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Allelopathic stimulatory effects of wheat differing in ploidy levels on *Orobanche minor* germination

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ABSTRACT

Orobanche minor seed germinates only when germination stimulants from host plants or some non-hosts become available. Wheat is trap crop to stimulate the *O. minor* germination, hence, wheat of different ploidy levels were evaluated for their impacts on *O. minor* germination. The wheat exudates collected hydroponically induced the *O. minor* germination. Wheat of different ploidy levels differed in their allelopathic stimulation on *O. minor* germination, with the hexaploid wheat being the most stimulatory. Root exudate activity was concentration-dependent, with no activity at 1 mg/L concentration. Likewise, the stimulation of *O. minor* germination also depended on wheat ploidy level, the growth stage and the concentrations of aqueous or methanolic extracts. The highest germination (41.6%) was achieved with aqueous extracts from the tillering stage with hexaploid *T. aestivum* var. Shaan No. 253, while the lowest rate (4.2%) was from maturity stage with diploid *T. boeoticum* L. Plant methanolic extracts resulted in higher *O. minor* germination than aqueous extracts. Thus wheat accessions of differed ploidy levels differed in their allelopathic stimulation of *O. minor* germination and the stimulation increased from diploid to hexaploid. The wheat allelopathic activity could be used as a non-chemical option for *O. minor* control.

Key words: Allelopathic stimulation, aqueous extract, exudates, genome, methonolic extract, *Orobanche minor*, ploidy level, *Triticum* spp., wheat

INTRODUCTION

Allelopathy is the beneficial and inhibitory effects of one plant (including microorganisms) on the growth of another plant through the release of chemical compounds into the environment (26). Wheat (*Triticum aestivum* L.) has been reported as an allelopathic plant (34). Aqueous extracts of its decomposing wheat residues in the soil, reduced the growth of rice and the maximum phytotoxicity was recorded 2-weeks after decomposition (13,14).

The "wild ancestors" of present crops possessed high allelopathic activity and this character was reduced or lost during the breeding of new varieties with useful characteristics (24). Recent researches have shown that allelopathic activity is quantitatively inherited in rice and wheat (4,33). The genetic diversity between and within the allelopathic accessions of *Triticum speltoides* L. has been estimated using the random amplified polymorphic DNA markers. The genetic similarity ranged from 44% to 88%,

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with mean of 55%, between all possible pairs of selected accessions (19). Substantial genetic variation in wheat allelopathy has also been reported (28). Wu *et al*. (36) identified two major QTLs on chromosome 2B associated with wheat allelopathy. It seems that plant breeding does not necessarily lead to a loss of genetic diversity (7). The breeders at the International Maize and Wheat Improvement Center (CIMMYT) successfully increased the genetic diversity through the introgression of novel wheat materials (25). Wu *et al.* (35) developed a screening bioassay method, the "equal-compartment-agar-method (ECAM)", and evaluated 92 wheat cultivars for their allelopathic activity on root growth of annual ryegrass. They found that wheat with higher levels of phenolic acids in roots were generally strongly allelopathic to the growth of annual ryegrass (32). There was a large variability in allelopathic effects among the various cultivars and genotypes of wheat (39,41). Wheat stubbles of different genotypes had various allelopathic potentials against weeds (42). Most recently, Bertholdsson (2) reported that wheat cultivars with high allelopathic activity were highly suppressive to the growth of black-grass (*Alopecurus myosuroides* Huds.) and such cultivars could be important in integrated weed management.

O. minor (Orobanchaceae family) is an parasitic angiosperm, whose host range includes Apiaceae, Asteraceae, Fabaceae and Solanaceae plant families (10,21,29). It is an obligate root-parasitic plant without chlorophyll, can parasitize many species and assimilate nutrition from the host plants and as an annual plant, produces >500,000 seeds per flowering stalk (23). The seeds of *O. minor* are so small and are easily spread by wind, water and farm machinery, hence, it is widely distributed in Mediterranean countries, Western Asia and East Africa and caused severe damages to various crops (8,12,21). The seeds have special requirements for germination, [after-ripening, pre-conditioning and germination stimulation by stimulants from host plants or some non-host plants (12)]. The seed remains viable in soil for more than 10-years until meeting the host plant(s) (21).

