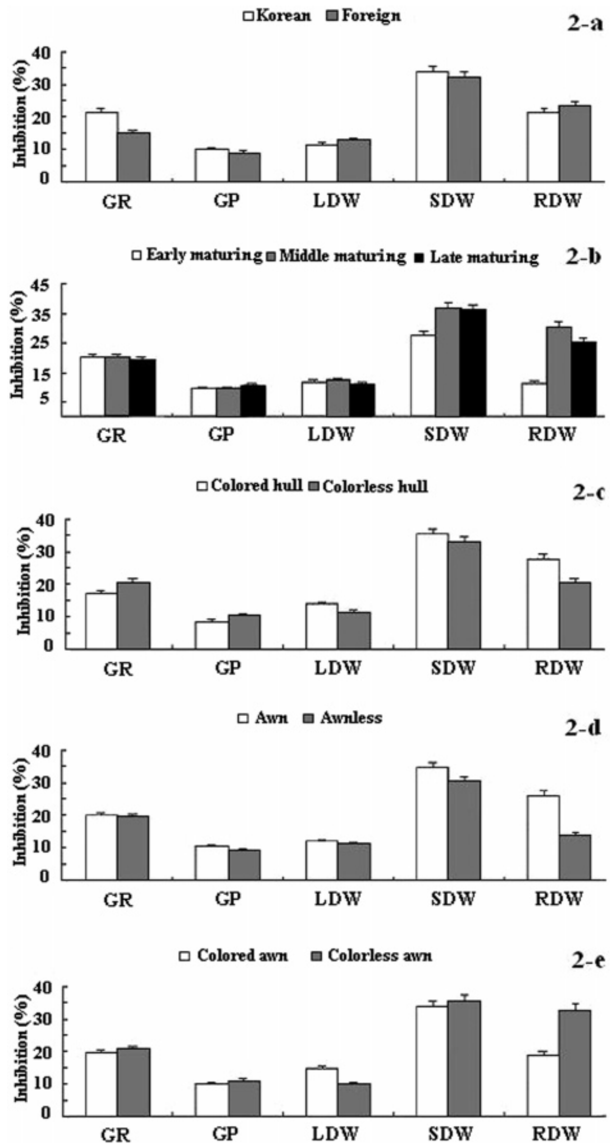


**Figure 2.** Allelopathic potential of rice hull extracts with genetic properties and morphological characteristics on barnyard grass.

maturing times, whereas the levels of momilactone B were similar (**Table 4** and **Figures 2b** and **3b**).

The region of origin of rice varieties was negatively correlated with the AIG ($r^2 = -0.23$***) and TM ($r^2 = -0.32$***), indicating that Korean rice varieties have higher allelopathic properties than foreign rice varieties. Maturing time for rice varieties showed positive correlations with SDW ($r^2 = 0.15$**), RDW ($r^2 = 0.19$**), and TM ($r^2 = 0.13$*). With the late-maturing time, the higher the momilactone level is, the greater the inhibition of SDW and RDW is. Chung et al. (*10*) reported that Korean rice varieties with late-maturing times had a higher average allelopathic effect on barnyard grass, and Jung et al. (*9*) found that rice hull residues generally show greater inhibition rates on the emergence, height, and dry weight of barnyard grass at late-maturing times.

In comparison with the present study, Chung et al. (*10*) reported that hull extracts from foreign and early-maturing rice varieties had a greater inhibition on GR, GP, and AIDW. In addition, rice hull residues from foreign varieties generally inhibited the emergence, height, and dry weight of barnyard grass to a greater extent that in the present study (*9*). However, these dissimilarities may be due to different weeds, bioassay methods, and the environment and cropping year analyzed. Thus,

