

## Inhibitory effects of Italian ryegrass (*Lolium multiflorum* Lam.) seedlings of rice (*Oryza sativa* L.)

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### ABSTRACT

The rice rotation in China and Korea encourages growth of Italian ryegrass (IRG) under moist conditions in rice fields. IRG in paddy fields is sown in end September/early October and harvested in May and its residues are ploughed in the field before the rice transplanting in June. The fermentation extract of IRG drastically inhibited the shoot and root length of rice cultivars, Ilmibyeo and Dongjinbyeo degree than boiling water extract and ethanol extracts. The fermentation extracts inhibited the shoot and root length of both cultivars during the seedling and development stages than in the harvest period. The inhibition of shoot and root length of rice by shoot and root extracts of various IRG cultivars was similar in all tested cultivars. The IRG shoot and root incorporations in direct seedling reduced the plant height and shoot fresh weight of rice cultivars than in transplanting cultivation. Caffeic acid (except in IRG root), *p*-coumaric acid, ferulic acid, and hydrocinnamic acid were detected in the water fraction of fermentation extract of IRG shoots and roots. Overall, the phenolic compounds of IRG reduced the rice growth.

**Key words:** Cultivars, extracts, growth inhibition, Italian ryegrass, *Lolium multiflorum* Lam., *Oryza sativa* L., phenolic compounds, plant residue, rice, roots, seedling growth inhibition, shoots, weed.

### INTRODUCTION

Italian ryegrass (*Lolium multiflorum* Lam., IRG) is major winter forage crop in temperate climates of China and Korea. It is rich in crude protein, digestible nutrients, sugar and prepares highest quality silage (23). However, it is also a troublesome weed, reduces the yield of wheat (*Triticum awstivum* L.) and barley (*Hordeum vulgare* L.) crops (24). For example, IRG densities of 100 plants m<sup>-2</sup> reduced the wheat yield by 30% (24). Residues of several crops and weeds are allelopathic to germination and early growth of crops and weeds (4,18,20). For example, annual fescue (*Vulpia myuros*) residues inhibited the germination and root growth of wheat, owing to the presence of some allelochemicals in crop residues (2,3,6). Additionally, volatile emissions from residues of winter legumes, [Berseem clover (*Trifolium alexandrinum* L.), hairy vetch [*Vicia hirsuta* (L.) S.F. Gray], and crimson clover (*Trifolium incarnatum* L.)], inhibited the germination and seedling development of onion, carrot and tomato (7). Incorporation of crimson clover as green manure suppresses the common lambsquarters (*Chenopodium album* L.) in succeeding corn (*Zea mays* L.) (12). The use of rapeseed residue as green manure decreased the occurrence of weed species [*Echinochloa crus-galli*, *Monochoria vaginalis* and *Cyperus amuricus* Max.] by > 50% than rice cropping systems (9).

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All plant roots secrete both low- and high-molecular weight compounds in the rhizosphere, in response to biotic and abiotic stresses (4). The phenolics are main allelopathic compounds (5), which inhibit the germination and growth of plants (25). For example, previous studies identified ferulic acid, *p*-coumaric acid, vanillic acid, *p*-hydroxybenzoic acid, salicylic acid, and syringic acid in wheat residues (15) and ferulic acid, *p*-coumaric acid, syringic acid, and vanillic acid in oat residues (13).

IRG in paddy fields is sown in end September/early October and harvested in May and its residues are ploughed in the field before the rice transplanting in June (26,27). IRG-rice rotation in China and Korea encourages good growth of IRG in rice fields. In Korea, the total growing area of IRG-rice rotation systems increased from 43,000 ha in 1990 to 155,000 ha in 2009, due to the introduction of cold resistant IRG cultivars such as Hwasan 101, Corwin master, and Corwinari (8). However, the number of rice tillers per plant were reduced drastically (44%) in paddy soil after IRG crop (17,27). The reduction in rice tillers may be related to the allelochemicals produced during the decomposition of soil incorporated IRG residues in rice fields. Although the allelopathic effects of IRG, including *Lolium* spp., have been investigated in IRG-rice rotation systems (17,20,22), but studies have not been conducted to investigate rice growth in response to residues of different IRG cultivars at various growth stages. Hence, this study aimed to (a) investigate the inhibition of rice growth by different plant parts, extraction methods, growth stages and cultivars of IRG, (b) determine % inhibition in rice growth after soil incorporation of IRG shoots and roots in to paddy soil, (c) Identify the inhibitory substances of rice growth by high-performance liquid chromatography (HPLC) analysis of extracts of fermented IRG shoots and roots and (d) confirm the magnitude of rice growth inhibition by phenolic compounds.

## MATERIALS AND METHODS

### Plant materials

On October 25, 2013, IRG (cv. Greenfarm) was sown in a paddy field in Naju, South Korea (N: 35° 0' 5", E: 127° 30' 30") to investigate the inhibitory effects of various IRG extracts on rice growth. On March 3, 2016, seven IRG cultivars (Greenfarm, Hwasan 101, Hwasan 104, Florida 80, Top 90, Corwin master, and Corwinari) were planted in greenhouse. Two rice cultivars (cvs. Ilmiby eo and Dongjinbyeo) were used for petri dish bioassay. The seeds of rice cultivars were sown on May 25 and were harvested on October 20, 2015. IRG cultivars were purchased from Korean Horticultural Company. All seeds used in this study were stored at 4 °C until used for experiments.

### Effect of IRG extracts on rice growth

Fifty days after sowing of IRG in field, its 50 g dried (cv. Greenfarm) shoots and roots were used to make extractions. The shoots and roots were extracted separately by different extraction methods (water, boiled water, ethanol and fermentation). Briefly, 50 g of dried shoots or roots of IRG were ground and put in 1,000 ml distilled water or ethanol for 24 h for making water or ethanol extracts. In addition, 50 g of dried shoots or roots of IRG were put in 1,000 ml distilled water and boiled at 100 °C for 30 min to make boiling water extracts, or in 1,000 ml distilled water and stored at room temperature for 20 days to make fermentation extracts. To maintain natural decomposition of shoots and roots of IRG, IRG growing soils were used as microbial inoculation. One hundred milliliters of fermentation

solution received 1 g of the air-dry IRG growing soils to make 1% w/v solution. Each extract was filtered through cheese cloth to remove fiber debris and centrifuged at 5,000 g for 30 minutes. The supernatant was completely evaporated using a vacuum dryer (Hanbaek Scientific Co. Korea). Each extract was then dissolved in distilled water to ensure that the final concentration was 50%, after which it was centrifuged at 10,000 g and 4 °C for 30 minutes. The supernatant was then filtered through a nylon syringe filter (13 mm diameter, 0.2 µm pore size). Petri dish bioassay experiments were conducted using 9 cm diameter petri dishes in a completely randomized design with three replications. Briefly, 10 seeds of rice (cvs, Ilmibyeo and Dongjinbyeo) were placed in a petri dish lined with two sheets of filter paper (Whatman #1) and filled with 10 ml of extracts at 0, 3 or 5%. The petri dish was then sealed with sealing tape and incubated for 3 days in the dark and 4 days in the light (50 µmol m<sup>-2</sup> s<sup>-1</sup> photosynthetically active radiation [PAR]) in a growth chamber at 25 °C. After incubation for 7 days, the plant height and root lengths of each seedling were measured. We selected a fermentation extract for subsequent experiments. Because rice growth was inhibited to a much greater degree in response to fermentation extract than water, boiling water, and ethanol extracts.

