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Evaluation of seedling allelopathy in 453 wheat (*Triticum aestivum*) accessions against annual ryegrass (*Lolium rigidum*) by the equal-compartment-agar method

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Abstract. Allelopathy has been receiving world-wide attention for its potential in integrated weed management. A newly developed screening bioassay, the 'equal-compartment-agar method' (ECAM), was used to evaluate seedling allelopathy against annual ryegrass in a collection of 453 wheat accessions originating from 50 countries. Significant differences in allelopathic potential were found in this worldwide collection, inhibiting root growth of ryegrass from 9.7% to 90.9%. Wheat seedling allelopathy also varied significantly with accessions from different countries. Wheat allelopathic activity was normally distributed within the collection, indicating the involvement of multiple genes conferring the allelopathic trait. Of the 453 wheat accessions screened, 2 distinct groups were identified. Condorderivatives were more allelopathic than Pavon-derivatives, with an average inhibition of root growth of ryegrass by 76% and 46%, respectively. Research was further extended to investigate the near isogenic lines derived from Hartog (Pavon-derivative) and Janz (Condor-derivative). Hartog and its backcrossed lines were less allelopathic than Janz and its backcrossed lines, inhibiting root length of ryegrass by 45% and 81%, respectively. These results strongly indicate that wheat allelopathic activity might also be controlled by major genes, depending on the particular populations. The present study demonstrates that there is a considerable genetic variation of allelopathic activity ity in wheat germplasm. It is possible to breed for cultivars with enhanced allelopathic activity for weed suppression.

Additional keywords: screening bioassay, in vivo, weed suppression, genetics, plant breeding.

Introduction

Cultural, mechanical, chemical, and biological methods are the major components in weed management. Among these, herbicides are the principal tool to manage weeds within many cropping systems (Powles *et al.* 1997). However, the continuing success of herbicide technology is threatened by the development of resistant weed biotypes as a result of the extensive and repetitive use of a particular class of herbicide.

Annual ryegrass has become an important weed in Australia, infesting crops in a wide variety of soil types and climatic conditions (Gill 1996). After the earliest report of annual ryegrass resistant to the aryloxyphenoxypropionate (APP) herbicide diclofop-methyl (Heap and Knight 1982), biotypes of this weed have developed resistance to the majority of herbicides currently used (Preston *et al.* 1996). The development of annual ryegrass resistance to glyphosate, a 5-*enol*pyruvylshikimate-3-phosphate synthase (EPSPS) inhibitor, was also reported (Pratley *et al.* 1996; Powles *et al.* 1998). The level of resistance has also been quantitatively evaluated (Pratley *et al.* 1999). In many cases, annual ryegrass that has developed resistance to one APP or CHD (cyclohexanedione) herbicide has automatic resistance to other APP and/or CHD herbicides, and can also have cross-resistance to acetolactate synthase (ALS) inhibitors, even though these have not been used. As a result, rotation of chemically distinct herbicides will not be effective (Powles and Howat 1990). With the development of resistance of ryegrass to herbicides (Heap and Knight 1982; Pratley *et al.* 1996, 1999; Powles *et al.* 1998), non-herbicidal innovations to manage weeds are increasingly needed (Wu *et al.* 1999b).

To date, biological methods are the least exploited area in weed management. The potential for using allelopathy in weed management has been well documented (Rice 1995; An *et al.* 1998). Allelopathy occurs through the addition of phytotoxic allelochemicals exuded by crop plants into the growth environment so that the growth of weeds in the close

vicinity is affected. The development of allelopathic crop cultivars for weed suppression has been increasingly recognised as one of the possible components in integrated weed management (Wu *et al.* 1999*b*). The use of crop cultivars with elevated allelopathic activity could reduce the need for commercial herbicides to early season application, with late season weed control provided by the heightened advantages of crop competitiveness.

Numerous reports have shown that many crops are allelopathic to certain weed species. During the last 3 decades, a number of field crops have been evaluated for their allelopathic potential in weed suppression, including cucumber (Putnam and Duke 1974), oat (Fay and Duke 1977), sorghum [Sorghum bicolor (L.) Moench] (Alsaadawi et al. 1986), sunflower (Leather 1983), wheat (*Triticum aestivum* L.) (Spruell 1984), and *Hordeum* spp. (Lovett and Hoult 1992). Extensive research has also been devoted to locating novel genotypes of rice (*Oryza sativa* L.) for weed suppression (Fujii 1992; Dilday et al. 1994, 1998; Olofsdotter and Navarez 1996). Some crop accessions have been shown to possess strongly allelopathic potential against the growth of certain weed species.