Wheat is a false-host of *O. minor* (12) and has been evaluated to control *O. minor* (15,30). Distilled water and methanolic extracts of rhizosphere soil of winter wheat could stimulate the *O. minor* seed germination (6). Wheat as a non-host crop could be used to control *Orobanche* weed by inducing suicidal germination. This biocontrol can potentially deplete *Orobanche* seed bank in the soil, providing an environmentally-friendly means to manage this parasitic weed. Wheat allelopathic activity induces the *Orobanche* germination (3,6,12), however, little is known about the effects of wheat with different ploidy levels on the induction of *O. minor* germination. This study aimed to determine whether the allelopathic stimulation on *Orobanche* seed germination varies with different ploidy levels of wheat.

MATERIALS AND METHODS

Pre-conditioning *O. minor* seeds

Seeds of *O. minor* were collected from the field near Utsunomiya University, Japan by Prof. Koichi Yoneyama. Seeds were disinfected by 1% NaClO solution for 3 min., and followed by 75% (V/V) ethanol for 3 min., respectively (22). The seed was pre-treated with 10^{-4} mol/L gibberellic acid (GA_3) to break the dormancy to improve the seeds germination of *O. minor* (8). Each Petri dish (90 mm) was lined with double filter papers

and then added with 5 mL 10^{-4} mol/L GA_3. Glass fiber filter paper (GFFP) (Whatman GF/A) disks (5 mm diameter) were arranged evenly on the filter paper. One hundred and fifty disks were put in each Petri dish (Fig. 1). About 20-40 seeds of *O. minor* were placed on each disk. A total of 30 Petri dishes were prepared for the pre-conditioning of seeds. Petri dishes were then sealed by parafilm to reduce evaporation and placed in an electrothermic constant-temperature incubator for 8 days at 25℃ in dark (37). Pre-conditioned seeds of *O. minor* were used in germination bioassays below.

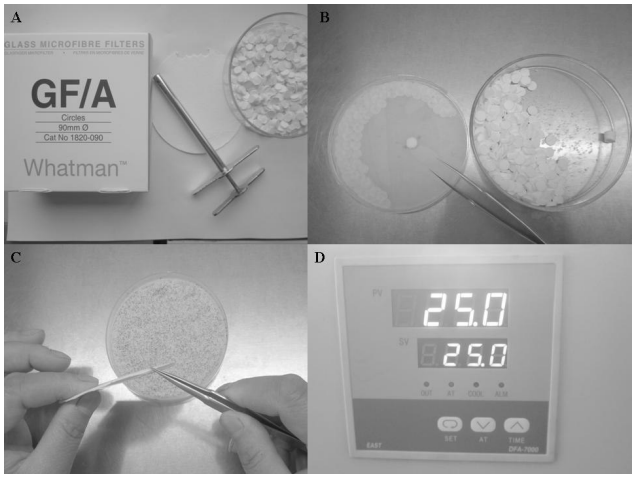

Figure 1. Pre-conditioning of *O. minor* seeds. A: Glass microfiber filters, punching bear and disks, B: Disks were put in Petri dish one by one with forceps, C: About 20-40 seeds of *O. minor* were placed on each disk, D: Petri dishes were placed in constant-temperature incubator at 25°C.

