Allelochemicals in Wheat (*Triticum aestivum* L.): Cultivar Difference in the Exudation of Phenolic Acids

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Analysis by GC-MS/MS showed that a worldwide collection of 58 wheat accessions differed significantly in the amounts of 7 known phenolic acids exuded by the living roots of 17-day-old wheat seedlings. The quantities of exuded allelochemicals varied with the specific compound and ranged from 2.3 to 18.6, from 0.6 to 17.5, from 0.1 to 4.9, from 0.0 to 52.7, from 0.33 to 12.7, from 1.5 to 20.5, and from 1.6 to 23.4 μg/L of water/agar for *p*-hydroxybenzoic, vanillic, *cis*-*p*-coumaric, syringic, *cis*-ferulic, *trans*-*p*-coumaric, and *trans*-ferulic acids, respectively. The concentrations of *p*-hydroxybenzoic and vanillic acids exuded by wheat seedlings were normally distributed in the 58 accessions. The level of each phenolic acid in root exudates did not correlate well to that previously observed in wheat. In comparison with weakly allelopathic accessions, strongly allelopathic accessions exuded larger quantities of allelochemicals into the growth medium. The chemical basis for wheat seedling allelopathy is an area for further investigation.

Keywords: *Allelopathy; allelochemicals; phenolic acids; root exudates; weed suppression; annual ryegrass (Lolium rigidum Gaud.)*

INTRODUCTION

Allelopathy has been well recognized as a biological means for integrated weed management (*1*−*3*). The phytotoxic compounds exuded by crop plants as root exudates could be employed to suppress weeds in the vicinity. Root exudates are defined as low molecular weight compounds that are released into the surrounding medium by living and intact roots (*4*). One of the basic criteria for a phytotoxic compound to be involved in allelopathic effects is that it must be released from the producing plant to the soil and absorbed by the target seed or plant (*5*). It is therefore necessary to investigate the exudation of allelochemicals prior to the implementation of crop seedling allelopathy for weed management.

Limited data have been reported on the identification of allelochemicals from root exudates. In a direct screening of 3000 accessions of *Avena* spp. for the ability to produce and exude scopoletin (6-methoxy-7-hydroxycoumarin), Fay and Duke (*6*) found that four accessions exuded up to 3 times as much scopoletin as a standard oat cultivar (*Avena sativa* L. cv. Garry). The accession PI 266281 produced the highest amount of scopoletin and significantly inhibited the growth of *Brassica kaber* (DC) Wheeler var. *pinnatifida* (stockes) Wheeler (wild mustard). Einhellig and Souza (*7*) reported that sorghum plants exuded sorgoleone, a hydroquinone that is quickly oxidized to a benzoquinone, which can inhibit weed growth at extremely low concentrations (0.01−0.125 mM).

In addition to the exudation of a single allelopathic compound, multiple compounds can also be simultaneously released by plant roots. Tang and Young (*8*) designed a trapping system for the continuous collection of root exudates and successfully identified 16 allelopathic compounds (mainly phenolic acids) in the root exudates of bigalta limpograss (*Hemarthria altissima*). In the search for allelochemicals in rice (*Oryza sativa*) that control ducksalad [*Hetheranthera limosa* (Sw.) Willd.], Mattice et al. (*9*) identified many phenolic acids and aliphatic acids from the soil previously supporting the growth of strongly allelopathic rice accessions.

Little information is available on the screening of multiple allelochemicals from root exudates in wheat germplasm, although single-compound screening has been done in *Avena* spp. (*6*). In many species, allelopathic activity does not appear to be due to the effects of a single compound but is most likely the result of the combination and interaction of many allelochemicals (*10*). Simultaneous screening of multiple allelopathic compounds responsible for weed suppression will therefore be most informative (*11*).

Wheat accessions have been shown to differ significantly in their allelopathic potential against the root growth of annual ryegrass (*Lolium rigidum* Gaud.) (*12*, *13*). It was further noted that wheat seedling allelopathy diminished after the addition of activated charcoal to the agar growth medium (*12*), indicating the involvement of allelochemicals exuded by wheat seedlings into the agar growth medium. Investigations were then carried out to determine the basis for the observed differential wheat allelopathic activity on ryegrass.