Before any plant breeding program can be initiated, genetic studies on crop allelopathy are necessary. A population of 400 F₂ plants derived from the cross of PI 312777 (allelopathic) and Lemont (non-allelopathic) was evaluated for rice allelopathic activities on ducksalad in the field. The normal distribution of allelopathic activity found in this F₂ population indicated that the rice allelopathic trait was quantitatively inherited (Dilday et al. 1998). Recently, the restriction fragment length polymorphism (RFLP) technique has been employed to investigate the genes controlling rice allelopathic activity on barnyard grass (Echinochloa crus-galli) in a population of 144 recombinant inbred lines (RILs) derived from a cross between IAC 165 (japonica upland variety, strongly allelopathic) and CO 39 (indica irrigated variety, weakly allelopathic). Six putative quantitative trait loci (QTLs) for allelopathy were located on chromosomes 2, 3, 4, 5, 6, and 9, using single marker analysis. Each QTL explained 5-10% of the phenotypic variation (Bach Jensen et al. 1999).

The application of crop allelopathy in weed suppression involves 2 crop growth stages, i.e. vegetative stage and postharvest stage. At the vegetative growth stage, crop seedling allelopathy could be exploited to suppress weeds. At the postharvest stage, crop residue allelopathy could be used for weed suppression, especially during the establishment period of the following crop. Wheat residue allelopathy on the growth of annual ryegrass has been reported previously and it has been found to vary with accessions (Wu *et al.* 1998). A screening bioassay method, the 'equal-compartment-agar method' (ECAM), has also been recently developed for the assessment of wheat seedling allelopathy on ryegrass (Wu *et al.* 2000). The objectives of this study were therefore to evaluate wheat seedling allelopathy in a world-wide collection of 453 wheat accessions for the suppression of annual ryegrass by the ECAM, and to investigate the genetic basis of wheat allelopathy in some selected wheat accessions.

Materials and methods

Tested species

A world-wide collection of 453 wheat (*T. aestivum* L.) accessions originating from 50 countries was obtained from various sources, mostly from the Australian Winter Cereals Collection. Seeds of annual ryegrass (*L. rigidum* Gaud.) were obtained commercially. Agar technical was purchased from Amyl Media Pty Ltd.

Sterilisation and pre-germination

The pretreatment of the seeds of both wheat and ryegrass has been previously described (Wu *et al.* 2000). Briefly, wheat seeds were surfacesterilised by soaking the seeds in 70% ethanol for 2.5 min, followed by 4 rinses in sterilised distilled water. They were then soaked in 2.5% sodium hypochlorite solution for 15 min followed by 5 rinses in sterilised distilled water. The surface-sterilised seeds of the wheat genotypes and ryegrass seeds were each soaked in sterilised water for the imbibition of water in light at 25°C for 24 h and then rinsed with fresh sterilised water. The wheat seeds were then incubated in light at 25°C for another 24 h, and ryegrass seeds incubated in light at 25°C for 48 h.

Biological screening of 453 wheat accessions for allelopathic potential against the growth of ryegrass

The ECAM described previously (Wu et al. 2000) was employed to screen 453 wheat accessions for their allelopathic potential on annual ryegrass. Briefly, 12 pre-germinated wheat seeds of each accession were uniformly selected and aseptically sown on the agar surface with the embryo up, in 3 rows on one-half of a glass beaker (500 mL) prefilled with 30 mL of 0.3% water agar. The beaker was wrapped with a piece of parafilm and placed in a controlled growth cabinet with a daily light/dark cycle of 13 h/11 h and a temperature cycle at 25°C /13°C. The fluorescent light intensity in the cabinet was $3.56\pm0.16 \times 10^3$ lux. After the growth of wheat seedlings for 7 days, 12 pre-germinated seeds of ryegrass were then sown on the other half of the agar surface in 3 rows. A piece of pre-autoclaved white paperboard was inserted across the centre and down the middle of the beaker with the lower edge of the paperboard kept 1 cm above the agar surface. The entire beaker was thereby divided into 2 equal compartments that were occupied separately by wheat and ryegrass seedlings. The beaker was again wrapped with Parafilm and placed back in the growth cabinet for continuous growth of 10 more days.

