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SCREENING METHODS FOR EVALUATING THE ALLELOPATHIC POTENTIAL OF WHEAT AND TRITICALE GENOTYPES

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Abstract

Of the many methods for weed management one of them is using allelopathy in weed management programs. Application of allelopathic wheat and triticale cultivars is thought a resources conservation and environmental friendly way of weed bio-control, and could promote the sustainable development of agriculture. Screening or evaluating the allelopathic potential of wheat and triticale variety is the first step. This experiment was done to develop a new simple and valid methods for allelopathy studies and facilitating the use of some physiological traits in such researchs. One method was to assessment the allelopathic potential of some winter wheat and triticale genotypes using aqueous extract from the leaves and shoot of each genotype. Evaluation of allelopathic potential of these genotypes was done by measuring the germination capacity of ryegrass seeds (Lolium rigidum). Results showed that the allelopathic activity of wheat was associated with extract concentrations and wheat/triticale cultivars. Germination percentage of ryegrass seeds ranged from 0.2 to 92 %. In average for all used concentrations, extract of Titan (triticale) reduced ryegrass germination by 61% and most suitable concentration to evidentiate allelopatic activity was 5%.
Another method consisted in incorporating in soil of the plant residues from winter wheat and triticale genotypes. Evaluation of allelopathic potential of these genotypes was done by measuring the length of root of amaranth (Amaranthus retrophlexus) and ryegrass (Lolium rigidum).
Results demonstrated that the root length of weed species were significantly reduced in the presence of some wheat cultivars. Wheat and triticale varieties differ in allelopathic potential against ryegrass and amaranth and the differences were evidentiate by both methods

Key words*: allelopathy, wheat, triticale, annual ryegrass (Lolium rigidum), amaranth (Amaranthus retrophlexus).*

INTRODUCTION

Allelopathy, an important ecological phenomenon explaining the interference among species through bio-chemical pathways, seems to be one such tool that can be manipulated to manage weeds in agro-ecosystems (Khanh et al., 2005).
The allelopathic activity of wheat has been attributed to hydroxamic acids (Wu et al., 2000) and phenolic acids (Wu et al., 2001; Andrew et al., 2015).
Wheat seedlings, straw, aqueous extracts of residues and root exudates exerted allelopathic effects on a number of agricultural weeds (Khaliq et al., 2011).
Wheat is the second crop in Romania and ryegrass (*Lolium* spp.), foxtail (*Setaria* spp.), lambsquarters (*Chenopodium album* L.), redroot amaranth (*Amaranthus retroflexus* L) and specially purslane, maybe thistle (*Cirsium arvense*) are widespread weeds in wheat field and substantially reduce the yield.
Their control has been performed by different management practices and high dose of herbicides. It is desirable to have an alternative weed control method in wheat fields.
Application of allelepathy to control weeds can avoid environmental contamination and improve crop yield.
Allelopathic control refers to the method that exploits allelochemicals from volatile, leachate, exudates of certain plant to control of weeds (Oueslati, 2003; Kashif et al., 2016).
To realise the potential of competitive crop cultivars as a tool in integrated weed management, a quick and simple-to-use protocol for assessing their competitive potential is required as it is likely that selection will not be based on a single trait, but will need to capture the

combined effect of multiple traits (Andrew et al., 2015).
In a field observation from NARDI Institute two wheat cultivars were found to be as allelopathic as the most allelopathic triticale and are now used in a conventional breeding program to improve the allelopathic proprieties of the Romanian winter wheat. Screening bioassays are essential tools in identifying crop accessions with allelopathic potential.
The objective of this study was to establish the screening methodology for determination wheat and triticale differences in allelopathic potential against some weeds.

MATERIALS AND METHODS

The wheat and triticale were considered as the donor plants and ryegrass and amaranth as acceptor or target plants.