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Table 1. Highest and Lowest Concentrations of Phenolic Acids in Wheat Root Exudates[a]

phenolic acid	accession with lowest contents		accession with highest contents	
p-hydroxybenzoic acid	Sudan 8	2.32 ± 0.29	Meering	18.57 ± 0.82
vanillic acid	Eretria	0.61 ± 1.15	AUS 12627	17.53 ± 0.12
cis-coumaric acid	Sunstate	0.07 ± 0.07	Wattines	4.89 ± 1.39
syringic acid	AUS 12627	nd	Dollarbird	52.72 ± 0.61
cis-ferulic acid	AUS 18364	0.33 ± 0.17	Khapli	12.69 ± 0.25
trans-coumaric acid	Sudan 8	1.50 ± 1.00	Canada 51	20.46 ± 2.85
trans-ferulic acid	Afghanistan 19	1.60 ± 0.90	AUS 18056	23.41 ± 0.15

[a] Mean ± SD, μg/L of water-agar. nd, not detected.

Chemical screening of multiple compounds in 58 wheat accessions has shown that accessions differed significantly in the production of 7 phenolic acids in the shoots (*11*) and in the roots (*14*). However, the presence of allelochemicals in plant tissues does not necessarily mean that these compounds can be exuded into the growth environment to affect neighboring plants. Wheat seedling allelopathy occurs only when allelochemicals present in the shoots and roots are eventually excreted by the living and intact roots into the growth environment. In this serial paper, we further report on the differential exudation of 7 phenolic acids from 17-day-old seedlings of 58 wheat accessions.

MATERIALS AND METHODS

Collection of Wheat Root Exudates from the Agar Medium. On the basis of previous experiments, a worldwide collection of 58 wheat accessions (*Triticum aestivum* L.) with varied allelopathic activities was selected and grown according to the procedure described previously (*12*). Twelve pregerminated wheat seeds (surface-sterilized) of each accession were uniformly selected and aseptically sown in a glass beaker (500 mL) prefilled with 30 mL of 0.3% water−agar. After the growth of wheat seedlings for 17 days, the 12 seedlings were uprooted from their nutrient-free agar medium and the roots rinsed twice with 5 mL portions of distilled water to remove the residual agar. The washings were pooled into the agar medium. The growth medium was then collected. This study was replicated three times so that three lots of agar medium were collected, pooled together, and stirred thoroughly. One-third of the pooled agar medium was then adjusted to pH 3.0 by dropwise addition of 0.06 M HCl, stirred, and sonicated at 5 °C for 15 min. The agar medium was extracted three times with 60 mL portions of diethyl ether. The combined ether layers were then evaporated under reduced pressure at 35 °C.

Derivatization, Quantification, and GC-MS/MS Analysis. The derivatization and quantification of wheat samples were identical to those described previously (*15*). Briefly, the silylation of wheat agar exudate samples was accomplished by the addition of 1.0 mL of MSTFA (Alltech Australia) at 60 °C for 30 min. The silylated samples were directly analyzed by GC-MS/MS (gas chromatography and tandem mass spectrometry), which was carried out on a Varian 3400 CX gas chromatograph coupled with a Varian Saturn 2000 ion trap mass spectrometer. The GC-MS/MS conditions for the analysis of wheat samples were identical to those reported previously (*15*). The phenolic acids were identified and quantified by comparing retention time and product ion spectra with those in the user library created from the standard compounds. Quantitative analysis was performed by the internal standard method (*15*). All samples were run in triplicate. Results were reported in units of micrograms per liter of water−agar.

Statistical Analysis. All experimental data were subjected to analysis of variance using Genstat 5 (release 3.2), and the treatment means were tested separately for least significant difference (lsd) at a 5% level of probability.