Because of the limited space in the controlled growth cabinet, the 453 wheat accessions were randomly separated into 19 sets of 23 wheat accessions and 1 set of 16 accessions. A nil-wheat control was included in each set. Each set was screened separately with 3 replicates.

Re-screening of 60 selected wheat accessions for allelopathic potential against the growth of ryegrass

Sixty wheat accessions, ranging from low to high in their allelopathic potential against the growth of annual ryegrass, were chosen from the 453 accessions screened above. In the following year, these accessions were equally divided into 2 sets and re-screened under the same experimental conditions with 4 replicates in order to confirm the experimental results. The 2 sets were screened separately with an interval of 4 days.

Screening of some selected near-isogenic lines derived from Hartog and Janz

Based on the biological screening results of 453 wheat accessions, 2 distinct groups of wheat accessions were identified. Accessions with

Condor background showed strongly allelopathic activity and with Pavon background weakly allelopathic activity. Some near isogenic lines (NILs) derived from Hartog (Pavon background) and Janz (Condor background) were then selected and screened in order to further the investigation on the genetic control of wheat allelopathy. These NILs were initially developed and selected for varied abilities in early vigour and competitiveness (Dr R. Richards and Dr G. J. Rebetzke, pers. comm.), but not for allelopathic activities. Hartog lines (3-H) were developed from the cross of Vigour 18/Hartog followed by 2 backcrosses to Hartog, and Janz lines (3-J) were derived from 2 backcrosses to Janz. Fifteen NILs and their parents (Hartog and Janz) were then screened by the ECAM to investigate the genetic control of allelopathy in these particular populations.

Experimental design and measurements

Each experiment or set of wheat accessions was arranged in a randomised complete block design with 3 replicates. After 10 days of cogrowth of ryegrass with wheat in the growth cabinet, the following measurements were conducted: the longest root length of ryegrass seedlings, and the longest root length and the plant height of wheat seedlings. In addition, the 1000-grain weight of wheat accessions was also measured from the original seed collection.

Statistical analysis

The biological screening data of 453 wheat accessions (20 sets in total) in terms of the root length of annual ryegrass were log_{10} transformed prior to data analysis. A simple fixed effects model was used:

log root length of ryegrass + GM + country + accession + set

where GM is the grand mean; country is the effect of country of origin; accession is the wheat accession effect within a country; and set is the set effect.

The set has been accounted for with a fixed set effect to allow comparison between sets. No significant effects have been found between different sets. The model was fitted using ASREML, a Fortran program used for fitting mixed models (Gilmour *et al.* 1998). The means of wheat accessions or of countries were tested separately for least significant difference (l.s.d.) at P = 0.05.

Other experimental data were subjected to analysis of variance using GENSTAT 5 Release 3.2 (1995) and the treatment means were tested separately for l.s.d. at P = 0.05 or 0.01 where appropriate. Percentage of the inhibition on root length of ryegrass was calculated as (control – raw data)/control*100.

Results

Wheat allelopathic activity on the root growth of ryegrass

Screening results of the 453 wheat accessions showed that accessions differed significantly in their allelopathic activities, inhibiting the root length of ryegrass across a range from 9.7% to 90.9%. A slightly skewed normal distribution of wheat allelopathic activity was found in this collection (Fig. 1). Of the 453 wheat accessions screened, 63 wheat accessions were strongly allelopathic, giving a root length of ryegrass of less than 10.0 mm, in comparison with the nil-wheat control of 55.0 mm. Twenty-one accessions were weakly allelopathic, giving a root length of ryegrass of more than 30.1 mm. Most wheat accessions were intermediate in allelopathy, with a root length of ryegrass ranging from 10.1 to 30.0 mm.

The 63 strongly allelopathic accessions inhibited root length of ryegrass by more than 81%, with an average root length of ryegrass of 8.6 ± 1.1 mm. These accessions originated from 23 countries, mostly from Australia (15 accessions), Mexico (11), South Africa (7), Brazil (4), Germany (3), and Russia (3).

The 21 least allelopathic accessions inhibited the root growth of ryegrass by <45%, with an average root length of ryegrass of 34.2±5.1 mm. These accessions originated from Afghanistan (1), Australia (7), Brazil (1), Canada (2), Chile (1), France (1), India (2), Sudan (1), Tunisia (1), United Arab Republic (1), and USA (3).