1. Hydroponic culture and collection of wheat root exudates

Six accessions of winter wheat (*T. boeoticum* L., *T. monococcum* L., *T. dicoccoides* K., *T. dicoccum* S., *T. aestivum* Shaan No. 167 and *T. aestivum* Shaan No. 253) differing in ploidy levels (Table 1) were provided by Prof. Xiping Deng of our University. One accession was each from *T. boeoticum* L., *T. monococcum* L., *T. dicoccoides* K. and *T. dicoccum* S. and two accessions from *T. aestivum* L. Four hundred seeds of each accession were put into a container (28 × 23.5 × 6 cm) lined with a sheet of gauze clothing placed inside a slightly larger container (40 × 29 × 11 cm) containing 4 L tap water. The plants were grown in a growth chamber (25℃ with 12/12 h photoperiod at 120 μmol photons $m^{-2}s^{-1}$) for 14 days, the root exudates released by wheat seedlings into the growing medium (water) during this period were adsorbed by activated charcoal. One hundred gram activated charcoal was added into a reagent bottle (1000 mL). The water was circulated through the bottle and the container using a circulation pump (China RS Electrical, RS-003 aquarium super pump). The tap water with 1 mM of $CaCl_2$ and activated charcoal were replaced every two days. The activated charcoal with the adsorbed root exudates was filtered to remove excess water and then was eluted with acetone (300 mL). The acetone

was removed by vacuum evaporation in a rotary evaporator at 40℃, and the residue was transferred into a 50-mL volumetric flask, adjusted to the required volume by adding distilled water, then partitioned three times with 50 mL of ethyl acetate (EtOAc) in a separating funnel according to our experiment results (6). The EtOAc fractions were combined and concentrated by vacuum evaporation to dryness. Dried substances were dissolved in acetone (5 mL) and stored in a sealed glass bottle at 4℃ (17).

2. Collection of rhizosphere soils and whole plants of tested materials

The six wheat accessions were sown in field plots (3 × 2 m) at Institute of Soil and Water Conservation in our University (108°08′E, 34°26′N, and elevation 500 m). The soil was dark loessial soil [pH: 8.2 (water), organic matter: 1.54%, available N: 0.54 g/kg and available P: 27.2 mg/kg]. All the wheat accessions were sown at 100 kg/ha on 5 October, 2009 and harvested on 10 June, 2010. The rhizosphere soils and the whole plant (both below and above ground parts) of wheat were collected at 5-growth stages (seedling, tillering, jointing, heading and maturity). The rhizosphere soil was defined as the soil not more than 5 mm between the root and soil (27). A completely randomized design was used with three replications. The rhizosphere soil (1 g) was dissolved in distilled water (10 mL) separately in conical flask (50 mL), and then extracted for 30 min by a sonicator. The solution was filtered by qualitative filter paper. The filtrate was used as the original aqueous extracts from the rhizosphere soil.

The whole plant of each cultivar was collected at different growth stages (carefully dug and washed with tap water). The plant materials were then freeze-dried for 48 h in a vacuum freeze dryer (FD-1A-50, Beijing Boyikang Experiment Instrument Co., Ltd) and ground by using an herbal medicine disintegrator (FW135-177, Tianjin Taisite Instrument Co., Ltd). The powder was passed through a sieve (0.45 mm in diameter). The powder (0.1 g) was extracted with either distilled water or methanol (1 mL) for 30 min by a sonicator. The mixture was centrifuged at 6,400 rpm for 2 min using a micro centrifugal machine (Millipore Cat. No. XX42 CF0, 60 Lot No. N8JMB042A, Nihon Millipore Ltd, Yonezawa, Japan). The supernatant was taken as the original aqueous or methanolic extracts (11).

3. *O. minor* germination bioassay

A standard germination-stimulating compound GR24 (Synthetic analogue of strigolactone) was provided by Prof. Binne Zwanenburg, Radboud University, Nijmegen, Netherlands. The acetone solutions containing the concentrated root exudates collected from the above mentioned hydroponic culture were diluted into 100 mg/L, 10 mg/L and 1 mg/L by distilled water. The original aqueous or methanolic extracts from the soil or the whole plant at the concentration of 100 g/L were diluted to 10 g/L, 1 g/L and 0.1 g/L by distilled water and methanol, respectively.