RESULTS AND DISCUSSION

Phenolic acids present in the shoots and roots can be exuded by young wheat seedlings through the roots. The 58 tested wheat accessions exuded differential amounts of phenolic acids into the growth medium (Table 1). The average levels of phenolic acids exuded by 17-day-old wheat seedlings into the agar growth medium were ranked in decreasing order as syringic acid at 21.09 ± 8.29 μg/L of water−agar, *trans*-ferulic acid at 9.87 ± 5.58 μg/L, vanillic acid at 7.33 ± 3.38 μg/L, *p*-hydroxybenzoic acid at 7.15 ± 3.50 μg/L, *trans*-*p*-coumaric acid at 6.22 ± 3.84 μg/L, *cis*-ferulic acid at 2.79 ± 2.57 μg/L, and *cis*-*p*-coumaric acid at 1.07 ± 1.11 μg/L.

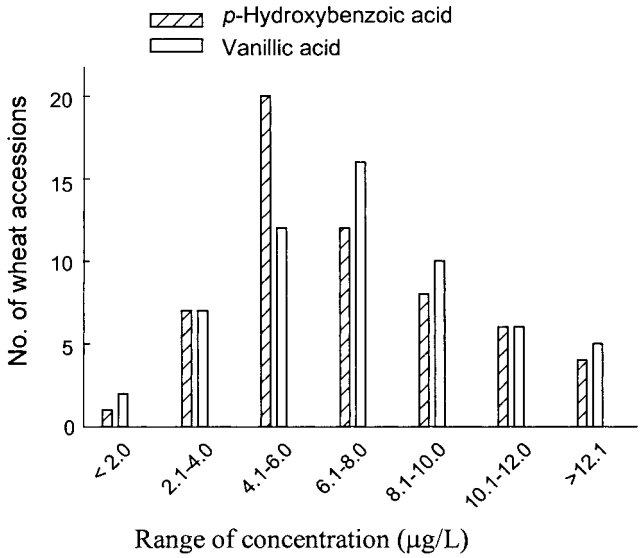

Figure 1. Distribution of allelochemicals exuded by wheat seedlings.

Table 2. Distribution of Phenolic Acids in Wheat Root Exudates[a]

phenolic acid	no. of accessions: wheat with low contents	no. of accessions: wheat with high contents
p-hydroxybenzoic acid	1 (<2.0)[b]	4 (>12.0)[c]
vanillic acid	2 (<2.0)	5 (>12.0)
cis-coumaric acid	9 (<0.2)	4 (>3.0)
syringic acid	5 (<10.0)	4 (>30.0)
cis-ferulic acid	12 (<1.0)	6 (>4.0)
trans-coumaric acid	5 (<2.0)	5 (>12.0)
trans-ferulic acid	8 (<4.0)	4 (>20.0)

[a] Values in parentheses are concentrations of phenolic acids (μg/L of water-agar) at the low[b] and high ends[c] of the distribution among 58 wheat accessions.

The distributions of exuded phenolic acids in 58 wheat accessions are outlined in Figure 1 and Table 2. The levels of *p*-hydroxybenzoic and vanillic acids exuded by living wheat roots were normally distributed in the 58 wheat accessions (Figure 1). Normal distribution of these two compounds was also found in the shoots and roots (*11, 14*), with the exception of vanillic acid (in which a binormal distribution was found in the roots).

Table 3. Correlations of Each Group of Phenolic Acids between Shoot and Agar Samples[a]

	shoots				
phenolic acid	benzoics	cinnamics	total COU	total FER	DIMBOA[b]
water−agar					
benzoics	0.273*	0.204	0.187	0.183	0.074
cinnamics	0.411**	0.438**	0.374**	0.404**	0.349**
total COU	0.317*	0.300*	0.220	0.291*	0.199
total FER	0.406**	0.454**	0.409**	0.411**	0.386**

[a] Data expressed as the correlation coefficient between phenolic acid groups. *, significant correlation at $P < 0.05$; **, significant correlation at $P < 0.01$. [b] Benzoics refers to the benzoic acid derivatives, including *p*-hydroxybenzoic, syringic, and vanillic acids; cinnamics refers to the cinnamic acid derivatives, including *cis*- and *trans*-*p*-coumaric and *cis*- and *trans*-ferulic acids; total COU includes the *cis*- and *trans*-*p*-coumaric acids; total FER includes the *cis*- and *trans*-ferulic acids; DIMBOA refers to 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one.