Bioassay with aqueous extracts of wheat and triticale

Three winter wheat and two triticale genotypes provided by Agricultural Research and Development Institute from Fundulea, Romania, and ryegrass seeds (Naki cv.) were used in order to establish/find a concentration level that allows sufficient germination of ryegrass for determining allelopathic potential.
Preparation of the extract from wheat plants
The donor plants seeds were sown during autumn in wooden boxes in soil:sand mixture and grown in vegetation house for five weeks. Than the above ground plant part (leaves and shoot) of each genotype were harvested, oven-dried at 40-50°C for 72 hours and ground to obtain a powder.
Different grams of powder from each wheat and triticale genotype (1.25, 2.5, 5, 7.5 and 10 g) were extracted with 100 ml of top water in a glass jad for 24 hours at room temperature.
The pulpy mixture was filtered through two layers of cheese cloth and the resulting filtrate was centrifuged at 10,000 rpm for 15 minutes. The supernatant was stored in a freezer prior to use. Beside those extract series as control was used top water.
Seed bioassay
Fifty seeds of ryegrass (*Lolium rigidum* L.) cv. "Naki" were sterilized with 10% sodium hypochlorite for 5 min., rinsed for 15 min. with top water and sown onto 10 cm Petri dishes lined with two layer of filter paper. Five ml of each concentration were then delivered to each Petri dish. All dishes were maintained in a control conditions, in darkness at 22-23°C for 14 days. The experiment was performed in five replications for each concentration.
Germination percentage was determined by counting germinated seeds after 24 hours of sowing till 10^{th} day.
The results are expressed as percent of sown seeds.
Pipper index (PI) was used for evaluated the dynamics of germination. PI refers to number of days needed for one seed to germinate and was calculated according to the following formula:

$$PI = x_1 s_1 + x_2 s_2 + \dots x_n s_n / s_1 + s_2 + \dots + s_n$$

$x_1, x_2,....x_n$ - day of germination
s – number of seeds that germinated at given day
n – last day of experiment

Bioassay with shoot residues incorporate into soil

Twenty four genotype of donor plants (wheat and triticale) were sown during autumn in plastic boxes in soil:sand mixture (20 seeds/plots and 1 kg soil:sand mixture) and grown in vegetation house up to maturity, than were cutting and shoot residues were incorporate into soil.
The same quantity of redroot amaranth seeds collected from spontan flore (approximately 0.5 g) and five ryegrass seeds were sown in each plot.
Alleopathy was studied in terms of root length inhibition. As control was used amaranth and ryegrass grows in clear soil mixture (without residues).

RESULTS AND DISCUSSIONS

Results revealed that the germination of ryegrass was inhibited by the extract of studied wheat and triticale genotypes.
The responses significantly differed depending on extract concentration.
Both the delay and the reduction in ryegrass germination are positive correlate with extract concentration, being the greatest at the highest concentration (Table 1).

Table 1. Germination of ryegrass (*Lolium rigidum* L.) (%) in water extracts of wheat and triticale

Genotype	Control (water)	Extract concentration (% DM w/v)					Average
		1.25	*2.5*	*5*	*7.5*	*10*	
Boema	91d	87cd	76 c	40 b	10 a	3.6 a	43 ab
GCO 3-22	88c	92c	84 c	51b	3 a	0.8 a	46 b
GDR 2863	88c	84 c	76 c	38 b	11 a	7.6 a	43 ab
Titan	90d	83 cd	71 c	36 b	4 a	0.8 a	39 a
Haiduc	98d	87 cd	76 c	50 b	14 a	4 a	46 b
Average	91d	87 d	76 c	43 b	8.4 a	3.3a	43

Ryegrass seeds, used in this test, were healthy having a high germinate capacity as in control conditions, ranking from 88% up to 98%

All the applied concentrations of shoot and leaves extract, except 1.25%, significantly suppressed the germination of the test weed.

The 5% concentration of shoot and leaves extracts highlights best the allelopathic potential of genotypes studied. At this concentration the germination of ryegrass seeds ranked from 36% (Titan) to 51% (GCO 3-22) In average for all used concentrations, extract of Titan (triticale) reduced ryegrass germination by 61% while of GCO 3 - 22 (wheat) by 54 % (Table 1). Our results are consistent with others that show that wheat accessions varied in their allelepathic activity in the fields, some accessions inhibited the weed growth up to 75 % (Rivzi et al., 2004).

Allelopathic effect of crop extracts was demonstrated also by changes in the dynamics of ryegrass germination. Pipper Index values ranged, on average for all studied concentrations, from 7.7 to 8.4 days (Table 2).