The pre-conditioned culture disks with *O. minor* seeds were transferred in each Petri dish and then 15 μL of different concentrations of root exudates collected from hydroponic culture and the aqueous extracts from the soil and the whole plant was added onto the disk, accordingly. However, the methanolic extracts (15 μL) were added onto the new glass fiber filter disk (no seeds) and then they were placed at room temperatures in a clean bench for 30 min to remove the methanol. The pre-conditioned culture disks with *O.*

minor seeds were transferred onto the glass fiber filter disk. Distilled water (30 μL) was added onto the double glass fiber filter disk (11). Three replications were used for each treatment. The distilled water, methanol and GR24 (1 mg/L) were used as controls. Petri dishes were then sealed by Parafilm to reduce evaporation and maintained in an electrothermic constant-temperature incubator for 10 days at a constant temperature of 25℃. The germination of *O. minor* was observed through a microscope (20 × 10).

Rhizosphere soils (10 g) was placed onto a culture dish (30 mm dia.) and leveled. Five pre-conditioned culture disks with *O. minor* seeds were carefully transferred onto the surface of soil, and 1 mL of distilled water was added to each disk to keep it moist. The soil in the absence of wheat growing during the season and GR24 (1 mg/L) were used as controls. Each soil sample had three replications. All the culture dishes were then placed in an electrothermic constant-temperature incubator at 25℃for 10 days. The *O. minor* germination was measured as described previously.

Statistical analyses: ANOVA analyses of all treatments were performed using SAS 8.1 and Excel 2003.

RESULTS

GERMINATION INDUCTION

Root exudates of different ploidy winter wheat: Wheat accessions of different ploidy levels produced variable quantities of root exudates (allelopathic residues i.e. concentrated allelopathic substances released in root exudates of winter wheat), collected from hydroponic culture (Table 1). The highest amount of residues (19 mg) was collected from the diploid *T. dicoccum* S. and the lowest (5.4 mg) from the haploid *T. monococcum* L.

GR24 at 10 mg/L concentration induced the germination (85.6%) of *O. minor* seeds (Table 1). Seeds treated by distilled water (or methanol) remained dormant and germination did not occur. *O. minor* germination increased gradually with the increasing ploidy levels of wheat. At exudate concentration of 100 mg/L the germination ranged from 49.4% (diploid *T. monococcum* L.) to 73.5% (hexaploid *T. aestivum* Shaan No. 167). Similarly, the root exudates of two hexaploid wheat induced higher germinations at 10 mg/L concentration than the two diploid wheat accessions. The wheat root exudates concentration significantly stimulated the germination of *O. minor* seeds. The allelopathic stimulation of root exudates declined sharply with the decreased exudate concentration. The root exudates at 1 mg/L concentration did not induce the germination irrespective of wheat accessions.

Rhizosphere soil of different ploidy winter wheat: The rhizosphere soil of different ploidy levels of wheat collected at 5-growth stages stimulated the *O. minor* seed germination (Fig. 2). At seedling stage, *O. minor* germination was higher in hexaploid wheat than in tetraploid or diploid wheat and the highest germination was 32.6% in *T. aestivum* Shaan No. 253. The germination showed decreasing trend at tillering stage, then again increased at the jointing stage except in *T. aestivum* Shaan No. 167. This increasing