Of the seven phenolic acids analyzed, only the content of *trans*-ferulic acid in root exudates was significantly associated with that in wheat shoots ($r = 0.45$**). There were no significant correlations for other phenolic acids. The content of each phenolic acid in root exudates also did not correlate well to that in wheat roots (data not shown), although significant correlation was found between the shoots and roots (*14*). Grouped data showed that the levels of benzoic acid derivatives, cinnamic acid derivatives, and total ferulic acid in wheat root exudates were significantly correlated with the levels of these phenolic acids in wheat shoots (Table 3). However, there were no significant correlations for each group of phenolic acids between the roots and water−agar medium. These results showed that the amount of each individual phenolic acid in the agar growth medium was not proportional to that of each phenolic acid in the roots or shoots of 17-day-old wheat seedlings, suggesting that the levels of allelochemicals in root exudates were not directly driven by those contained in wheat roots. The present study supports the idea that exudation of allelochemicals is an active metabolic process (*16*, *17*).

The exudation of allelopathic compounds is an essential process for wheat seedlings to exhibit allelopathic activity against the growth of annual ryegrass. Allelopathic compounds in wheat shoot tissues may be translocated, accumulated in the roots, and then released into the environment through the root system. Wheat roots of 17-day-old seedlings accumulate higher levels of allelochemicals than the shoots (*14*). The present study demonstrated that wheat seedling roots were able to exude varied amounts of phenolic acids into their agar growth medium and that the quantities exuded depended on the particular compounds and wheat accessions evaluated. The exudation of phenolic compounds and aliphatic acids by plant roots occurs in a number of crops, including alfalfa (*Medicago sativa*), cotton (*Gossypium hirsutum*), pea (*Pisum sativum*), and wheat (*T. aestivum*) (*18*−*20*).

Previous research has shown that strongly allelopathic wheat accessions, such as Tasman, exuded higher levels of allelochemicals than weakly allelopathic accessions, such as HY-65 (*21*). Mattice et al. (*9*) found that soils growing allelopathic rice accessions contained higher levels of allelochemicals than those growing nonallelopathic ones. Similar results were also found in the present study. Among the seven wheat accessions with the highest contents of the seven phenolic acids (Table 1), five accessions were previously found to be strongly allelopathic to the growth of annual ryegrass as screened by the equal-compartment-agar method (*13*). These accessions are AUS 12627, AUS 18056, Khapli, Meering, and Wattines, giving an average of ryegrass root length of 7.0 mm, compared to 55.0 mm root length of a no-wheat control. Similarly, among the six wheat accessions with the lowest contents of the seven phenolic acids (Table 1), four accessions were previously found to be weakly allelopathic to the growth of annual ryegrass (*13*). These accessions are Afghanistan 19, Eretria, Sudan 8, and Sunstate, giving an average ryegrass root length of 31.0 mm. These results indicate that there is a strong chemical basis for the differential allelopathic activity among wheat accessions against the growth of ryegrass.

Phenolic compounds are known to be of great significance in allelopathy (*22*). They are one of the main groups of phytotoxic substances associated with wheat allelopathy (*21*, *23*). Many phenolic compounds are inhibitory to germinating seeds and growing plants (*5*). The mechanisms and modes of action of these allelopathic agents have been summarized (*14*). Further research is underway to investigate the close association between wheat seedling allelopathy and phenolic compounds together with other groups of allelopathic agents identified in wheat root exudates.

Consistent differences in wheat allelopathic activity and in the contents of phenolic acids between wheat accessions indicate that genetic factors are involved in the production of allelochemicals. Rice (*5*) stated that little work has been done on the genetics of allelopathic agents. Chemical analysis coupled with DNA technology will facilitate the identification of genetic markers conferring the allelopathic trait. Attempts have been made to locate the chromosomal position of genes conferring the accumulation of hydroxamic acids in wheat (*24*). The possibilities of genetic manipulation of crop allelopathic potential have also been discussed previously (*3*). Genetic enhancement of allelochemical production and exudation offers potential implications for future weed management.

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