Allelochemicals in Wheat (*Triticum Aestivum* L.): Variation of Phenolic Acids in Root Tissues

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Analysis by GC/MS/MS showed that a worldwide collection of 58 wheat accessions differed significantly in the production of seven phenolic acids in the roots of 17-day-old wheat seedlings. The allelochemical contents among wheat accessions ranged from 24.5 to 94.5, 19.9 to 91.7, 3.7 to 15.4, 2.2 to 38.6, 1.0 to 42.2, 19.3 to 183.6, and 11.7 to 187.6 mg/kg of root dry weight for *p*-hydroxybenzoic, vanillic, *cis*-*p*-coumaric, syringic, *cis*-ferulic, *trans*-*p*-coumaric, and *trans*-ferulic acids, respectively. *trans*-Ferulic acid was identified as the most predominant phenolic acid in the roots. Phenolic acids, with the exception of syringic acid, were more concentrated in roots than in shoots. Significant correlation was found between the roots and the shoots in the contents of vanillic, *cis*-*p*-coumaric, syringic, *trans*-*p*-coumaric, and *trans*-ferulic acids, and in the content of each structural group of phenolic acids. Wheat accessions with high levels of total identified phenolic acids in the roots were generally strongly allelopathic to the growth of annual ryegrass.

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

INTRODUCTION

It has been documented that allelopathy has several implications for integrated weed management (Altieri and Doll, 1978; Putnam et al., 1983; Leather, 1983; Rice, 1984 and 1995; Weston, 1996; An et al., 1998). Allelopathic suppression of weeds is caused by biologically active allelochemicals that are actively released from living plants into the environment through root exudation, leaching, and volatilization, and passively liberated through decomposition of plant residues. A number of field crops, including wheat, have been reported to possess allelopathic potential for weed suppression (Putnam and Duke, 1974; Fay and Duke, 1977; Spruell, 1984; Dilday et al., 1994; Wu et al., 1999a and 2000).

Allelopathic wheat accessions strongly inhibit the growth of certain weed species (Spruell, 1984; Wu et al., 1998, 1999a, and 2000). A number of allelopathic agents have been identified from wheat (Guenzi and McCalla, 1966; Lodhi et al., 1987; Lynch, 1978; Niemeyer, 1988; Gaspar and Neves, 1995). The involvement of *p*-hydroxybenzoic, vanillic, *p*-coumaric, syringic, and ferulic acids in wheat allelopathy has been demonstrated (Guenzi and McCalla, 1966; Salomonsson et al., 1978; Lodhi et al., 1987). Studies on the mechanisms and modes of action of these allelopathic agents have revealed that phenolic compounds affected nutrient uptake (Kobza and Einhellig, 1987; Baziramakenga et al., 1994), membrane permeability (Baziramakenga et al., 1995), protein synthesis (Mersie and Singh, 1993), photosynthesis (Mersie and Singh, 1993; Baziramakenga et al., 1994), respiration (Penuelas et al., 1996), enzyme activity (Rama Devi and Prasad, 1992), hormone balance (Holappa and Blum, 1991), and plant water potential (Einhellig, 1986).

The production of allelochemicals by the plant is the intrinsic determinant for the allelopathic effect. Crop accessions containing higher levels of allelochemicals are more likely to possess stronger allelopathic capability (Wu et al., 1999a). Plant roots play pivotal roles in allelopathy. Allelochemicals contained in plant roots could partly contribute to residue allelopathy after plant harvest. More important, plant roots may regulate the exudation of allelochemicals into the growth environment to exert seedling allelopathy during the growing season. Therefore, in the exploration of a crop's allelopathic potential, it is important to examine the allelochemical content in plant roots. To date, only limited research has been conducted to screen crop accessions for the differential production of responsible allelochemicals within roots. Two alkaloids, gramine and hordenine, have been implicated in insect resistance in barley (Lovett et al., 1994). Differential levels of hordenine were found in root tissues of 43 lines of *Hordeum* spp, ranging from 133 to 241 mg/kg fresh weight. In their study of sorghum allelopathic potential, Ben-Hammouda et al. (1995) found that three sorghum hybrids contained varied concentrations of *p*-hydroxybenzoic, syringic, vanillic acids, ferulic acid, and *p*-coumaric acids in the roots. Sorgoleone {2-hydroxy-5-methoxy-3-[(8′*Z*,11′*Z*)-8′,11′,14′-pentadecatrienyl]-*p*-benzoquinone}, identified from sorghum, has been found to possess herbicidal activity (Nimbal et al., 1996). It was found that 25 sorghum genotypes varied considerably in the production of sorgoleone in the roots of 5-day-old seedlings, ranging from 0.6 to 14.2 mg/g of root fresh