#### **Effects of fermentation extracts of IRG shoots and roots on rice growth**

To confirm the levels of rice growth inhibition in response to fermentation extracts of IRG (cv. Greenfarm) shoots and roots at the seedling stages (50 days after sowing), maturation period (80 days after sowing), and harvesting time (120 days after sowing) grown in paddy field, a similar experiment was done in petri dish bioassay using the same method as described above. In addition, seeds of seven cultivars (Greenfarm, Hwasan 101, Hwasan 104, Florida 80, Top 90, Corwin master, and Corwinari) of IRG were sown in greenhouse, grown till 50 days after sowing to determine their allelopathic potential. Other conditions and procedures were same as described previously for the petri dish bioassay.

#### **Effect of soil incorporated Italian ryegrass shoots and roots on rice growth**

Pots (17 × 11 cm) were filled with paddy soils to determine the levels of inhibition on rice growth in response to soil incorporation of shoots and roots of IRG (cv. Greenfarm) at seedling stages (50 days after seeding) grown in a greenhouse under different cropping patterns. Briefly, pots received IRG shoots and roots mixed in to a depth of 5 cm by hand at 0, 200, and 400 g m<sup>-2</sup>. Nine soaked seeds of rice (cvs. Ilmibyeo and Dongjinbyeo) were planted in the pots for direct seedling on water paddy conditions. Additionally, nine eight-day-old rice plants were transplanted in the pot for transplanting cultivation condition. After planting, the pots were placed in a greenhouse at 30 °C/25 °C under a 14/10 h day/night regime and a light intensity of 500 µmol m<sup>-2</sup> s<sup>-1</sup>. Plant height and shoot fresh weight were recorded at 7, 14 and 21 days after seeding or transplanting.

#### **Effect of extracts obtained by different solvents of fermentation on rice growth**

A total of 260 g of ground powder of IRG (cv. Greenfarm) shoots or roots at seedling stages (50 days after seeding) grown in a greenhouse were mixed with 2.6 L of distilled water. The mixture was then incubated in a growth chamber at 26 °C with dark condition for 30 days for natural fermentation. Thereafter, the mixture was filtered through a Miracloth and concentrated under reduced pressure to a final volume of 1,000 ml. The

concentrated extracts were subsequently separated by hexane, chloroform, ethyl acetate, and water layers. Each organic layer was then concentrated under reduced pressure and the pellet was completely evaporated using a vacuum dryer (Hanbaek Scientific Co. Korea). Each of the solvent fractions was dissolved in 0.1% Tween 20 to ensure that the final concentration was 50%, after which they were diluted with distilled water to attain concentrations of 0, 1, 3 and 5%. Petri dish bioassays were as described in the previous section.

#### **Phenolic compound analysis by HPLC**

A 10 g equivalent from the water layer of the solvent fraction of the fermentation extract of IRG shoots and roots was concentrated under reduced pressure and dissolved in 10 ml of methanol (HPLC grade). The suspension was filtered through a 0.45  $\mu\text{m}$  syringe filter, after which 10  $\mu\text{L}$  of filtrate was loaded onto the HPLC system (Agilent 1200 series). Separation was achieved on a 205  $\times$  4.60 mm, Spherclone 5  $\mu$  ODS (2) column. The absorbance of each sample solution was measured by a UV detector at 210 nm. The mobile phase was 0.1% glacial acetic acid in water (solvent A) and 0.1% glacial acetic acid in acetonitrile (solvent B). The gradient was 50 min, 0% to 40% (B); 2 min, 40% to 100% (B). The run time was 60 min using a flow rate of 1 mL/min. Phenolic compound standards, hydrocinnamic acid, *p*-coumaric acid, ferulic acid, and caffeic acid were purchased from Sigma Aldrich (St. Louis, MO, USA) to generate standard curves. All standard calibration curves showed high degrees of linearity ( $r^2 > 0.99$ ) (data not shown).

#### **Phenolic compounds bioassay**

To confirm the growth inhibition of rice by phenolic compounds, caffeic acid, *p*-coumaric acid, ferulic acid, and hydrocinnamic acid were applied at 0, 0.3, 0.5, and 1 mM for petri dish bioassay. The petri dish bioassay was then conducted as described in the previous section.

#### **Statistical analysis**

All experiments were conducted twice with three replicates for each treatment. Data were expressed as percentage of the untreated control to standardize comparisons between treatments. Significant difference were determined using analysis of variance (ANOVA). Analyses were performed using Statistical Analysis Systems (21) software. In the case of significant difference, means were separated using Duncan's multiple range test at  $P \leq 0.05$ .

## **RESULTS AND DISCUSSION**

#### **Effects of IRG shoots and roots extracts on rice growth**

There was a significant interaction between extraction method and concentration on inhibition of shoot and root lengths of rice cultivars in response to IRG shoot and root extracts (Table 1). The shoot and root length of the rice cultivars Ilmibyeo and Dongjinbyeo were inhibited to a much greater degree in response to fermentation extract than water, boiling water, and ethanol extracts. However, there were no differences among water, boiled water, and ethanol extracts. The shoot of Ilmibyeo and Dongjinbyeo was inhibited by 68% and 49%, respectively, in response to fermentation extract of IRG shoots at a 5% concentration. Additionally, the root length of Ilmibyeo and Dongjinbyeo was inhibited by 79% and 91%, respectively, in response to fermentation extract of IRG shoots at 5%. In the

case of fermentation extract of IRG roots, shoot length of Ilmibyeo and Dongjinbyeo was inhibited by 51% and 39% in response to 5% fermentation extract, respectively, while their root lengths were inhibited by 52% and 63%. The shoot and root length of the two rice cultivars showed a greater degree of inhibition in response to IRG shoot extracts than IRG root extracts. The IRG shoot and root extracts led to greater inhibition of the root length than shoot length of the two rice cultivars. In another study, co-growth of annual ryegrass and wheat reduced the root and shoot length of wheat, and annual ryegrass grown for 6-8 days had the greatest inhibitory effect against wheat root growth (1).

Table 1. Inhibitory effects of various Italian ryegrass (IRG) shoot and root extracts on shoot and root lengths of rice (cvs. Ilmibyeo and Dongjinbyeo).