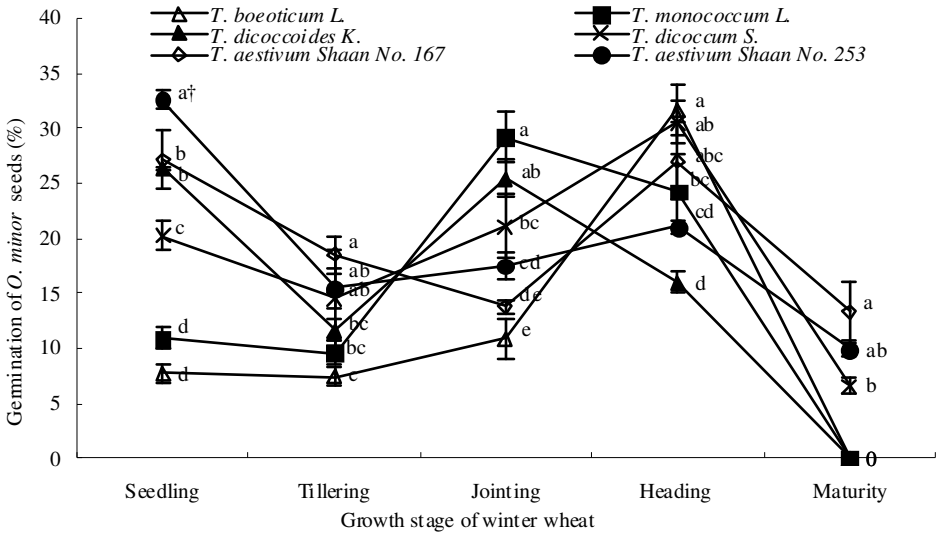

Figure 2. *O. minor* germination (%) induced by rhizosphere soil of wheat differing in ploidy levels. Note: † Means in the same growth stage followed by different letters indicate significant difference according to Fisher's protected least significant difference (LSD) ($P < 0.05$).

trend continued till heading stage in *T. boeoticum* L., *T. dicoccum* S. and the two accessons of *T. aestivem* L., while after the heading stage, a decreasing trend was in *T. monococcum* L. and *T. dicoccoides* K. The allelopathic stimulation in *O. minor* seed germination declined sharply from the heading to maturity stage in all six wheat accessions, with little stimulation at the maturity stage.

GERMINATION STIMULATION

Aqueous extracts of rhizosphere soil (different ploidy winter wheat): The concentration of the aqueous soil extracts had significant impact on the germination of *O. minor* (Table 2). *O. minor* seeds did not germinate at 0.1 and 1.0 g/L extract concentrations (data not shown). Higher germination occurred at 100 g/L concentration than 10 g/L concentration. At 10 g/L concentration, only the extracts collected at seedling and heading stages stimulated the *O. minor* seed germination, with the exception of two hexaploid wheat accessions at joining stage.

At 100 g/L concentration, the aqueous extracts of rhizosphere soil stimulated the *O. minor* germination at all 5-growth stages. At seedling stage, the hexaploid wheat had higher stimulatory activities than the tetraploid or diploid wheat, while there was no significant difference between the tetraploid and diploid wheat. Two hexaploid *T. aestivum* var. Shaan No. 167 and var. Shaan No. 253 had the highest germination of 36.6% and 27.1%, respectively. The allelopathic stimulation was variable at the 5-growth stages. The stimulation decreased from seedling stage to tillering and jointing stages, peaked at the heading stages and then decreased at the maturity stage.

Table 1. *O. minor* germination (%) induced by root exudates of wheat

Varieties	Ploidy level	Concentrated root exudates (mg)	Concentration of root exudates		
			100 mg/L	10 mg/L	1 mg/L
T. boeoticum L.	AA	10.8	52.1^{d†}	29.4^{c}	0.0
T. monococcum L.	AA	5.4	49.4^{d}	21.0^{d}	0.0
T. dicoccoides K.	AABB	16.4	55.8^{cd}	21.7^{d}	0.0
T. dicoccum S.	AABB	19.8	65.7^{bc}	34.7^{bc}	0.0
T. aestivum Shaan No. 167	AABBDD	15.5	73.5^{b}	37.8^{b}	0.0
T. aestivum Shaan No. 253	AABBDD	14.4	70.9^{b}	40.4^{b}	0.0
GR24 (Control)			85.6^{a}	85.6^{a}	74.2

GR24: a synthetic strigolactone analogue, † Means in the same column followed by different letters indicate significant difference according to Fisher's protected least significant difference (LSD) ($P < 0.05$).