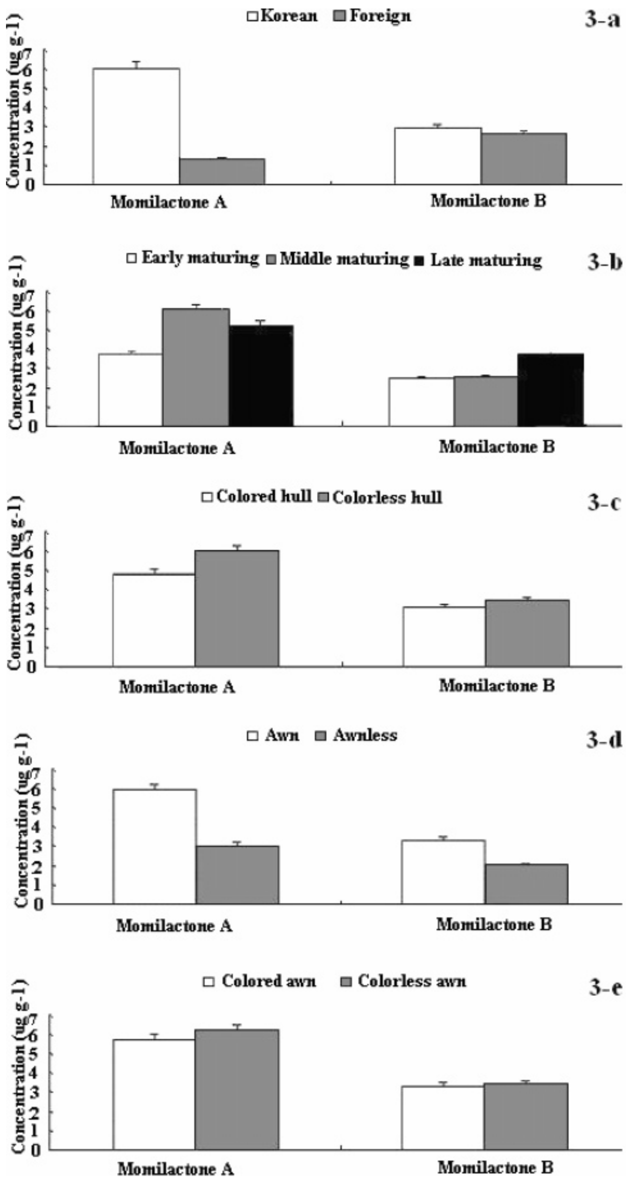


**Figure 3.** Quantification of momilactones A and B in rice hulls with genetic properties and morphological characteristics.

**Table 4.** Evaluation of Allelopathic Effect and TMs in Rice Hull Extracts with Maturing Time on Barnyard Grass

| | inhibition (%) | | | $\mu$g g$^{-1}$ |
|---|---|---|---|---|
| rice varieties | AIG[a] | AIDW[b] | TIR[c] | TMs |
| early maturing (33)[d] | 14.9 | 17.9 | 16.4 | 3.1 |
| middle maturing (30) | 14.9 | 23.1 | 19.0 | 4.3 |
| late maturing (36) | 15.0 | 20.7 | 17.8 | 4.4 |
| $LSD_{0.05}$ | 1.9 | 4.1 | 2.5 | 1.2 |

[a] (GR + GP)/2. [b] (LDW + SDW + RDW)/3. [c] (AIG + AIDW)/2. [d] number of varieties.

we suggest that further study is needed to elucidate the correlation between the genetic properties of rice varieties and the cultivation environments. Other researchers also found that allelopathic potential varies with region of origin, as well as with traditional and improved rice varieties (*4*, *30*). Fujii (*31*) found that japonica Korean rice varieties had a greater allelopathic activity than foreign varieties, Indica varieties, and hybrids.

**Table 5.** Evaluation of Allelopathic Effect and TMs in Rice Hull Extracts with the Existence or Nonexistence of Hulls Color on Barnyard Grass

| | inhibition (%) | | | $\mu$g g$^{-1}$ |
|---|---|---|---|---|
| rice varieties | AIG[a] | AIDW[b] | TIR[d] | TMs |
| colored hulls (20)[d] | 12.8 | 21.0 | 16.9 | 3.4 |
| colorless hulls (79) | 15.4 | 20.1 | 17.8 | 4.1 |
| $LSD_{0.05}$ | 2.0 | 4.2 | 2.6 | 1.3 |

[a] (GR + GP)/2. [b] (LDW + SDW + RDW)/3. [c] (AIG + AIDW)/2. [d] Number of varieties.

**Table 6.** Evaluation of Allelopathic Effect and TMs in Rice Hull Extracts with the Existence or Nonexistence of Awns on Barnyard Grass

| | inhibition (%) | | | $\mu$g g$^{-1}$ |
|---|---|---|---|---|
| rice varieties | AIG[a] | AIDW[b] | TIR[c] | TMs |
| awn (61)[d] | 15.2 | 21.4 | 18.3 | 4.7 |
| awnless (38) | 14.5 | 18.7 | 16.6 | 2.6 |
| $LSD_{0.05}$ | 1.6 | 3.5 | 2.1 | 1.0 |

[a] (GR + GP)/2. [b] (LDW + SDW + RDW)/3. [c] (AIG + AIDW)/2. [d] Number of varieties.