Extraction method (E)	Conc. <sup>1)</sup> (%)	Shoot extracts of IRG				Root extract of IRG			
		Ilmibyeo		Dongjinbyeo		Ilmibyeo		Dongjinbyeo	
		Shoot length	Root length	Shoot length	Root length	Shoot length	Root length	Shoot length	Root length
		% of control							
Water	1	5.2±6.2 <sup>h</sup>	19.0±10.3 <sup>d</sup>	8.0±6.7 <sup>cd</sup>	22.2±6.2 <sup>e</sup>	28.4±13.4 <sup>bcd</sup>	30.8±13.1 <sup>bc</sup>	16.5±3.7 <sup>def</sup>	24.1±1.5 <sup>d</sup>
	3	24.5±1.5 <sup>de</sup>	39.0±1.3 <sup>c</sup>	26.2±5.6 <sup>bc</sup>	58.6±9.2 <sup>bc</sup>	26.0±0.9 <sup>bcdde</sup>	29.3±2.5 <sup>bc</sup>	14.8±1.6 <sup>def</sup>	25.6±5.9 <sup>d</sup>
	5	42.1±1.2 <sup>c</sup>	56.8±3.3 <sup>b</sup>	32.3±1.8 <sup>ab</sup>	66.9±7.5 <sup>b</sup>	16.6±4.2 <sup>cde</sup>	25.7±4.0 <sup>cd</sup>	11.0±4.1 <sup>efg</sup>	25.2±6.4 <sup>d</sup>
Mean		23.9	38.27	22.17	49.23	23.67	28.6	14.1	25.0
Boiled water	1	10.1±6.4 <sup>gh</sup>	20.6±13.4 <sup>d</sup>	-0.4±16.6 <sup>d</sup>	18.8±7.2 <sup>e</sup>	10.5±1.3 <sup>e</sup>	19.7±5.0 <sup>cd</sup>	4.7±5.6 <sup>e</sup>	22.6±5.4 <sup>d</sup>
	3	19.4±8.1 <sup>efg</sup>	26.6±3.4 <sup>cd</sup>	20.9±13.6 <sup>bc</sup>	42.6±7.1 <sup>d</sup>	12.7±2.7 <sup>de</sup>	26.3±1.3 <sup>cd</sup>	26.0±3.1 <sup>bc</sup>	29.9±15.0 <sup>cd</sup>
	5	28.3±6.4 <sup>de</sup>	39.0±3.1 <sup>c</sup>	31.9±10.4 <sup>ab</sup>	57.0±1.5 <sup>bc</sup>	21.9±5.2 <sup>bcdde</sup>	28.3±1.0 <sup>bc</sup>	18.1±2.1 <sup>de</sup>	33.2±2.6 <sup>bcd</sup>
Mean		19.3	28.7	17.5	39.5	15.0	24.8	16.3	28.6
Ethanol	1	12.9±6.2 <sup>gh</sup>	17.2±4.4 <sup>d</sup>	10.7±8.3 <sup>cd</sup>	41.9±3.5 <sup>d</sup>	16.5±12.7 <sup>cde</sup>	12.7±4.9 <sup>d</sup>	9.2±5.6 <sup>fg</sup>	39.4±1.6 <sup>bc</sup>
	3	22.3±7.3 <sup>def</sup>	19.1±2.2 <sup>d</sup>	17.8±4.1 <sup>bcd</sup>	43.4±4.2 <sup>d</sup>	19.8±5.7 <sup>bcdde</sup>	17.7±2.2 <sup>cd</sup>	13.6±5.1 <sup>def</sup>	40.6±2.3 <sup>bc</sup>
	5	28.4±4.9 <sup>de</sup>	21.1±3.5 <sup>d</sup>	27.9±3.6 <sup>bc</sup>	46.8±6.8 <sup>cd</sup>	30.7±7.7 <sup>bc</sup>	18.2±2.7 <sup>cd</sup>	19.0±1.5 <sup>cd</sup>	42.4±3.1 <sup>b</sup>
Mean		32.9	19.1	18.8	44.0	22.3	16.2	13.9	40.8
Fermentation	1	30.2±6.4 <sup>d</sup>	38.2±7.8 <sup>c</sup>	23.4±9.2 <sup>bc</sup>	65.4±14.6 <sup>b</sup>	32.9±4.8 <sup>bc</sup>	25.0±6.8 <sup>cd</sup>	20.6±6.2 <sup>cd</sup>	37.6±4.1 <sup>bc</sup>
	3	57.3±4.5 <sup>b</sup>	76.6±13.8 <sup>a</sup>	34.3±11.7 <sup>ab</sup>	84.6±7.8 <sup>a</sup>	36.4±11.6 <sup>ab</sup>	41.6±13.5 <sup>ab</sup>	30.7±1.5 <sup>b</sup>	54.5±4.5 <sup>a</sup>
	5	67.6±3.6 <sup>a</sup>	78.6±9.2 <sup>a</sup>	49.2±18.2 <sup>a</sup>	90.8±8.2 <sup>a</sup>	50.7±18.5 <sup>a</sup>	51.6±15.2 <sup>a</sup>	39.0±3.7 <sup>a</sup>	63.3±6.2 <sup>a</sup>
Mean		51.7	64.5	35.6	80.3	40.0	39.4	30.1	51.8
Significance (E x Conc.)		***	***	***	***	***	***	***	***

<sup>1)</sup> Conc., Concentration.

<sup>2)</sup> Data are means ± SD (n=3) \*\*\* P ≤ 0.001

<sup>3)</sup> Means within a column followed by the same letters are not significantly different at 5% level according to Duncan's Multiple Range Test.

### Effects of fermentation extracts of IRG shoots and roots on rice growth

There was a significant interaction between growth stage and concentration on inhibition of shoot and root lengths of rice cultivars in response to the fermentation extracts of IRG shoot or root (Table 2). Greater inhibition of shoot and root length of the two cultivars was observed in shoot and root extracts of IRG of seedling and mature stages but not in that of the harvest period. The inhibition of shoot and root length of the two rice cultivars was increased with increasing concentrations of shoot and root extracts of IRG of all growth stages tested. Shoot and root length of the two rice cultivars were inhibited by

33-70% and 49-96% in response to the 5% extract of seedling and mature stages, respectively. Generally, shoot length of Ilmibyeo was more sensitive than those of Dongjinbyeo in shoot and root extracts of IRG of all growth stages tested while root length of Dongjinbyeo was more sensitive than those of Ilmibyeo in shoot and root extracts of IRG of all growth stages tested. Lee (16) conducted a study in which the effects of extracts of cover crops of different growth stages (vegetative growth, flowering, and fruiting) on root lengths of soybean, radish, rape, and lettuce were investigated. In their investigation, the inhibitory effects were found to be higher in response to treatment with the extracts of crops obtained during the flowering and fruiting period.

Table 2. Inhibitory effects of fermentation extraction of Italian ryegrass (IRG, cv. Greenfarm) shoot and root at different growth stages on shoot and root lengths of rice (cvs. Ilmibyeo and Dongjinbyeo).