Table 2. Pipper Index for ryegrass (*Lolium rigidum* L.) seeds germinated in water extracts of wheat and triticale

Genotype	Control (water)	Extract concentration (% DM w/v)					Average
		1.25	2.5	5	7.5	10	
Boema	4.5a	5.9b	6.7 b	8.7 c	8.42c	8.70 c	7.7 ab
GCO 3 -22	4.5a	6.4b	7.5 bc	8.5 cd	8.6d	11.0 e	8.4c
GDR 2863	4.6a	6.4b	7.02bc	8.1 cd	8.6d	7.68 d	7.5 ab
Titan	4.6a	6.1 ab	7.8 bc	9.1 c	8.8c	9.0 c	8.1 bc
Haiduc	4.7a	6.3 ab	7.1 ab	8.64 b	8.4b	7.0 ab	7.5a
Average	4.6a	6.2b	7.2c	8.63 d	8.61d	8.68 d	7.89

Regarding the allelopathic effect of extracts from wheat the greatest delay was caused by extract from GCO3-22 genotype, as one seed to germinate, in average for all concentrations, needed 8.4 days more than in water (control).

Aqueous extract bioassays, which are conducted in Petri-dishes and seedling screening bioassays with the "equal compartment agar method"(ECAM), are two bioassay tests widely used in laboratory screening bioassays (Wu et al., 2001). Despite many advantages of these methods, there are also criticisms. For example, the release of certain salts, amino acids and nitrogen compounds, all may not be released under natural circumstances, inconsistent results due to non-uniform wetting of growth medium were mentioned as strong criticism of extract bioassays in Petri-dishes (Mardani et al., 2014). In order to determine the allelopathic potential of other 24 wheat and triticale genotypes the 5% extract concentration was used (Table 3).

Table 3. Germination and Pipper Index for ryegrass (*Lolium rigidum* L.) in water extracts of wheat and triticale

Genotype	Germination (%)	Pipper Index
Control	90	4.5
Dropia	52	6.9
Boema	38	7.3
Glosa	32	7.6
Izvor	31	7.6
00628G34-20	36	7.2
00628G34-1M01	51	6.4
00628G34-2M02	34	7.3
Titan	24	8.2
Stil	34	7.5
Gorun	53	6.7
Haiduc	42	7.0
Cascador	57	6.2
Impuls	30	7.8
Lotru	44	6.6
Migrator	46	6.9
Metropol	36	7.3
Matroz	49	6.8
00596 T1-2	61	6.0
00596 T1-101	30	8.0
114 T1-10101	34	7.9
01234T1-2	33	7.7
99574 T1-10201	33	7.7
00153 T5-12301	33	7.6
02511 T6-2	38	8.1

Our results indicated that the 5% concentration of extract was toxic enough than varietal differences in the inhibition of the germination of ryegrass could hardly be detected. At this level of concentration, the germination rate ranging from 24 (Titan) to 61% (00596 T1-2), compared to water control with a germination rate of 90%. The Pipper index ranging from 8.2 (Titan) to 6 (00596 T1-2), compared to water control with a Pipper index of 4.5 (Table 3).

Because the aqueous extract bioassays offer only information on behaviour of weed seed at allelopathic potential of wheat, screening

bioassays using plant residues have been developed. In fact the combination of several screening bioassays in sequence is therefore essential in order to establish conclusive proof of crop allelopathy on weeds (Wu et al., 2001).
The data indicated that the degree of phytotoxicity of the residues differed among varieties. Of the 24 wheat and triticale cultivar tested, three cultivars (Izvor, 00596T1-101 and Matroz) were able to inhibit length of ryegrass root on by 40 %. Migrator cultivar gaves less than 5% root length reduction in ryegrass (Figure 1).

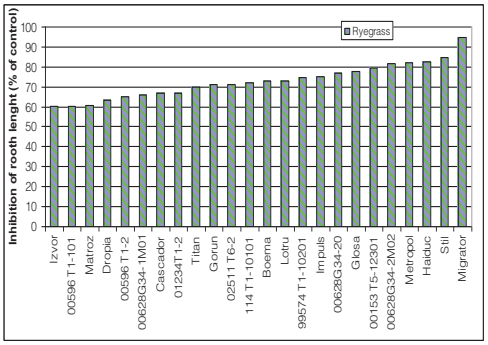

Figure 1. Effect of residues incorporate into soil on length of ryegrass root

Our results show that the degree of inhibition for root growth of ryegrass, and that the plant responses to the phytotoxic substances are genotype dependent (Figure 2).