In this study, momilactone derivatives tended to increase in Korean rice varieties or in varieties with later maturing times. However, there is little information on the relationship between momilactones and genetic properties. Therefore, we need to conduct further studies on genetic properties and momilactones in order to select high allelopathic rice varieties.

**Evaluation of Allelopathic Potential with Morphological Characteristics in Rice Germplasm.** Colored or colorless hulls exhibited no significant effect on the TIR, although colorless hulls had a slightly higher inhibition rate (17.8%) than colored hulls (16.9%). The AIG (15.4%) was significantly higher for colorless hulls than for colored hulls, whereas the AIDW (21.0%) was slightly higher for colored hulls. Overall, colored hulls showed greater inhibitory effects on LDW, SDW, and RDW, while colorless hulls showed a higher inhibitory effect on GR and GP. Levels of TM (4.1 $\mu$g g$^{-1}$) and momilactones A (5.1 $\mu$g g$^{-1}$) and B (2.9 $\mu$g g$^{-1}$) were higher in colorless hulls than in colored hulls. However, these differences were not significant (**Table 5** and **Figures 2c** and **3c**).

With regard to awn or awn color, varieties with an awn had a higher TIR (18.3%) than varieties without an awn. However, these differences were not significant. In particular, awn varieties showed a significant inhibitory effect on RDW as compared with awnless varieties. Awn varieties also had higher TM level (4.7 $\mu$g g$^{-1}$) as compared with awnless varieties (2.6 $\mu$g g$^{-1}$). The TM levels, including both momilactone A and B levels, were statistically significant between awn and awnless varieties (**Table 6** and **Figures 2d** and **3d**). Rice varieties with awn color had a higher inhibition rate on LDW (14.6%), whereas varieties without awns showed higher inhibitory effects on the AIG (15.7%), AIDW (22.3%), and in particular RDW (33.0%). Awn existence had no significant effect on momilactone A and B levels (**Table 7** and **Figures 2e** and **3e**). Chung et al. (*10*) reported that colored hulls are less likely to inhibit germination and dry weight than colorless hulls. This partially agrees with our results, which show that both colored and colorless hulls were negatively correlated with GR ($r^2 = -0.14$*) and GP ($r^2 = -0.12$*). We also found that rice varieties with awns had greater inhibitory effects than awnless varieties and that rice varieties without awns had higher allelopathic activities on the

**Table 7.** Evaluation of Allelopathic Effect and TMs in Rice Hull Extracts with the Existence or Nonexistence of Awn Color on Barnyard Grass

| | inhibition (%) | | | $\mu$g g$^{-1}$ |
|---|---|---|---|---|
| rice varieties | AIG[a] | AIDW[b] | TIR[c] | TMs |
| colored awns (27)[d] | 14.6 | 20.4 | 17.5 | 4.5 |
| colorless awns (72) | 15.7 | 22.3 | 19.0 | 4.8 |
| $LSD_{0.05}$ | 1.9 | 4.1 | 2.5 | 1.2 |

[a] (GR + GP)/2. [b] (LDW + SDW + RDW)/3. [c] (AIG + AIDW)/2. [d] Number of varieties.

**Table 8.** Inhibitory Bioactivity on *E. crus-galli* P. Beauv. Var. *oryzicola* Ohwi of Momilactones A and B in the Field

| | | | toxicity (0–9)[a] | | inhibitory effect (0–100)[b] | |
|---|---|---|---|---|---|---|
| | | | transplanted rice | | *E. crus-galli* P. Beauv. var. *oryzicola* Ohwi | |
| treated compounds | shape type | concn (g a.i/ha) | 7DAA[c] | 14DAA | 7DAA | 14DAA |
| momilactone A | Tech. | 0 | 0 | 0 | 0 | 0 |
| | | 250 | 0 | 0 | 20 c | 15 e[d] |
| | | 500 | 0 | 0 | 30 b | 20 d |
| | | 1000 | 0 | 0 | 40 a | 30 c |
| | | 2000 | 0 | 0 | | 35 b |
| | | 4000 | 0 | 0 | | 50 a |
| momilactone B | Tech. | 0 | 0 | 0 | 0 | 0 |
| | | 250 | 0 | 0 | 0 | 0 |
| | | 500 | 0 | 0 | 0 | 0 |
| | | 1000 | 0 | 0 | 0 | 0 |
| | | 2000 | 0 | 0 | | 5 b |
| | | 4000 | 0 | 0 | | 15 a |
| cyhalofop-B | Tech. | | 0 | 0 | 100 | 100 |

[a] 0, no toxicity of rice; 9, all of the rice dead. [b] 0, no effect of natural extracts; 100, all of the weed plants dead. [c] DAA, days after application. [d] Means within a column followed by the same letter are not significantly different based on the LSD test ($P < 0.05$).