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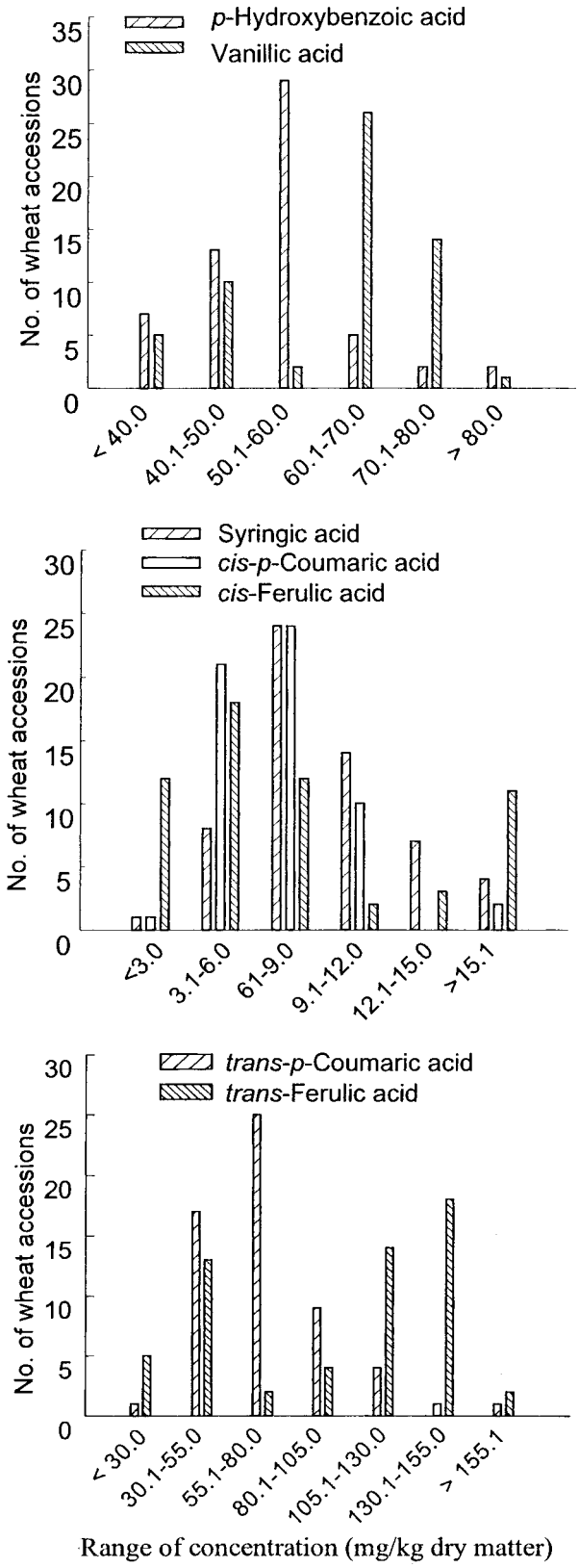

Figure 1. Frequency distribution of phenolic acids in the roots of 58 wheat accessions.

weight. Most other genotypes contained sorgoleone at levels ranging between 1.5 and 10 mg/g of root fresh weight.

Little research has been done on the identification and screening of root allelochemicals in wheat germplasm. Variations in allelochemical production may determine the difference in allelopathic activities between crop accessions. Our previous research has shown that wheat accessions differed significantly in their seedling allelopathy against the growth of annual ryegrass (Wu et al., 1999a and 2000). Further research was then carried out to investigate the allelochemical basis for the varied allelopathic activity. In this paper we report on the differential production of seven known phenolic acids in the roots of 17-day-old wheat seedlings from a genetically diverse collection of 58 wheat accessions originating from 20 countries.