The mean value of wheat allelopathic activity in terms of root length of ryegrass is listed in decreasing order by the country of origin in Table 1. There were significant differences in wheat allelopathic activity between countries. On average, 11 accessions from Afghanistan (Afghanistan 2, Afghanistan 6, Afghanistan 7, Afghanistan 9, Afghanistan 19, Afghanistan 55-623, Afghan 2, Afghan 6, Afghan 7, Afghan 19, and PI 181261) showed the weakest allelopathic activity, with a root length of ryegrass of 25.0 mm, whereas the accession from Hungary (Karcagi 21) showed strong allelopathic activity. The 10 countries with the strongest allelopathic activity were listed in descending order as Hungary, Peru, Germany, Bangladesh, Israel, South Africa, Kenya, Malta, Mexico, and the United Kingdom. The 10 countries with the weakest allelopathic activity were listed in ascending order as



Fig. 1. Frequency distribution of the allelopathic activity in 453 wheat accessions. The root length of ryegrass was 55.0 mm for the nil-wheat control.

Country of origin	Entry	Root length of ryegrass (mean ± s.d.; mm)	Country of origin	Entry	Root length of ryegrass (mean ± s.d.; mm)
Afghanistan	11	25.0±4.4	Algeria	3	17.1±1.2
Greece	3	24.0±4.5	Japan	3	17.1±3.5
Finland	1	23.0 ^A	Spain	3	16.8±3.9
Sudan	3	22.9±7.3	Italy	5	16.8±5.9
United Arab Rep.	4	22.6±8.3	Russia	15	16.7±6.0
Poland	6	22.6±7.5	Bulgaria	7	16.6 ± 4.0
Chile	5	21.3±7.3	Tunisia	3	16.3±12.5
Iran	6	21.1±2.8	Portugal	3	15.8 ± 2.4
Canada	13	20.6±9.7	Ecuador	1	15.7 ^A
Korea	3	20.0±2.9	Cypress	4	15.4±3.5
Denmark	3	19.9±4.1	Argentina	4	15.1±6.7
Liberia	1	19.3 ^A	Pakistan	10	14.9 ± 4.5
India	8	19.1±10.6	Uruguay	3	14.8 ± 4.9
Yugoslavia	5	19.0±5.2	Venezuela	3	14.8 ± 3.1
New Zealand	4	19.0±9.6	Syria	3	14.4 ± 2.8
Czechoslovakia	3	18.9 ± 5.7	UK	6	13.8±5.6
France	6	18.8 ± 8.3	Mexico	29	13.7±5.6
Ethiopia	3	18.7±3.2	Malta	5	13.5±5.0
Turkey	8	18.6±4.9	Kenya	3	13.5±5.8
Angola	3	18.6±3.9	South Africa	13	12.7±6.1
Brazil	13	$18.4{\pm}11.6$	Israel	5	12.6±2.8
Switzerland	3	18.4 ± 5.4	Bangladesh	1	12.3 ^A
United States	28	18.2 ± 7.0	Germany	5	10.3±3.0
Australia	145	18.0±6.9	Peru	3	9.4±2.1
China	24	17.1±5.1	Hungary	1	8.0 ^A

Table 1. Differences of wheat allelopathic activity between fifty countriesl.s.d. (P = 0.05) = 6.1 mm for root length

^A No s.d., one sample only.



Fig. 2. Allelopathic activity in 145 Australian wheat accessions.



Fig. 3. Experimental results of 60 wheat accessions in Year 1 v. Year 2.

Afghanistan, Greece, Finland, Sudan, United Arabic Republic, Poland, Chile, Iran, Canada, and Korea.

Wheat allelopathic activity in 145 Australian accessions

The allelopathic activity of 145 Australian accessions is presented in Fig. 2. Significant differences in allelopathic activity were found in the Australian wheat accessions, with root length of ryegrass ranging from 5.0 mm with Tasman to 42.7 mm with 3-H 21. The 10 Australian wheat accessions with strongest allelopathic activity were Tasman, Triller, Wilgoyne, Meering, 3-J 27, Nabawa, Sunstar, 3-J 67, CH 31, and AUS#375. The 10 Australian wheat accessions with weakest allelopathic activity were Robin, 3-H 64, Baroota wonder, Sunstate, Rac 710, Excalibur, 3-H 75, 3-H 94, 3-H 46, and 3-H 21.