AIG and AIDW of barnyard grass. Although these results differ from previous studies, the allelopathic effect of rice varieties on barnyard grass in the latter studies used extracts and residues from different rice plant parts (*9*, *10*). Rice varieties without awns or with colored awns showed greater inhibitory effects on the growth of barnyard grass than varieties with colorless awns. This result disagrees with the current work.

In this study, awn existence was positively correlated with momilactone A ($r^2 = 0.23$***) and B ($r^2 = 0.14$**). Therefore, rice varieties with awns appear to have more momilactones as compared with awnless varieties. The results of this study were only partially in accordance with previous studies, possibly because of the complex allelopathic reactions between plants, weeds, and various environmental factors.

**Inhibitory Bioactivity Study in the Field.** In the field, the inhibitory bioactivity study of the momilactones A and B showed no toxicity on rice plants (**Table 8**). However, *E. crus-galli* P. Beauv. var. *oryzicola* Ohwi was inhibited by momilactone A (50% inhibition with 4000 g a.i./ha momilactone A). Also, *S. juncoides* and *E. kuroguwai* were suppressed by 75 and 90%, respectively, in the presence of momilactone A (**Table 9**). Momilactone B showed no toxicity in all treatments. Momilactone A showed a greater inhibition than momilactone B at all concentrations. Our study showed that momilactone A had a greater inhibitory effect than momilactone B on *E. crus-galli* P. Beauv. var. *oryzicola* Ohwi, *M. vaginalis* var. *plantaginea*, *S. juncoides*, *E. kuroguwai.* and *E. crus-galli* var. *formosensis* Ohwi (**Tables 8** and **9**). This contrasts with the results of Kato et al. (*19*) and Kato-noguchi and Ino (*23*), who observed that the inhibitory activity of momilactone B was much greater than that of momilactone A on the growth of cress and lettuce seedlings. The difference of inhibitory activity may be caused by interactions of compounds or the sensitivity of test species between laboratory and field conditions. For example, momilactones made in Tween-20 solution may be different in allelopathic effect as compared with solutions containing one momilactone compound. Fourteen days after the application of 4000 g a.i./ha of momilactone A showed a 50% reduction of *E. crus-galli* P. Beauv. var. *oryzicola* Ohwi. The inhibitory effect of momilactone A was directly proportional to its concentration. Early application corresponds to a higher reduction of weeds. Therefore, the growth inhibition of *E. crus-galli* P. Beauv. var. *oryzicola* Ohwi using momilactone A was stronger on early application.

Results of inhibitory activity of broad leaf weeds such as *M. vaginalis* var. *plantaginea*, *S. juncoides*, and *E. kuroguwai* are shown on **Table 9**. The responses of three broad leaf weeds species to momilactone A and B showed that momilactone A had a higher inhibitory activity than momilactone B. The inhibitory effects of momilactones A and B on broad leaf weed species were similar to the one on *E. crus-galli* P. Beauv. var. *oryzicola* Ohwi. In broad leaf weeds, early application of momilactones A and B had a higher effect as compared to later

**Table 9.** Inhibitory Bioactivity of Three Weed Species of Momilactones A and B in the Field

| | | toxicity (0–9)[a] | | inhibitory effect (0–100)[b] | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | transplanted rice | | *M. vaginalis* var. *plantaginea* | | *Scirpus juncoides* | | *Eleocharis kuroguwai* | |
| treated compounds | concn (g a.i/ha) | 7 DAA | 14 DAA | 7 DAA | 14 DAA | 7 DAA | 14 DAA | 7 DAA | 14 DAA[c] |
| momilactone A | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 250 | 0 | 0 | 20 | 0 | 0 | 0 | 30 | 20 e[d] |
| | 500 | 0 | 0 | 30 | 20 | 20 | 10 b | 60 | 55 d |
| | 1000 | 0 | 0 | 37.5 | 20 | 30 | 10 b | 70 | 60 c |
| | 2000 | 0 | 0 | | | | 80 a | | 70 b |
| | 4000 | 0 | 0 | | | | 90 a | | 75 a |
| momilactone B | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 250 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 500 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 1000 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 2000 | 0 | 0 | | | | 5 | | 0 |
| | 4000 | 0 | 0 | | | | 5 | | 10 |
| carfentrazone | | 1 | 1 | 98 | 92 | 91 | 88 | 94 | 90 |
| pyrazosulfuron | | 1 | 1 | 99 | 95 | 95 | 98 | 97 | 90 |