Growth stages (G) (DAS) <sup>1)</sup>	Conc. <sup>2)</sup> (%)	Shoot extracts of IRG				Root extracts of IRG			
		Ilmibyeo		Dongjinbyeo		Ilmibyeo		Dongjinbyeo	
		Shoot length	Root length	Shoot length	Root length	Shoot length	Root length	Shoot length	Root length
		% of control							
Young (50)	1	32.3±14.0 <sup>c</sup>	39.0±0.6 <sup>d</sup>	33.6±5.0 <sup>c</sup>	81.1±2.9 <sup>c</sup>	27.3±12.3 <sup>c</sup>	29.0±12.1 <sup>cde</sup>	19.1±1.0 <sup>de</sup>	41.5±3.6 <sup>de</sup>
	3	60.7±2.8 <sup>ab</sup>	83.0±1.7 <sup>a</sup>	46.4±7.1 <sup>b</sup>	84.8±6.6 <sup>bc</sup>	26.9±3.2 <sup>c</sup>	47.6±10.6 <sup>bc</sup>	30.1±5.5 <sup>c</sup>	49.6±2.5 <sup>cd</sup>
	5	68.8±0.9 <sup>a</sup>	83.7±2.6 <sup>a</sup>	66.5±6.3 <sup>a</sup>	95.9±1.9 <sup>a</sup>	33.0±7.7 <sup>bc</sup>	49.4±16.6 <sup>ab</sup>	34.9±5.5 <sup>abc</sup>	64.4±2.6 <sup>ab</sup>
	Mean	53.9	68.6	48.8	87.3	29.0	42.0	28.0	51.8
Adult (80)	1	23.0±3.8 <sup>c</sup>	30.0±4.5 <sup>d</sup>	21.0±8.9 <sup>de</sup>	52.1±4.1 <sup>c</sup>	36.1±14.2 <sup>bc</sup>	28.8±2.2 <sup>cde</sup>	15.3±5.7 <sup>e</sup>	33.4±4.2 <sup>e</sup>
	3	52.2±3.0 <sup>b</sup>	86.0±4.7 <sup>a</sup>	33.6±7.7 <sup>c</sup>	92.2±5.3 <sup>ab</sup>	49.4±11.9 <sup>b</sup>	51.0±11.9 <sup>ab</sup>	32.4±1.8 <sup>bc</sup>	58.4±10.9 <sup>abc</sup>
	5	70.4±2.5 <sup>a</sup>	84.1±16.6 <sup>a</sup>	50.7±5.7 <sup>b</sup>	95.1±6.9 <sup>a</sup>	49.0±5.9 <sup>b</sup>	67.8±12.5 <sup>a</sup>	39.7±5.7 <sup>ab</sup>	68.8±4.2 <sup>a</sup>
	Mean	48.5	66.7	35.1	79.8	44.8	49.2	29.1	53.5
Harvest (120)	1	35.2±13.5 <sup>c</sup>	45.6±9.7 <sup>cd</sup>	15.6±1.5 <sup>e</sup>	63.0±3.9 <sup>d</sup>	27.7±19.0 <sup>c</sup>	17.2±10.8 <sup>e</sup>	27.4±3.1 <sup>c</sup>	38.0±10.5 <sup>de</sup>
	3	59.1±12.5 <sup>ab</sup>	60.8±13.2 <sup>bc</sup>	23.0±1.3 <sup>de</sup>	76.6±5.1 <sup>c</sup>	32.9±7.8 <sup>bc</sup>	26.1±4.7 <sup>de</sup>	29.6±5.7 <sup>c</sup>	55.5±5.7 <sup>bc</sup>
	5	63.6±8.6 <sup>ab</sup>	68.0±17.1 <sup>ab</sup>	30.2±1.3 <sup>cd</sup>	81.3±4.9 <sup>c</sup>	70.0±1.1 <sup>a</sup>	37.7±7.5 <sup>bcd</sup>	42.3±6.1 <sup>a</sup>	56.7±10.1 <sup>abc</sup>
	Mean	52.6	58.1	22.9	73.6	43.5	27.0	33.1	50.1
	Significance (G × Conc.)	***	***	***	***	**	***	***	***

<sup>1)</sup> DAS, days after seeding.

<sup>2)</sup> Conc., Concentration.

<sup>3)</sup> Data are means ± SD (n=3) \*\*  $P \leq 0.01$  \*\*\*  $P \leq 0.001$

<sup>4)</sup> Means within a column followed by the same letters are not significantly different at 5% level according to Duncan's Multiple Range Test.

### Effects of fermentation extraction of seven IRG cultivars on rice growth

There was a significant interaction between cultivar (Greenfarm, Hwasan 101, Hwasan 104, Florida 80, Top 90, Corwinmaster, and Corwinari) and concentration on inhibition of shoot and root lengths of rice cultivars in response to IRG shoot and root extracts (Table 3).

However, inhibition of shoot and root lengths of rice as affected by shoot and root extracts of different IRG cultivars was generally similar in all cultivars tested. The shoot and root length of Ilmibyeo and Dongjinbyeo were inhibited by 37-77% and 85-98%, respectively, in response to 5% shoot fermentation extracts of all IRG cultivars. Generally,

inhibition of shoot and root lengths of rice as affected by shoot and root extracts of different IRG cultivars was similar between two rice cultivars. However, the root fermentation extracts of all cultivars inhibited shoot and root length of these rice cultivars by 12-50% and

Table 3. Inhibitory effects of fermentation extraction of Italian ryegrass (IRG) cultivars at 50 days after seeding on shoot and root lengths of rice (cvs. Ilmibyeo and Dongjinbyeo).