Performance of wheat allelopathic activity in different years

Based on the screening results of 453 wheat accessions, 60 accessions with allelopathic activity ranging from weakly allelopathic to strongly allelopathic were selected and rescreened in the following year. Results showed that wheat allelopathic activity was consistent in different years, with a correlation coefficient of $r = 0.909^{**}$ (Fig. 3). Some accessions found to be strongly allelopathic in the first year also significantly inhibited the root growth of ryegrass in the following year. Such accessions included SST 6, SST 16, Tasman, AUS#18364, SST 17, Triller, Khapli, Wattines, Tunis 2, and AUS#18056. Similarly, some accessions always exhibited weak allelopathic activity, such as Afghanistan 9, Sudan 8, Tunis 3, Afghanistan 19, L 1512-2721, HY-65, Canada 4125, Canada 51, PF 8716, and Thesee.



Fig. 4. Differences in allelopathic activity between 2 distinct types of wheat accessions derived from Condor and Pavon [l.s.d. (P = 0.01) = 2.2].

Genetic control of allelopathic activity in wheat

The normal distribution of wheat allelopathic activity in the collection of 453 wheat accessions indicates that this weed-suppressing ability is quantitatively inherited (Fig. 1). Among the 145 Australian accessions screened, 30 accessions shared the same parent, Condor, and 12 accessions shared the same parent, Pavon. Results showed that the 2 distinct types of accessions differed significantly in their allelopathic activity (Fig. 4). Those with Condor back-



Fig. 5. Genetic variation of wheat allelopathic activity in some selected near-isogenic lines.

ground were more allelopathic than those with Pavon background, giving a mean root length of ryegrass of 13.4 ± 5.1 mm and 29.6 ± 5.9 mm, respectively, with l.s.d. (P = 0.01) = 2.2 mm. These results indicate that allelopathic activity is genetically controlled.

Allelopathic activity varied significantly (P < 0.01) in some selected near-isogenic lines derived from Hartog and Janz. Of the 2 parents, Hartog was weakly allelopathic, with a root length of ryegrass of 29.7 mm, whereas Janz was strongly allelopathic, with a root length of ryegrass of 11.0 mm (Fig. 5). The allelopathic activity of BC₂-Hartog lines (backcrossed to Hartog) was very weak and was similar to that of parent Hartog, with a mean root length of ryegrass of 30.2 ± 7.1 mm. Janz lines had strongly allelopathic activity, similar to that of Janz, with a mean root length of ryegrass 10.2 ± 2.4 mm. These results indicate that simple genetic control might be involved in conferring the allelopathic activity in this cross.

Discussion

The present research has been focused on the utilisation of wheat seedling allelopathy for weed suppression at wheat early vegetative growth stage. Natural allelopathic compounds released by intact living roots of wheat seedlings may be important in determining the natural balances between wheat and weed plants. Wheat plants may suppress weeds through seedling allelopathy and gain a subsequent advantage over the weeds (Wu *et al.* 2000).

There were significant differences in allelopathic activity against annual ryegrass in a collection of 453 wheat accessions originating from 50 countries, giving a root length of ryegrass of 5.0 mm with Tasman and 49.7 mm with PF 8716, with the nil-wheat control of 55.0 mm. Among the 453 accessions, 63 accessions were strongly allelopathic, inhibiting the root growth of ryegrass by >81%, whereas the 20 weakest allelopathic accessions inhibited ryegrass by <45%. Wheat accessions originating from different countries also differed significantly in their allelopathic activity. Sixty wheat accessions with varied allelopathic activity were selected and screened in different years. Consistent results were obtained between the years ($r = 0.909^{**}$).

The present research also found that wheat allelopathic activity showed little correlation to 3 wheat competitive parameters, i.e. 1000-grain weight, plant height, or root length of wheat seedlings (data not shown). For example, the wheat 1000-grain weight for the 63 strongly allelopathic accessions ranged from 21.27 to 55.70 g with a mean of 38.82 ± 7.3 g, and for the 21 weakly allelopathic accessions ranged from 25.86 to 53.49 g with a mean of 39.45 ± 7.4 g. These results indicate that wheat accessions with vigorous growth parameters do not necessarily show strong allelopathic activity and *vice versa*. Similar findings have also been reported (Lockerman and Putnam 1981; Olofsdotter and Navarez 1996; Bach Jensen *et al.* 1999). Lockerman and