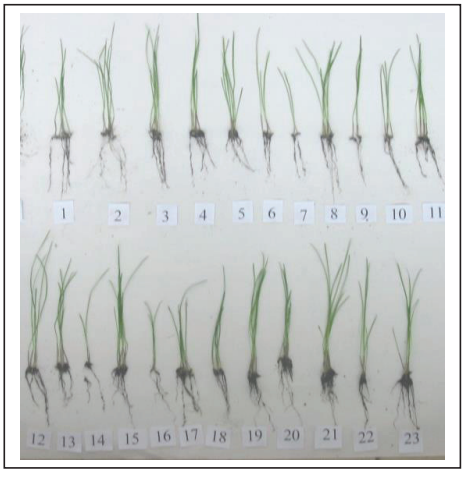

Figure 2. Effect of residues of 24 wheat and triticale and incorporate into soil on ryegrass

Of the 24 wheat and triticale cultivar tested, several cultivars have significantly reduced the amaranyh root elongation by more than 40% (02511 T6-2, Izvor, 99574T1-10201, Impuls, Titan and Glosa). Two genotypes (Boema and 00628G34-20) gave less than 5% root length reduction in amaranth (Figures 3 and 4).

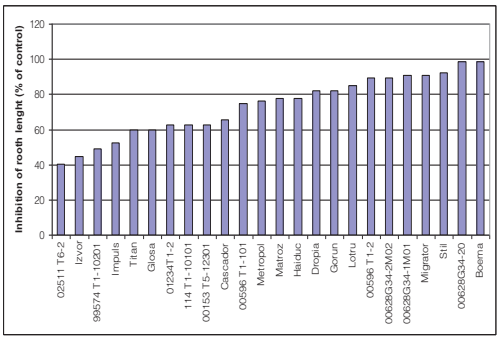

Figure 3. Effect of residues from wheat and triticale genotypes incorporate into soil on length of amaranth root

Growth of root was influenced different by the plant residues beeing genotypes which could be more toxic for ryegrass and less for amaranth (ex. Boema - Figure 3). Other researchers indicated there existed a significant difference among different bioassay methods, and the same rice line had a different allelopathic potential depending on bioassay methods employed (Lu et al., 2016).
Laboratory screening of 453 wheat genotypes showed continous variation in wheat allelopathy against annual ryegrass *(Lolim rigidum)* and among wheat genotypes studied, 63 were highly allelopathic, inhibiting root growth by more than 80% (Olasdotter and Andersen, 2004).

Figure 4. Effect of residues of several wheat genotypes incorporate into soil on amaranth

There was a positive correlation between germination response and root (r = 0.43, r = 0.67 for P 5% and 1%) (Table 4).

The positive correlation between seed germination and root growth may indicate that extracts that allowed rapid germination also allowed more time for root growth compared to extracts that delayed germination. This suggests that the reduction in root growth may have been a reflection of delayed germination rather than due to a direct effect of an allelochemical.

Table 4. The relationships between of the studied traits

Specification	Inhibition of rooth	
	Rygrass	Amaranth
Pipper index	r = - 0.43*	r = - 0.42*
Seed germination	r = 0.21 NS	r = 0.43*
Inhibition of root		r = 0.67**

This are in concordance with the recent discussion about the problems and proposal for future research directions in this field to provide a useful reference for future studies on plant allelopathy (Fang Cheng and Zhihui Cheng, 2015).

CONCLUSIONS

Compounds contained in water extracts of GCO 3-22 and GDR 2863 wheat genotypes have allelopathic activity against ryegrass comparable with triticale genotype.

These results are in concordance with Romanian breeders' expectation because these winter wheat genotypes have rye (*Secale cereale*) in their genetic background.

The plant extracts of these two winter wheat genotypes evaluated in this study act by inhibiting seed germination and may have potential for preemergence weed control.

Wheat and triticale varieties differ in allelepathic potential against ryegrass and amaranth and the differences were evidentiate by both methods.

The genotypes with higher allelophatic potential can be used for breeding purposes.

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