Cultivar (C)	Conc. <sup>1)</sup> (%)	Shoot extracts of IRG				Root extracts of IRG			
		Ilmibyeo		Dongjinbyeo		Ilmibyeo		Dongjinbyeo	
		Shoot length	Root length	Shoot length	Root length	Shoot length	Root length	Shoot length	Root Length
		% of control							
Green farm	1	2.6±3.4 <sup>i</sup>	65.9±1.9 <sup>f</sup>	14.9±7.0 <sup>fg</sup>	46.8±15.9 <sup>ef</sup>	-11.1±3.7 <sup>e</sup>	25.4±7.3 <sup>efg</sup>	10.8±12.2 <sup>f</sup>	17.5±6.0 <sup>g</sup>
	3	40.2±1.5 <sup>de</sup>	88.4±13.3 <sup>bc</sup>	29.2±3.7 <sup>defg</sup>	79.4±1.4 <sup>bc</sup>	20.9±3.7 <sup>bcd</sup>	41.9±5.8 <sup>cdef</sup>	19.7±6.4 <sup>cdef</sup>	50.8±5.0 <sup>abcde</sup>
	5	45.7±13.0 <sup>cd</sup>	93.8±5.4 <sup>ab</sup>	36.5±8.4 <sup>bcd</sup>	84.8±0.5 <sup>abc</sup>	19.7±8.7 <sup>bcd</sup>	60.3±9.3 <sup>abc</sup>	31.1±4.1 <sup>abcd</sup>	61.6±10.0 <sup>abc</sup>
	Mean	29.5	80.9	26.9	70.3	9.8	42.5	20.5	43.3
Hwasan 101	1	12.8±8.4 <sup>hi</sup>	71.3±6.4 <sup>ef</sup>	7.6±15.0 <sup>g</sup>	21.4±4.1 <sup>g</sup>	12.8±6.7 <sup>bcd</sup>	23.4±6.7 <sup>fg</sup>	24.9±2.5 <sup>cde</sup>	33.8±6.9 <sup>efg</sup>
	3	47.3±3.4 <sup>cd</sup>	100.0±0.0 <sup>a</sup>	37.8±4.7 <sup>bcd</sup>	67.5±19.5 <sup>cc</sup>	28.2±16.9 <sup>b</sup>	41.9±10.5 <sup>cdef</sup>	31.9±2.9 <sup>abcd</sup>	66.2±17.3 <sup>a</sup>
	5	50.7±0.8 <sup>bcd</sup>	100.0±0.0 <sup>a</sup>	55.4±0.0 <sup>abc</sup>	84.6±2.8 <sup>abc</sup>	27.4±8.5 <sup>bc</sup>	74.1±1.3 <sup>a</sup>	40.5±2.3 <sup>a</sup>	65.4±16.3 <sup>ab</sup>
	Mean	36.9	90.4	33.6	57.8	22.8	46.5	32.4	55.1
Hwasan 104	1	11.5±3.4 <sup>hi</sup>	44.5±10.8 <sup>g</sup>	18.9±10.7 <sup>efg</sup>	37.3±11.0 <sup>f</sup>	15.8±15.5 <sup>bcd</sup>	13.8±7.3 <sup>g</sup>	14.3±3.3 <sup>ef</sup>	46.0±5.5 <sup>gabcde</sup>
	3	56.5±0.2 <sup>abc</sup>	83.0±9.1 <sup>cd</sup>	57.1±2.8 <sup>abc</sup>	81.0±1.9 <sup>bc</sup>	6.8±1.5 <sup>d</sup>	27.3±19.1 <sup>efg</sup>	21.6±2.3 <sup>cdef</sup>	42.9±4.8 <sup>cde</sup>
	5	55.4±2.2 <sup>abc</sup>	100.0±0.0 <sup>a</sup>	57.5±1.6 <sup>abc</sup>	100.0±0.0 <sup>a</sup>	16.7±11.1 <sup>bcd</sup>	47.5±3.8 <sup>bcd</sup>	21.6±4.7 <sup>cdef</sup>	64.6±2.9 <sup>abc</sup>
	Mean	41.1	75.8	44.5	72.7	13.1	29.5	19.2	51.2
Florida 80	1	20.9±6.6 <sup>gh</sup>	88.6±1.3 <sup>bc</sup>	23.5±13.0 <sup>fg</sup>	49.2±17.9 <sup>ef</sup>	11.5±5.9 <sup>bcd</sup>	12.8±13.3 <sup>g</sup>	8.1±6.2 <sup>f</sup>	19.8±8.4 <sup>fg</sup>
	3	65.2±5.5 <sup>a</sup>	87.9±4.3 <sup>bc</sup>	52.4±5.7 <sup>ab</sup>	36.5±11.7 <sup>at</sup>	20.9±5.2 <sup>bcd</sup>	54.1±15.2 <sup>abcd</sup>	25.7±2.3 <sup>cde</sup>	58.7±11.0 <sup>abc</sup>
	5	62.1±2.3 <sup>a</sup>	95.9±0.5 <sup>ab</sup>	58.4±36.6 <sup>ab</sup>	36.8±17.5 <sup>at</sup>	29.1±12.1 <sup>b</sup>	66.3±12.1 <sup>ab</sup>	25.9±11.4 <sup>bcd</sup>	62.9±4.6 <sup>abc</sup>
	Mean	49.4	90.8	44.8	74.2	20.5	44.4	19.9	47.1
Top 90	1	6.4±2.2 <sup>i</sup>	68.0±9.0 <sup>ef</sup>	27.6±4.9 <sup>efg</sup>	67.9±9.1 <sup>cd</sup>	-12.4±4.9 <sup>e</sup>	28.3±9.3 <sup>efg</sup>	20.0±10.8 <sup>cdef</sup>	31.7±15.9 <sup>efg</sup>
	3	26.0±1.5 <sup>fg</sup>	97.9±0.2 <sup>ab</sup>	37.3±5.9 <sup>bcd</sup>	96.8±1.4 <sup>ab</sup>	10.3±4.4 <sup>cd</sup>	37.0±7.3 <sup>def</sup>	21.6±6.2 <sup>cdef</sup>	39.5±5.4 <sup>def</sup>
	5	65.5±4.0 <sup>a</sup>	96.4±1.6 <sup>ab</sup>	71.9±26.2 <sup>a</sup>	97.1±2.5 <sup>ab</sup>	12.0±10.8 <sup>bcd</sup>	46.7±16.0 <sup>bcd</sup>	21.9±2.6 <sup>bcd</sup>	45.6±8.7 <sup>abcde</sup>
	Mean	32.6	87.4	45.6	87.3	3.3	37.3	21.2	38.9
Corwin master	1	11.1±15.9 <sup>hi</sup>	76.6±1.9 <sup>de</sup>	16.5±9.0 <sup>fg</sup>	50.3±13.1 <sup>de</sup>	16.7±7.1 <sup>bcd</sup>	33.5±8.1 <sup>defg</sup>	18.1±17.4 <sup>def</sup>	43.7±22.9 <sup>bcd</sup>
	3	42.4±4.2 <sup>de</sup>	93.6±2.4 <sup>ab</sup>	55.1±9.1 <sup>abc</sup>	97.5±2.4 <sup>ab</sup>	24.8±0.7 <sup>bc</sup>	66.1±11.0 <sup>ab</sup>	33.0±8.7 <sup>abc</sup>	51.0±9.4 <sup>abcde</sup>
	5	55.9±2.3 <sup>abc</sup>	98.4±0.9 <sup>ab</sup>	77.3±14.1 <sup>a</sup>	97.0±4.8 <sup>ab</sup>	49.6±3.0 <sup>a</sup>	73.8±4.7 <sup>a</sup>	33.2±4.9 <sup>abc</sup>	65.1±21.9 <sup>ab</sup>
	Mean	36.5	89.5	49.6	84.9	30.4	57.8	28.1	53.3
Corwinari	1	32.5±8.7 <sup>ef</sup>	73.1±7.4 <sup>ef</sup>	31.9±1.6 <sup>cdefg</sup>	93.2±1.1 <sup>ab</sup>	15.8±6.6 <sup>bcd</sup>	47.7±24.8 <sup>bcd</sup>	33.5±6.1 <sup>abc</sup>	34.9±7.7 <sup>efg</sup>
	3	55.5±3.3 <sup>abc</sup>	88.2±0.5 <sup>bc</sup>	53.8±24.6 <sup>abc</sup>	94.1±1.5 <sup>ab</sup>	4.3±5.3 <sup>d</sup>	42.8±11.7 <sup>cdef</sup>	31.1±1.4 <sup>abcd</sup>	52.4±4.8 <sup>abcde</sup>
	5	61.1±3.1 <sup>ab</sup>	97.8±0.3 <sup>ab</sup>	54.1±2.5 <sup>abcd</sup>	94.1±3.2 <sup>ab</sup>	19.7±18.5 <sup>bcd</sup>	43.8±16.0 <sup>bcd</sup>	39.5±5.5 <sup>ab</sup>	62.4±7.1 <sup>abc</sup>
	Mean	49.7	83.4	46.6	93.8	13.3	44.8	34.7	49.9
Significance (C x Conc.)		***	***	***	***	***	***	***	***

<sup>1)</sup> Conc., Concentration. <sup>2)</sup> Data are means ± SD (n=3) \*\*\* P ≤ 0.001

<sup>3)</sup> Means within a column followed by the same letters are not significantly different at 5% level according to Duncan's Multiple Range Test.