Table 2. *O. minor* germination (%) induced by rhizosphere soil aqueous extracts of wheat varieties

Varieties	Growth stage									
	Seedling		Tillering		Jointing		Heading		Maturity	
	100 g/L	10 g/L	100 g/L	10 g/L	100 g/L	10 g/L	100 g/L	10 g/L	100 g/L	10 g/L
T. boeoticum L.	19.4^{c†}	11.4^{bc}	4.8^{e}	0.0^{b}	7.2^{b}	0.0^{c}	28.5^{c}	19.5^{b}	5.2^{b}	0.0
T. monococcum L.	17.9^{c}	15.3^{ab}	10.3^{bc}	0.0^{b}	10.4^{b}	0.0^{c}	26.3^{c}	10.7^{c}	12.3^{a}	0.0
T. dicoccoides K.	20.1^{c}	16.8^{a}	6.0^{de}	0.0^{b}	9.6^{b}	0.0^{c}	26.0^{c}	15.0^{cd}	5.8^{b}	0.0
T. dicoccum S.	20.8^{c}	8.8^{c}	9.8^{cd}	4.6^{a}	12.2^{b}	0.0^{c}	49.6^{a}	26.2^{a}	7.2^{b}	0.0
T. aestivum Shaan No. 167	36.6^{a}	15.2^{ab}	14.1^{ab}	0.0^{b}	19.2^{a}	9.3^{b}	28.3^{c}	13.5^{de}	11.1^{a}	0.0
T. aestivum Shaan No. 253	27.1^{b}	12.0^{bc}	14.7^{a}	0.0^{b}	20.7^{a}	12.8^{a}	37.3^{b}	18.8^{bc}	14.1^{a}	0.0
Distilled water (Control)	0.0^{d}	0.0^{d}	0.0^{f}	0.0^{c}	0.0^{c}	0.0^{c}	0.0^{d}	0.0^{f}	0.0^{c}	0.0

Note: † Means in the same column followed by different letters indicate significant difference according to Fisher's protected least significant difference (LSD) ($P < 0.05$).

Aqueous or methanolic extracts (whole plant of different ploidy winter wheat): The aqueous extracts of whole wheat plant of all 6-wheat accessions stimulated the *O. minor* germination at 100 g/L concentration (Table 3). Similarly, the allelopathic stimulatory effects were stronger in hexaploid wheat than in tetraploid and diploid wheat with a few exceptions. The germination was higher at tillering and heading stages than at the seeding, jointing and maturity stages. The highest germination was 41.6% at the tillering stage of hexaploid *T. aestivum* var. Shaan No. 253, while the lowest (4.2%) was at maturity stage of diploid *T. boeoticum* L. The allelopathic stimulation of aqueous extracts was also concentration-dependent, with limited or no stimulation at the lower concentration of 10 g/L, depending on the wheat accessions and growth stages (Table 3).

The methanolic extracts at 100 g/L concentration induced higher germination in *O. minor* seeds than the aqueous extracts (Tables 3 and 4). At seedling stage, there was significant difference between the hexaploid and tetraploid wheat, with the highest rate being 34.1% by methanolic extracts of *T. aestivum* var. Shaan No. 167 at 100 g/L concentration. The stimulation effect differed among the wheat genomes, generally with hexaploid > tetraploid > diploid. The whole plant methanolic extracts collected at the heading stage from *T. aestivum* var. Shaan No. 253 induced in the highest germination (58.7%) in *O. minor* seeds (Table 4).

DISCUSSION

Bertholdsson (1) used a multivariate analysis to determine the relative impact of wheat allelopathy and competitiveness on weed suppression. He reported that the combined effects of wheat allelopathy and competitiveness reduced the weed biomass by 60%, while improved allelopathy alone caused reduction of 18-28%. The allelopathic effects of winter wheat (6), cotton (17), medicinal herbs (18) and maize (16) have been studied on the germination of *Orobanche* spp. The inhibitory effects of wheat allelopathy could be exploited for its biological weed management (6). The allelopathy of winter wheat at the seedling and heading stages induced the *O. minor* germination. The wheat allelopathy could be also used to stimulate the germination of *O. minor*, thereby rapidly depleting its seed bank (6). This is the first report that wheat of different ploidy levels differed in the stimulation of *O. minor* seed germination under both hydroponic culture and field experiments.