MATERIALS AND METHODS

Wheat Growth. On the basis of previous experiments, a worldwide collection of 58 wheat accessions (*Triticum aestivum* L.) with varied allelopathic activity was selected, and was grown according to the procedure described previously (Wu et al., 2000). Briefly, twelve pre-germinated wheat seeds (surface-sterilized) of each accession were uniformly selected and aseptically sown on an agar surface, with the embryo up, in three rows, on one-half of a glass beaker (500 mL) that had been pre-filled with 30 mL of 0.3% water agar. The beaker was wrapped with a piece of Parafilm paraffin wax film and placed in a controlled-growth cabinet with a daily light/dark cycle of 13 h/11 h and a temperature cycle of 25 °C /13 °C. After the growth of wheat seedlings for 7 days, a piece of pre-autoclaved white paperboard was inserted across the center and down the middle of the beaker with the lower edge of the paperboard kept 1 cm above the agar surface. The beaker was again wrapped with Parafilm and placed back in the growth cabinet for continuous growth of 10 more days.

Preparation of Root Samples. The procedure previously described for the preparation of root samples was used (Wu et al., 1999b). Briefly, roots of 17-day-old wheat seedlings were harvested for each accession and immediately freeze-dried. An amount of 0.100 g of roots was cut into 2-mm lengths, ground into powder, macerated with 3 mL of 0.001 M HCl, sonicated at 5 °C for 15 min, and then centrifuged at 20 000 rpm at 10 °C for 15 min. The supernatant was then collected and extracted three times with 10-mL portions of diethyl ether. The ether was then evaporated under reduced pressure at 35 °C.

Derivatization and Quantitation. The derivatization and quantitation of wheat samples were identical to those described previously (Wu et al., 1999b). Briefly, the silylation of wheat root samples was accomplished by the addition of 1.0 mL of *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide (MSTFA) (Alltech Australia) at 60 °C for 30 min. The silylated samples were directly analyzed by GC/MS/MS. Each phenolic acid was identified and quantified by comparing retention time and product ion spectrum with that in the user library created from the standard compound. Quantitative analysis was performed by the internal standard method (Wu et al., 1999b) and is reported in units of milligrams per kilogram of dry matter.

GC/MS/MS Analysis. Gas chromatographic and tandem mass spectrometric (GC/MS/MS) analysis was carried out on a Varian 3400 CX gas chromatograph coupled with a Varian Saturn 2000 ion trap mass spectrometer. *p*-Hydroxybenzoic acid (PHB), vanillic acid (VAN), syringic acid (SYR), *p*-coumaric acid (COU), ferulic acid (FER), and the internal standard (*p*-chlorobenzoic acid) were obtained from Sigma-Aldrich Chemical Co. Silylated samples or standard compounds were introduced via a DB-5MS fused-silica capillary column of 30 m × 0.25 mm × 0.25 μm (J and W Scientific, Alltech Australia). The GC/MS/MS conditions for the analysis of wheat samples were identical to those reported previously (Wu et al., 1999b). Mass spectral scan time from *m/z* 50 to 450 was 1.0 s (using 3 microscans). Nonresonant collision-induced dissociation (CID) was used for MS/MS. All samples were run in triplicate.

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.