44-74%, respectively. Dissimilar to our study, the potential allelopathic effects of

*Echinochloa crus-galli* toward 47 varieties of rice were found to differ significantly (11). These results indicate that there are genetic differences among rice varieties. Additionally, 58 wheat accessions differed significantly in the amounts of allelochemicals exuded by the living roots of 17-day-old wheat seedlings (25). Thus, this result suggests that there is genetic similarity among IRG cultivars.

#### Effects of soil incorporated Italian ryegrass shoots and roots on rice growth

There was a significant cultivation condition by IRG treatment interaction on inhibition of plant height and shoot fresh weight of rice cultivars in response to the soil incorporation of IRG shoot and root (Table 4). In the direct seeding on water paddy treatment, the plant height of Ilmibyeo and Dongjinbyeo was significantly reduced by IRG shoot incorporation at 200 and 400 g/m<sup>2</sup> compared with the control. Although the shoot fresh weight of Ilmibyeo and Dongjinbyeo was not significantly different in response to IRG shoot incorporation of 200 g/m<sup>2</sup> when compared with the control, the shoot fresh weight of both cultivars was reduced by 41-60% in response to IRG shoot incorporation at 400 g/m<sup>2</sup>. The plant height of Ilmibyeo and Dongjinbyeo was also significantly reduced by IRG root incorporation at 200 and 400 g/m<sup>2</sup> compared with the control. The shoot fresh weight of both cultivars was inhibited by 26-40% in response to IRG root application at 200 and 400 g/m<sup>2</sup> compared with the control, but there were no significant differences between 200 and 400 g/m<sup>2</sup>. Generally, plant height and shoot fresh weight of Ilmibyeo were more reduced by IRG shoot and root incorporation than those of Dongjinbyeo.

Table 4. Inhibitory effects of soil incorporated Italian ryegrass (IRG) shoot and root on plant height and shoot fresh weight of rice (cvs. Ilmibyeo and Dongjinbyeo) under different cultivation conditions.

Cultivation condition (C)	IRG treatment (g/m <sup>2</sup> )	Ilmibyeo		Dongjinbyeo	
		Plant height	Shoot fresh weight	Plant height	Shoot fresh weight
		21 DAT <sup>1)</sup>	21 DAT	21 DAT	21 DAT
% of control					
Direct seeding on water paddy	Shoot 200	28.8±13.2 <sup>a</sup>	14.9±6.5 <sup>cde</sup>	12.3±3.9 <sup>b</sup>	9.5±6.1 <sup>bcd</sup>
	Shoot 400	29.5±12.0 <sup>a</sup>	59.8±3.6 <sup>a</sup>	19.5±6.3 <sup>a</sup>	41.1±24.0 <sup>a</sup>
	Root 200	16.4±3.1 <sup>bc</sup>	39.7±14.7 <sup>b</sup>	9.1±3.0 <sup>b</sup>	28.9±4.7 <sup>ab</sup>
	Root 400	24.0±4.1 <sup>ab</sup>	25.9±22.5 <sup>bc</sup>	11.7±6.0 <sup>b</sup>	26.5±15.4 <sup>ab</sup>
Transplanting culture	Shoot 200	0.8±4.1 <sup>d</sup>	3.0±1.9 <sup>de</sup>	5.8±2.3 <sup>bc</sup>	2.5±1.6 <sup>cd</sup>
	Shoot 400	1.9±1.1 <sup>d</sup>	-0.7±6.1 <sup>e</sup>	-0.4±3.3 <sup>c</sup>	-5.3±4.3 <sup>c</sup>
	Root 200	12.9±0.7 <sup>bcd</sup>	19.1±1.3 <sup>cd</sup>	7.6±2.7 <sup>b</sup>	24.4±4.9 <sup>ab</sup>
	Root 400	6.8±0.7 <sup>cd</sup>	17.2±5.0 <sup>cde</sup>	9.1±3.3 <sup>b</sup>	22.5±8.4 <sup>abc</sup>
Significance (C x IRG trt.)		***	***	**	**

<sup>1)</sup> DAT, days after treatment.

<sup>2)</sup> Data are means ± SD (n=3) \*\*  $P \leq 0.01$  \*\*\*  $P \leq 0.001$

<sup>3)</sup> Means within a column followed by the same letters are not significantly different at 5% level according to Duncan's Multiple Range Test.

On the other hand, the plant height of Ilmibyeo cultivated under transplanting culture condition was significantly reduced by IRG shoot incorporation at 7 and 14 days (data not shown) after seeding compared with the control, but there was no significant difference at 21 days after seeding. Additionally, the height of Dongjinbyeo was

significantly reduced by IRG shoot application at 7 days after seeding compared with the control, but there were no significant differences at 14 and 21 days (except for the 200 g treatment/m<sup>2</sup>) after seeding. The shoot fresh weight of both Ilmibyeo and Dongjinbyeo in the IRG shoot application was not significantly different from that of the control. For the incorporation of IRG roots treatment, the plant height of Ilmibyeo and Dongjinbyeo differed significantly at 7, 14, and 21 days after seeding compared with the control. The shoot fresh weight of Ilmibyeo and Dongjinbyeo at 21 days after seeding was reduced significantly by 17-24% in response to IRG root application. However, there were no significant differences between plants treated with 200 and 400 g/m<sup>2</sup> of IRG roots. Additionally, there were no differences between two rice cultivars treated with IRG shoot and root. These results indicate that plant height and shoot fresh weight of rice cultivars showed greater reductions in response to IRG shoot and root applications in direct seedling on water paddy conditions than in those under transplanting cultivation conditions. The reduction of rice growth may have been caused by decomposition products occurring during the decay of IRG shoot and roots. Similar to our study, a greenhouse experiment showed that incorporation of IRG residues in the soil reduced the number of tillers in rice by 36% (17). Additionally, total weed biomass and weight of *Setaria viridis*, *Amaranthus retroflexus*, and *Portulaca olearacea* were consistently reduced in response to residues of barley (*Hordeum vulgare* L.), oat (*Avena sativa* L.), wheat (*Triticum aestivum* L.) and rye (*Secale cereale* L.), as well as sorghum (19).

#### **Effects of different solvents fermentation extracts on rice growth**

There was a significant interaction between solvent fraction and concentration on rice growth in response to IRG shoot and root extracts (Table 5). The shoot and root length of Ilmibyeo and Dongjinbyeo treated with 5% of the water fraction of the fermentation extract of IRG shoots were inhibited 70-72% and 98-99%, respectively, when compared with the control. Additionally, 5% of the water fraction of the fermentation extract of IRG root inhibited shoot and root length by 28-40% and 72-83%, respectively, when compared with the control. The inhibition of shoot and root length was greater in response to treatment with the water fraction of the fermentation extract of IRG shoots than in response to treatment with the fermentation extract of IRG root. Additionally, the inhibition of shoot and root length was increased with increasing concentrations of IRG shoot and root extracts. However, the inhibition of shoot and root length in chloroform, ethyl acetate and butanol fractions was lower than those in water fraction.

Taken together, these findings indicate that the allelochemicals present in the fermentation extract of IRG shoots and roots may be water soluble.