Putnam (1981) demonstrated that allelopathic activity of cucumber plants was not correlated to 2 competitive parameters of plant seedlings, i.e. the net assimilation rate (NAR) and relative growth rate (RGR). The allelopathic accession PI 169391 did not exceed the NAR or RGR of a non-allelopathic accession Pioneer during the 10-day period of association with proso millet (*Panicum miliaceum*). Similarly, it was found that rice allelopathic potential did not correlate to rice height (Olofsdotter and Navarez 1996), and to the root biomass of rice plants (Bach Jensen *et al.* 1999). These findings indicate that the competitive trait and allelopathic trait may not be genetically linked and that the breeding for crop cultivars with enhanced competitiveness coupled with allelopathy may be accomplished for weed suppression.

Crop allelopathic activity might also not be genetically linked to other important agronomic traits. Dilday *et al.* (1998) successfully developed a high-yielding rice line (Stg 94L42-130) with moderate allelopathic activity. The Stg 94L42-130 was allelopathic, with significantly fewer ducksalad plants than a non-allelopathic check, Rexmont. The successful example suggests that allelopathic activity may be weakly correlated with yield or other important agronomic traits, and high-yielding crop cultivars with enhanced allelopathic weed-suppressing ability can be developed without sacrificing other desirable agronomic traits.

Study of the genetic control of crop allelopathic activity is receiving increasing attention. More knowledge about the genetic control of crop allelopathic activity is essential before the development of crop cultivars with allelopathic potential for weed suppression occurs. Several attempts have been made to understand the genetic basis of allelopathy and to locate the genetic markers governing the production of allelochemicals (Niemeyer and Jerez 1997; Dilday et al. 1998; Bach Jensen et al. 1999). The present study shows that there are substantial genetic variations in allelopathic activity in wheat, thereby providing a sufficient gene pool for the development of allelopathic wheat cultivars in order to suppress weeds. The normal distribution of allelopathic activity in the collection of 453 wheat accessions was similar to that reported in rice (Dilday et al. 1998), indicating that wheat allelopathic activity is a quantitative trait.

Studies on wheat accessions with Condor or Pavon background, and on the near isogenic lines derived from Hartog and Janz, strongly indicate that allelopathic activity might also be controlled by major genes depending on the particular crosses. Similarly, the pedigree analysis of allelopathic rice accessions from the International Rice Research Institute (IRRI) has shown that 4 parents, BPI 76, IR8, Peta, and TN-1, were commonly found in those rice accessions allelopathic to both ducksalad (*Hetheranthera limosa*) and redstem (*Ammannia coccinea*) (Dilday *et al.* 1998).

An important economic potential of allelopathy is to enhance crop allelopathic activity to suppress weeds. Disease and insect resistance as well as stress adaptation have been genetically incorporated into crop cultivars. Therefore, possibilities exist for plant breeders to develop allelopathic genotypes for weed suppression. Incorporation of the allelopathic trait into commercial cultivars may enable crop plants to gain an advantage over weeds through the exudation of active allelochemicals and subsequent competitiveness (Lockerman and Putnam 1979).

Evaluating thousands of crop accessions for allelopathic activity in weed suppression is extremely time-, space-, and labour-consuming. To tackle these problems, an approach from biological screening under laboratory conditions to glasshouse experiment and in further field testing is recommended. After the preliminary screening by laboratory bioassays, a small proportion of promising genotypes with strongly allelopathic activity could be selected for glasshouse and further field testing in order to verify laboratory results, thereby substantially reducing the requirements for time, labour, and space in a large screening project. However, the approach from field screening to laboratory testing has also been successfully employed in rice allelopathy research (Olofsdotter and Navarez 1996). Olofsdotter and Navarez (1996) demonstrated that rice accessions able to suppress the growth of E. crus-galli in field experiments were also able to inhibit root elongation of this weed in the laboratory bioassay.

Further research is necessary to verify laboratory results under field conditions and to identify the responsible allelochemicals in wheat. An analytical technique employing gas chromatography and tandem mass spectrometry (GC/MS/-MS) has been recently developed for the identification and quantification of allelochemicals in wheat (Wu *et al.* 1999*a*). A comprehensive chemical analysis would consolidate the biological screening results and facilitate the genetic study of the allelopathic trait.

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