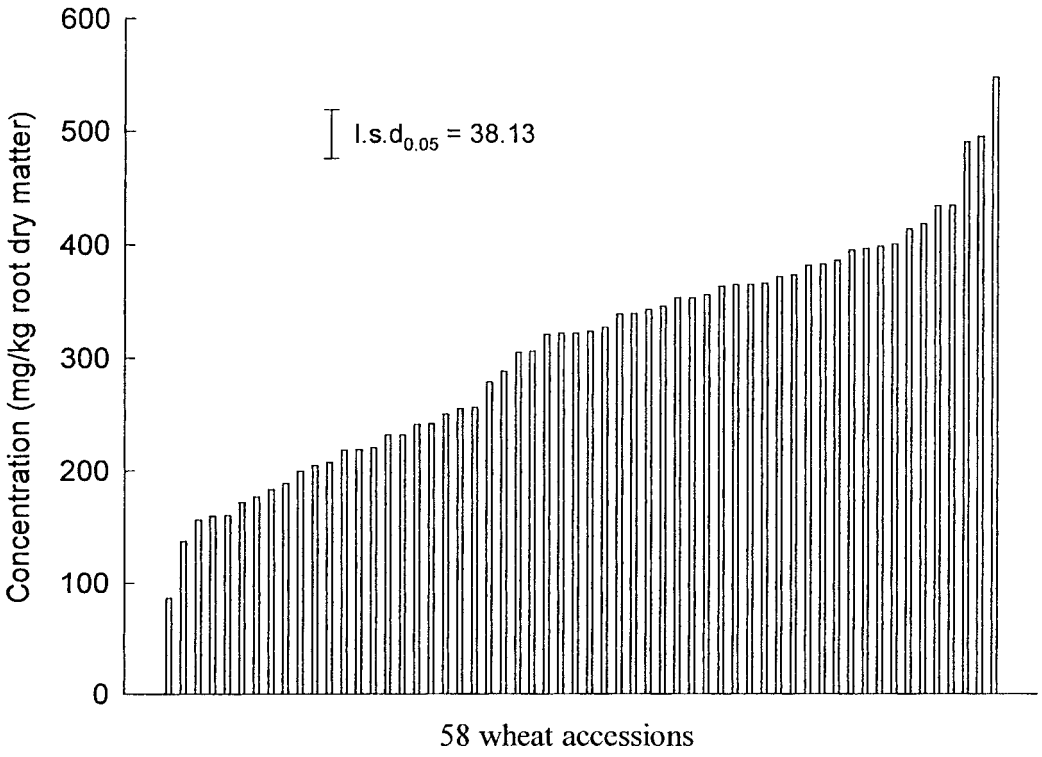

Figure 2. Content of total identified phenolic acids in wheat roots.

RESULTS AND DISCUSSION

The contents of phenolic acids in the roots of 17-day-old wheat seedlings differed significantly among accessions (Figure 1). Accession Hartog produced as little as 24.5 mg/kg of *p*-hydroxybenzoic acid (root dry weight), whereas AUS# 12788 produced 94.5 mg/kg. The content of vanillic acid in wheat roots ranged from 19.9 mg/kg of accession Hartog to 91.7 mg/kg of Tasman; *cis*-*p*-coumaric acid ranged from 3.7 mg/kg of Hartog to 15.4 mg/kg of Meering; syringic acid ranged from 2.2 mg/kg of Canada 51 to 38.6 mg/kg of Cadoux; *cis*-ferulic acid ranged from 1.0 mg/kg of Robin to 42.2 mg/kg of Tunis 2; *trans*-*p*-coumaric acid ranged from 19.3 mg/kg of Hartog to 183.6 mg/kg of Tasman; and *trans*-ferulic acid ranged from 11.7 mg/kg of Hartog to 187.6 mg/kg of Meering.

The levels of *p*-hydroxybenzoic, syringic, *cis*-*p*-coumaric, and *trans*-*p*-coumaric acids were normally distributed in the 58 wheat accessions, whereas a binormal distribution was found for vanillic, *cis*-ferulic, and *trans*-ferulic acids (Figure 1). There were seven accessions that produced a level of *p*-hydroxybenzoic acid at less than 40.0 mg/kg, and four accessions that produced levels at more than 70.0 mg/kg. Five accessions produced vanillic acid at less than 20.0 mg/kg, and one accession produced more than 90.0 mg/kg. Nine accessions produced syringic acid at less than 6.0 mg/kg, and four accessions produced more than 30.0 mg/kg. There were five accessions that produced a level of *trans*-ferulic acid at less than 30.0 mg/kg, and two accessions that produced more than 155.0 mg/kg. Eight accessions produced *trans*-*p*-coumaric acid at less than 40.0 mg/kg, and two accessions produced more than 130.0 mg/kg. There was one accession that produced a level of *cis*-*p*-coumaric acid at less than 4.0 mg/kg, and two accessions that produced more than 6.0 mg/kg. Twelve accessions produced *cis*-ferulic acid at less than 3.0 mg/kg, and eleven accessions produced more than 15.0 mg/kg dry weight.