#### **Analysis of phenolic compounds by HPLC**

HPLC analysis of the water fraction of fermentation extract of IRG shoots and roots revealed caffeic acid (not in the roots), *p*-coumaric acid, ferulic acid, and hydrocinnamic acid (Table 6). Caffeic acid in the water fraction of fermentation extract of IRG shoots was present in greater amounts among phenol compounds, but was not found in the IRG roots. The contents of *p*-coumaric acid and ferulic acid were similar to those of IRG shoots and roots. The levels of hydrocinnamic acid in the IRG shoots were higher than in the roots. In addition, the total phenolic compounds were higher in the IRG shoots than the IRG roots.

Table 5. Inhibitory effects of different solvent fractions by fermentation extracts of Italian ryegrass (IRG) shoots and roots on shoot and root lengths of rice (cvs. Ilmibyeyo and Dongjinbyeyo).

Fraction layer (F)	Conc. <sup>1)</sup> (%)	Shoot extracts of IRG				Root extracts of IRG			
		Ilmibyeyo		Dongjinbyeyo		Ilmibyeyo		Dongjinbyeyo	
		Shoot length	Root length	Shoot length	Root length	Shoot length	Root length	Shoot length	Root length
		% of control							
Hexane	1	15.8±3.6 <sup>ef</sup>	25.7±5.0 <sup>g</sup>	6.2±1.2 <sup>fg</sup>	22.3±0.9 <sup>ijk</sup>	-9.8±19.4 <sup>g</sup>	12.5±5.2 <sup>ef</sup>	11.7±2.0 <sup>fg</sup>	10.8±0.0 <sup>f</sup>
	3	19.6±3.4 <sup>def</sup>	26.0±4.4 <sup>g</sup>	33.2±2.2 <sup>de</sup>	29.5±5.0 <sup>hi</sup>	27.0±5.2 <sup>bcd</sup>	34.6±4.4 <sup>c</sup>	21.6±7.5 <sup>ef</sup>	25.9±2.5 <sup>e</sup>
	5	26.7±0.6 <sup>cd</sup>	41.8±7.5 <sup>f</sup>	40.3±0.9 <sup>cd</sup>	40.4±4.9 <sup>fg</sup>	34.0±4.9 <sup>ab</sup>	20.5±9.1 <sup>de</sup>	33.2±0.4 <sup>cde</sup>	26.0±7.5 <sup>e</sup>
Mean		20.7	31.2	26.6	30.7	17.1	22.5	22.2	20.9
Chloroform	1	11.2±10.6 <sup>fg</sup>	17.7±2.1 <sup>g</sup>	-4.2±7.7 <sup>h</sup>	19.0±8.8 <sup>ik</sup>	9.1±8.4 <sup>f</sup>	12.2±2.6 <sup>ef</sup>	8.6±5.8 <sup>g</sup>	28.3±6.3 <sup>e</sup>
	3	18.9±3.6 <sup>def</sup>	15.8±5.7 <sup>g</sup>	29.1±6.7 <sup>e</sup>	25.4±6.6 <sup>ij</sup>	13.3±4.3 <sup>ef</sup>	9.0±1.3 <sup>f</sup>	9.4±14.7 <sup>g</sup>	24.2±1.0 <sup>e</sup>
	5	22.8±0.6 <sup>de</sup>	24.1±2.6 <sup>g</sup>	39.2±8.9 <sup>cd</sup>	34.6±1.2 <sup>gh</sup>	18.6±2.2 <sup>def</sup>	15.0±2.6 <sup>ef</sup>	24.4±1.6 <sup>e</sup>	24.2±2.0 <sup>e</sup>
Mean		17.6	19.2	21.4	26.3	13.7	12.1	14.1	25.6
Ethyl acetate	1	2.5±1.6 <sup>g</sup>	4.1±2.1 <sup>h</sup>	-2.1±6.7 <sup>gh</sup>	14.3±4.6 <sup>k</sup>	15.4±0.6 <sup>def</sup>	13.4±4.4 <sup>ef</sup>	24.7±1.2 <sup>e</sup>	40.6±8.0 <sup>cd</sup>
	3	18.2±2.2 <sup>def</sup>	48.1±7.8 <sup>ef</sup>	38.7±7.8 <sup>cd</sup>	47.7±4.8 <sup>ef</sup>	28.8±8.0 <sup>bcd</sup>	35.6±1.7 <sup>c</sup>	26.8±11.3 <sup>de</sup>	40.0±1.0 <sup>d</sup>
	5	21.1±5.9 <sup>de</sup>	56.4±16.3 <sup>de</sup>	42.1±2.5 <sup>cd</sup>	51.7±4.8 <sup>de</sup>	32.6±10.9 <sup>bc</sup>	64.8±7.3 <sup>a</sup>	51.9±4.4 <sup>a</sup>	41.6±5.2 <sup>cd</sup>
Mean		13.9	36.2	26.2	37.9	25.6	37.9	34.5	40.7
Butanol	1	5.6±9.8 <sup>g</sup>	17.6±6.0 <sup>g</sup>	7.5±6.7 <sup>f</sup>	26.6±3.0 <sup>ij</sup>	10.5±5.5 <sup>f</sup>	19.5±1.3 <sup>def</sup>	28.3±5.6 <sup>cde</sup>	37.3±4.0 <sup>d</sup>
	3	32.3±2.4 <sup>bc</sup>	76.5±2.8 <sup>c</sup>	36.9±3.6 <sup>cde</sup>	56.2±4.7 <sup>d</sup>	28.8±2.4 <sup>bcd</sup>	27.1±11.9 <sup>cd</sup>	36.4±1.6 <sup>bcd</sup>	40.0±8.3 <sup>cd</sup>
	5	38.6±8.0 <sup>b</sup>	86.0±0.9 <sup>bc</sup>	44.2±2.2 <sup>c</sup>	67.0±4.9 <sup>c</sup>	46.7±7.4 <sup>a</sup>	31.7±3.2 <sup>c</sup>	46.0±1.2 <sup>ab</sup>	49.5±1.8 <sup>bc</sup>
Mean		25.5	60.0	29.5	49.9	28.7	26.1	36.9	42.3
Water	1	23.5±5.2 <sup>cde</sup>	63.8±11.4 <sup>d</sup>	45.2±0.9 <sup>c</sup>	88.8±3.5 <sup>b</sup>	4.2±12.1 <sup>f</sup>	49.2±7.9 <sup>b</sup>	23.6±0.8 <sup>e</sup>	39.7±3.1 <sup>cd</sup>
	3	63.5±1.2 <sup>a</sup>	91.0±2.1 <sup>ab</sup>	61.6±1.8 <sup>b</sup>	92.1±0.7 <sup>ab</sup>	11.2±1.2 <sup>f</sup>	52.6±2.3 <sup>b</sup>	29.1±1.3 <sup>cde</sup>	56.9±10.5 <sup>b</sup>
	5	70.3±1.1 <sup>a</sup>	98.5±0.3 <sup>a</sup>	71.9±2.1 <sup>a</sup>	97.8±1.6 <sup>a</sup>	27.7±3.4 <sup>bcd</sup>	71.9±8.8 <sup>a</sup>	39.2±8.1 <sup>bc</sup>	83.1±3.7 <sup>a</sup>
Mean		52.4	84.4	59.6	92.9	14.4	57.9	30.6	59.9
Significance (F x Conc.)		***	***	***	***	***	***	***	***

<sup>1)</sup> Conc., Concentration. <sup>2)</sup> Data are means ± SD (n=3) \*\*\*  $P \leq 0.001$

<sup>3)</sup> Means within a column followed by the same letters are not significantly different at 5% level according to Duncan's Multiple Range Test.