Wheat root exudates from hydroponic culture can stimulate the *O. minor* seed germination. The stimulatory potential of wheat was increased by its evolution from diploid, tetraploid to hexaploid. The hydroponic culture could be used to efficiently and quickly collect the root exudates and to determine their biological activities on target organisms such as *O. minor* germination.

The allelopathic effects are influenced by biotic and abiotic stresses and other environmental factors (soil, water, temperature, photoperiod and so on) (10,20). The gradual-decrease in temperature at seedling stage could impose temperature stress on seedlings, resulting in greater allelopathic activities. The *O. minor* seed germination was stimulated by the rhizosphere soil aqueous extracts of 6- winter wheat varieties (except *T. aestivum* Shaan No. 167) at heading stage than other growth stages at 100 g/L concentration (Table 2).

Table 3. *O. minor* germination (%) induced by whole plant aqueous extracts of wheat*

Varieties	Growth stage									
	Seedling		Tillering		Jointing		Heading		Maturity	
	100 g/L	10 g/L	100 g/L	10 g/L	100 g/L	10 g/L	100 g/L	10 g/L	100 g/L	10 g/L
T. boeoticum L.	14.1^{bc†}	0.0^{c}	12.2^{c}	6. 5^{d}	7.9^{d}	0.0^{d}	14.4^{d}	5.4^{c}	4.2^{d}	0.0^{c}
T. monococcum L.	9.9^{c}	0.0^{c}	16.2^{c}	7.0^{d}	9.4^{d}	0.0^{d}	11.5^{d}	5.4^{c}	10.6^{c}	0.0^{c}
T. dicoccoides K.	13.2^{bc}	0.0^{c}	39.3^{a}	23.5^{b}	10.9^{d}	4.4^{c}	23.3^{c}	10.0^{b}	14.5^{b}	0.0^{c}
T. dicoccum S.	18.4^{ab}	8.9^{b}	30.2^{b}	30.4^{a}	19.5^{c}	6.2^{b}	29.4^{bc}	10.6^{b}	14.2^{b}	0.0^{c}
T. aestivum Shaan No. 167	16.6^{b}	7.4^{b}	35.2^{ab}	17.3^{c}	34.0^{a}	11.8^{a}	34.4^{ab}	13.1^{b}	18.6^{a}	4.3^{b}
T. aestivum Shaan No. 253	24.0^{a}	11.7^{a}	41.6^{a}	31.0^{a}	27.1^{b}	10.6^{a}	38.3^{a}	21.1^{a}	18.4^{a}	7.5^{a}
Distilled water (Control)	0.0^{d}	0.0^{c}	0.0^{d}	0.0^{e}	0.0^{e}	0.0^{d}	0.0^{e}	0.0^{d}	0.0^{e}	0.0^{c}

Note: *No germination occurred at the extract concentrations of 0.1 and 1.0 g/L (data not shown). † Means in the same column followed by different letters indicate significant difference according to Fisher's protected least significant difference (LSD) ($P < 0.05$).