The concentration of total identified phenolic acids varied from 85.9 to 547.4 mg/kg in the roots of 58 accessions (Figure 2). There were 10 accessions that produced a level of total identified phenolic acids at less than 200.0 mg/kg, including Hartog, Canada 51, Federation, Janz, Emika, Eretria, Excalibur, L 1512−2721, Canada 4125, and Currawong. Correspondingly, these accessions generally contained lower levels of individual phenolic acids in the root tissues. Eight accessions contained total identified phenolic acids at more than 400.0 mg/kg, including RAC 820, Sunstar, Tunis 2, Khapli, Canada 56, Meering, Kite, and Tasman. Generally, higher concentrations of individual phenolic acids were also found in these accessions. Forty accessions produced total identified phenolic acids within the range of 200.0 and 400.0 mg/kg of root dry weight.

Different phenolic acids occurred in varied quantities in the roots of the same wheat accession. On average, *trans*-ferulic acid was produced predominantly at 97.7 ± 47.8 mg/kg in the roots across the 58 accessions, whereas the cis isomers of *p*-coumaric and ferulic acids were produced at levels as little as 7.1 ± 2.4 and 8.7 ± 8.4 mg/kg, respectively (Table 1). The levels of phenolic acids in wheat root tissues are ranked in decreasing order of the concentration of each phenolic acid in the roots of 17-day-old wheat seedlings as: *trans*-ferulic acid, *trans*-*p*-coumaric acid, vanillic acid, *p*-hydroxybenzoic acid, syringic acid, *cis*-ferulic acid, and *cis*-*p*-coumaric acid.

The contents of phenolic acids in wheat root tissues were significantly correlated with each other except for *cis*-*p*-coumaric and syringic acids, where no significant correlation was obtained (Table 2). Significant correlation was also found between the structural groups of phenolic acids (Table 3).

There was significant correlation between the roots

Table 1. Distribution of Phenolic Acids in Wheat

	no. of accessions			concentration (mg/kg root dry matter)		
phenolic acid	roots[a]	shoots[a]	no difference	shoots[b]	roots[b]	l.s.d$_{0.01}$
PHB	56	2	0	31.1 ± 7.3	52.5 ± 12.1	1.70
VAN	51	5	2	42.0 ± 12.1	61.0 ± 14.0	1.61
cis-COU	53	5	0	4.2 ± 1.6	7.1 ± 2.4	0.11
SYR	11	39	8	22.9 ± 18.2	10.4 ± 7.0	1.04
cis-FER	31	21	6	5.2 ± 3.6	8.7 ± 8.4	0.44
trans-COU	53	4	1	38.5 ± 18.3	69.4 ± 28.8	2.28
trans-FER	42	13	3	74.1 ± 48.1	97.7 ± 47.8	2.40

[a] No. of accessions with higher levels of phenolic acids detected in the roots or shoots. [b] Mean of 58 wheat accessions ± standard deviation (SD).

Table 2. Correlation between Contents of Phenolic Acids Extracted from Wheat Roots[a]

phenolic acid	PHB	VAN	*cis*-COU	SYR	*cis*-FER	*trans*-COU	*trans*-FER
PHB	1						
VAN	0.505[c]	1					
cis-COU	0.445[c]	0.401[c]	1				
SYR	0.296[b]	0.288[b]	0.086	1			
cis-FER	0.365[c]	0.464[c]	0.711[b]	0.301[b]	1		
trans-COU	0.578[c]	0.721[c]	0.622[c]	0.331[b]	0.552[c]	1	
trans-FER	0.584[c]	0.590[c]	0.443[c]	0.383[b]	0.439[c]	0.739[c]	1

[a] Data expressed as the correlation coefficient between phenolic acids. [b] Significant correlation at $p < 0.05$. [c] Significant correlation at $p < 0.01$.

Table 3. Correlation between the Groups of Phenolic Acids Extracted from Wheat Roots[a]

phenolic groups[b]	benzoics	cinnamics	total COU	total FER
benzoics	1			
cinnamics	0.773**	1		
total COU	0.747**	0.904**	1	
total FER	0.721**	0.969**	0.770**	1

[a] Data expressed as the correlation coefficient between phenolic acid groups. [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.