Table 6. Comparison of phenolic compounds in shoot and root of Italian ryegrass (IRG). (Unit: mg%)

Phenolic compounds	Shoot of IRG	Root of IRG
Caffeic acid	184.7±37.7	*N. D
<i>p</i> -Coumaric acid	7.3±0.6	11.0±0.1

Ferulic acid	18.8±2.2	20.7±0.2
Hydrocinnamic acid	11.9±0.5	1.0±0.0

<sup>1)</sup>N. D; Not detected.

The greater inhibition of shoot and root length of rice in the fermentation extract of IRG shoots than in IRG roots may be caused by higher levels of caffeic acid and hydrocinnamic acid, and the total phenolic compounds. Allelochemicals were isolated and identified from the aqueous leachates of decaying Italian ryegrass residues (17). One allelochemicals was identified as hydrocinnamic acid based on MS, <sup>1</sup>H NMR and <sup>13</sup>C NMR data.

Table 7. Inhibitory effects of phenolic compounds on shoot and root lengths of rice (cvs. Ilmibyeo and Dongjinbyeo).

Phenolic compounds (P)	Conc. <sup>1)</sup> (mM)	Ilmibyeo		Dongjinbyeo	
		Shoot length	Root length	Shoot length	Root length
		% of control			
Caffeic acid	0.1	-9.8±3.1 <sup>gh</sup>	-13.0±5.1 <sup>cd</sup>	15.1±4.1 <sup>lg</sup>	28.1±10.1 <sup>cdet</sup>
	0.3	13.4±6.9 <sup>bcd</sup>	34.2±15.9 <sup>b</sup>	23.7±4.0 <sup>detlg</sup>	39.0±1.6 <sup>c</sup>
	0.5	24.0±14.8 <sup>ab</sup>	39.8±8.7 <sup>b</sup>	44.4±12.3 <sup>a</sup>	60.3±8.6 <sup>b</sup>
	1.0	25.6±15.9 <sup>ab</sup>	74.1±13.9 <sup>a</sup>	43.2±6.6 <sup>ab</sup>	82.2±6.2 <sup>a</sup>
Mean		13.3	33.8	31.6	52.4
<i>p</i> -Coumaric acid	0.1	-16.5±5.3 <sup>h</sup>	-19.9±3.3 <sup>d</sup>	11.0±4.3 <sup>g</sup>	13.1±2.1 <sup>g</sup>
	0.3	-2.0±10.9 <sup>etg</sup>	-8.5±2.0 <sup>cd</sup>	17.6±5.3 <sup>etg</sup>	19.1±5.1 <sup>etg</sup>
	0.5	3.9±7.9 <sup>detg</sup>	33.2±16.6 <sup>b</sup>	22.2±5.7 <sup>detg</sup>	30.9±5.2 <sup>cde</sup>
	1.0	15.0±6.2 <sup>bcd</sup>	68.0±3.5 <sup>a</sup>	33.4±2.6 <sup>abcd</sup>	61.0±3.4 <sup>b</sup>
Mean		0.1	18.2	21.1	31.0
Ferulic acid	0.1	-6.7±1.8 <sup>gh</sup>	-1.6±1.3 <sup>c</sup>	28.0±7.3 <sup>cdte</sup>	21.8±8.8 <sup>detg</sup>
	0.3	12.6±1.2 <sup>bcd</sup>	3.2±3.0 <sup>c</sup>	15.1±9.1 <sup>lg</sup>	16.2±13.0 <sup>lg</sup>
	0.5	9.1±5.4 <sup>cde</sup>	0.5±8.1 <sup>c</sup>	25.9±4.0 <sup>det</sup>	19.8±8.0 <sup>etg</sup>
	1.0	0.8±3.5 <sup>detg</sup>	0.2±11.2 <sup>c</sup>	31.5±3.0 <sup>bcd</sup>	29.8±7.1 <sup>cde</sup>
Mean		4.0	0.6	25.1	21.9
Hydrocinnamic acid	0.1	4.7±3.6 <sup>det</sup>	2.8±5.6 <sup>c</sup>	-0.2±8.8 <sup>n</sup>	24.6±5.9 <sup>detg</sup>
	0.3	19.3±7.2 <sup>abc</sup>	41.2±7.0 <sup>b</sup>	15.1±6.4 <sup>lg</sup>	34.0±1.0 <sup>cd</sup>
	0.5	25.6±4.3 <sup>ab</sup>	27.5±6.2 <sup>b</sup>	23.9±7.2 <sup>def</sup>	32.3±2.7 <sup>cd</sup>
	1.0	33.1±6.5 <sup>a</sup>	67.3±5.4 <sup>a</sup>	39.0±8.4 <sup>abc</sup>	88.2±1.0 <sup>a</sup>
Mean		20.7	34.7	19.5	44.8
Significance (P × Conc.)		***	***	***	***

<sup>1)</sup> Conc., Concentration.

<sup>2)</sup> Data are means ± SD (n=3) \*\*\*  $P \leq 0.001$

<sup>3)</sup> Means within a column followed by the same letters are not significantly different at 5% level according to Duncan's Multiple Range Test.

### Inhibitory effects of phenolic compounds on rice growth

There was a significant interaction between phenolic compound and concentration on growth inhibition of rice in response to phenolic compounds (Table 7). The shoot and root length of Ilmibyeo did not differ significantly from that of the control following treatment with ferulic acid at 1 mM, but those of Dongjinbyeo were inhibited by 32% and

30%, respectively. Greater inhibition of Ilmibyeo and Dongjinbyeo was observed in response to hydrocinnamic acid, caffeic acid, and *p*-coumaric acid than in response to ferulic acid. When hydrocinnamic acid, caffeic acid, and *p*-coumaric acid were applied at 1 mM, the shoot and root length of Ilmibyeo and Dongjinbyeo were inhibited by 15-43% and 61-88%, respectively. The shoot and root length of Dongjinbyeo by treatments of phenolic compounds were more inhibited than those of Ilmibyeo. In addition, the inhibition of shoot and root length of Ilmibyeo and Dongjinbyeo was increased with increasing concentrations of phenolic compounds. Similar to fermentation extract of IRG shoots and roots, phenolic compounds led to greater inhibition of the root length than shoot length of rice. These results were in agreement with those of other studies in which hydrocinnamic acid significantly inhibited the root and shoot growth of rice seedlings and barnyard grass, even though barnyard grass is much more sensitive than rice (10,17).

## CONCLUSIONS

The fermentation extract of IRG severely inhibited the shoot and root length of the rice cultivars Ilmibyeo and Dongjinbyeo than water, boiling water, and ethanol extracts. The growth reduction of rice could have been caused by the phenol compounds in the IRG. Our results suggested that inhibitory allelopathic effects of decaying IRG residues might play a key role in the rice growth reduction. Thus, if the interval between IRG residues incorporation and rice transplanting is long enough, the phytotoxic effects of IRG residues on rice plants could be decreased.

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