[a] 0, no toxicity of rice; 9, all of the rice dead. [b] 0, no effect of natural extracts; 100, all of the weed plants dead. [c] DAA, days after application. [d] Means within a column followed by the same letter are not significantly different based on the LSD test ($P < 0.05$).

application. Seven days after the application of 1000 g a.i./ha of momilactone A, the highest inhibition (70%) was seen in *Eleocharis*. However, 14 days after application, momilactone A 4000 g a.i./ha showed the highest inhibitory effect (90%) on *S. juncoides*. Results in **Table 9** indicated that the inhibitory effects of momilactones A and B on weed species were different according to the application stage and weed species. These experiments showed that the different responses to momilactones A and B are weed species-dependent. Therefore, further studies will be needed for all plant species at various concentrations of momilactone A and momilactone B. Furthermore, we will evaluate the biochemical mode of action of momilactones A and B in herbicides.

The evaluation or selection of rice varieties with allelopathic effects is an arduous task because of complex reactions between plants and cultivation environments. The main objectives of this study were to determine rice varieties with allelopathic potential using rice hull extracts and to quantify momilactones A and B in rice hulls germplasm. Rice varieties were separated according to genetic properties such as region of origin and maturation time, as well as morphological characteristics such as hull color, awn, and awn color. In this study, the selection pressure for allelopathic rice varieties by morphological characteristics was slightly weak when it was adapted to TIR on barnyard grass. However, because inhibition of GR, GP, LDW, and RDW of barnyard grass occurred, it is possible that allelopathic rice varieties can be selected based on morphological characteristics such as hull color. One limitation of this bioassay is that the concentration of allelopathic substances in extracts may be greater than in paddy field. On the basis of the bioassay hull extracts (50 g/L) used in this bioassay, 14850 kg hulls $ha^{-1}$ would be theoretically required for allelopathic effects to occur in the paddy field (*8*). However, because factors such as soil components related with the positive and negative effects other than extract concentration are involved in allelopathic activity, it is more appropriate to generalize that the more rice hulls remaining in the paddy soil, the greater the concentration of allelopathic compounds, momilactones, phenolic compounds released during the decomposition. This study has implications for the use of agricultural residues because farmers in Korea generally leave rice hulls in the field after harvesting. Further investigations are needed to analyze the relationship between allelopathic varieties and momilactones concentrations under field conditions.

This study will greatly contribute in that as the cultural practices for paddy rice change from hand or machine transplanted to direct-seeded to reduce production costs, weed problems such as barnyard grass, one of the greatest yield-limiting weeds in the rice field, will be more predominant because rice and weeds can emerge together. Thus, direct-seeded rice with aerobic conditions is expected to have a greater reliance on herbicides to control weeds. Therefore, with an increase in direct-seeded rice and the implementation of conservation tillage practices, the breeding and development of a rice variety with proven allelopathic characteristics and contained high allelopathic compounds could provide an environmentally acceptable and low-cost approach for barnyard grass control.

In conclusion, we first evaluated the correlation between momilactones and genetic properties and morphological characteristics among rice hulls germplasm and assessed the inhibitory bioactivity of the compounds for weed control in the paddy field. These results may be useful in providing basic information for breeding allelopathic varieties through the extension of databases. We should be able to efficiently control paddy weeds such as barnyard grass and manipulate our rice germplasm resources to successfully select for enhanced allelopathic compounds production. Momilactones may be partially involved in allelopathic effects and may also be used as useful selection makers to identify allelopathic rice varieties capable of controlling paddy weeds. Also, the inhibitory bioactivity of momilactones A and B suggests that the compounds might be used as a natural herbicide for paddy weed control. In the future, a broader range of doses and application times of momilactones A and B and environmental impact in paddy soil on rice needs to be investigated to improve the efficacy of these compounds for weed control.

## ABBREVIATIONS USED

AIDW, average inhibition of dry weight; LDW, leaf dry weight; SDW, straw dry weight; RDW, root dry weight; GR, germination weight; GP, germination percentage; AIG, average inhibition of germination; TM, total momilactones; GC/MS, gas chromatography/mass spectroscopy.

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