Table 4. Correlation of Each Group of Phenolic Acids between Shoot and Root Samples[a]

phenolic groups	shoots			
	benzoics	cinnamics	total COU	total FER
roots				
benzoics	0.527**	0.525**	0.414**	0.498**
cinnamics	0.566**	0.616**	0.490**	0.582**
total COU	0.523**	0.588**	0.518**	0.536**
total FER	0.541**	0.578**	0.431**	0.558**

[a] Data expressed as the correlation coefficient between groups.

and the shoots in the contents of vanillic, *cis*-*p*-coumaric, syringic, *trans*-*p*-coumaric, and *trans*-ferulic acids, and the total identified phenolic acid, with correlation coefficients (r) of 0.46**, 0.35**, 0.36**, 0.52**, 0.56**, and 0.63**, respectively. However, no significant correlation was found for *p*-hydroxybenzoic or for *cis*-ferulic acids between the roots and the shoots. Grouped data showed that each structural group of phenolic acids in the roots was significantly associated with those in the shoots (Table 4). These results suggest that higher levels of phenolic acids could be expected in the roots when the contents of phenolic acids are high in the shoots, and that phenolic compounds produced in wheat shoots could be translocated to the roots to be exuded into the growth environment or to be microbially liberated after the termination of plant growth.

Phenolic acids were found to be distributed differentially in young wheat seedlings, with the roots generally containing the higher concentrations (Table 1). The number of wheat accessions containing higher amounts of phenolic acids in the roots than in the shoots was 56 for *p*-hydroxybenzoic acid, 51 for vanillic acid, 53 for *cis*-*p*-coumaric acid, 31 for *cis*-ferulic acid, 53 for *trans*-*p*-coumaric acid, and 42 for *trans*-ferulic acid. However, there were 39 accessions that contained higher levels of syringic acid in the shoots rather than in the roots. On average over the 58 accessions, *trans*-ferulic, vanillic, *trans*-*p*-coumaric, *p*-hydroxybenzoic, *cis*-ferulic, and *cis*-*p*-coumaric acids were present in higher amounts in the root tissues than in the shoots, while the concentration of syringic acid was higher in the shoots than the roots (Table 1). Information on the distribution of phenolic acids within plants is very limited. Ben-Hammouda et al. (1995) found that mature plants of three sorghum hybrids generally contained higher levels of total phenolic contents in the leaves than in the roots, glumes, culms, or seeds. Sorghum roots also produced less *p*-hydroxybenzoic, vanillic, syringic, *p*-coumaric, and ferulic acids than the shoots. Similarly, Waniska et al. (1988) reported that sorghum contained higher amounts of phenolic compounds in leaves and glumes than in culms and caryopses. Cherney et al. (1991) claimed that phenolic acids, such as coumaric acid and ferulic acid, were accumulated more in culms than in leaf blades or leaf sheaths.

The preferential accumulation of phenolic acids in wheat roots could facilitate their exudation into the growth environment. A worldwide collection of 453 wheat accessions has been shown to differ significantly in seedling allelopathy on the growth of annual ryegrass (*Lolium rigidum* Gaud.) (Wu et al., 1999a). The eight wheat accessions with total identified phenolic acids levels > 400.0 mg/kg of root dry weight, with the exception of Kite (in which a weak allelopathic activity was found), were strongly allelopathic to the growth of *L. rigidum*. Similarly, the 10 accessions with the total identified phenolics levels < 200.0 mg/kg, with the exceptions of Janz and Currawong (in which strong and intermediate levels of allelopathic potential were found), were weakly allelopathic to the growth of *L. rigidum*. Accessions from Mexico were found to be strongly

allelopathic in our previous experiment. Chemical analysis found that these accessions contained high levels of total identified phenolic acids (>300.0 mg/kg).

Allelochemicals present in the plants have to be exuded via living roots into the environment to affect the growth of other plants in vicinity. It is therefore necessary to further investigate the exudation of phenolic acids by wheat seedlings, and to determine the allelochemical relationship between the contents of phenolic acids present in the roots and those exuded by the roots or released by decaying roots into the growth environment. This logical extension of the research would significantly assist in the understanding of the chemical basis of wheat seedling allelopathy for weed suppression.

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