Table 4. *O. minor* germination (%) induced by whole plant methanolic extracts of wheat

Varieties	Growth stage									
	Seedling		Tillering		Jointing		Heading		Maturity	
	100 g/L	10 g/L	100 g/L	10 g/L	100 g/L	10 g/L	100 g/L	10 g/L	100 g/L	10 g/L
T. boeoticum L.	31.7^{a†}	9.6^{ab}	18.5^{d}	0.0^{c}	17.7^{d}	5.4^{c}	22.0^{c}	7.5^{d}	14.5^{b}	5.1^{ab}
T. monococcum L.	30.0^{ab}	8.8^{ab}	20.9^{d}	0.0^{c}	23.4^{c}	9.1^{c}	24.8^{c}	11.1^{cd}	10.5^{b}	0.0^{c}
T. dicoccoides K.	21.7^{b}	7.9^{ab}	16.5^{d}	0.0^{c}	29.9^{b}	16.3^{b}	31.7^{b}	14.0^{bc}	10.1^{b}	0.0^{c}
T. dicoccum S.	23.0^{b}	7.2^{b}	32.6^{c}	0.0^{c}	30.3^{b}	14.3^{b}	35.2^{b}	16.2^{b}	15.4^{b}	3.9^{b}
T. aestivum Shaan No. 167	34.1^{a}	11.3^{a}	45.4^{a}	14.0^{b}	32.7^{b}	14.4^{b}	37.3^{b}	14.0^{bc}	13.6^{b}	5.7^{a}
T. aestivum Shaan No. 253	29.5^{a}	10.1^{ab}	38.4^{b}	16.4^{a}	41.9^{a}	21.1^{a}	58.7^{a}	21.6^{a}	21.0^{a}	5.5^{ab}
Methanol (Control)	0.0^{c}	0.0^{c}	0.0^{e}	0.0^{c}	0.0^{e}	0.0^{d}	0.0^{d}	0.0^{e}	0.0^{c}	0.0^{c}

Note: † Means in the same column followed by different letters indicate significant difference according to Fisher's protected least significant difference (LSD) ($P < 0.05$).

The *O. minor* seed germination was higher with whole plant extracts (Table 3 and 4) than rhizosphere soil extracts (Table 2) at the same concentration and same growth stage. It indicated that the stimulant(s) were partly exuded into the soil through root exudates.

Majority of aqueous extracts of soil and plant sample at 100 g/L concentration stimulated the *O. minor* seed germination and at each growth stage the germination was higher than at 10 g/L concentration. The methanolic extracts of whole plant stimulated the *O. minor* seed germination, resulting in higher germination than water extracts at the corresponding growth stage and concentration. The germination was very low and even negligible at 10 g/L concentration at maturity stage (Table 3), which could be partly because the root growth stopped and could not actively exude stimulant(s). Besides, the leaves gradually senesced and transport of secondary metabolites to the roots was decreased.

The allelopathic potential of cucumber gradually decreases during the breeding process (24). However, this study indicated that wheat allelopathic potential on *O. minor* germination increased with the genome evolution and artificial breeding from diploid (AA) and tetraploid (AABB) to hexaploid (AABBDD). These results were in agreement with Zuo *et al.* (40) who reported that the allelopathy of mature aerial parts of different wheat genomes increased with the genome evolution from AA (2n), AABB (4n) to AABBDD (6n).

Similarly, Siddique *et al.* (31) reported that water use efficiency (WUE) for grains increased substantially from old to modern varieties, with little difference among the modern cultivars (31). Zhang *et al.* (38) also reported that during the wheat evolution from 2n to 6n, WUE at whole plant level increased with increase in chromosome ploidy levels. Among 453 wheat cultivars from 50 countries, Wu *et al.* (33) found significant differences in seedling allelopathy against annual ryegrass (*Lolium rigidum* L.). They concluded that it should be possible to breed for cultivars with enhanced allelopathic activity. These studies supported that allelopathic potential may not necessarily decrease due to the breeding.

Root exudates of different wheat genomes exhibited variable stimulatory effects on *O. minor* seed germination (Table 1 to 4). Our earlier study (5) also showed that aqueous or methanolic extracts from wheat rhizosphere soil stimulated the *O. minor* germination. Especially, *T. aestivum* Xiaoyan No. 22 had stimulatory effects on *O. minor* at all growth stages. The results from this study suggest that wheat allelopathic activity can be utilized as a biological means to control *O. minor*.

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