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Table: 1, Fig : 5

Biological induction of DIMBOA in wheat seedlings by weeds

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ABSTRACT

We investigated the induction effects of DIMBOA by 6 weed species in wheat seedling in hydroponic and in pot culture. In hydroponic experiments, DIMBOA content in both root exudates and shoots of wheat seedlings were significantly increased by *Digitaria sanguinalis* L., *Avena fatua* L. and *Amaranthus retroflexus* L. weeds. While *Poa annua* L., *Lolium multiflorum* Lam. and *Capsella bursa-pastoris* L. showed less production of DIMBOA in wheat root exudates and shoots. In pot culture the biological induction of DIMBOA in wheat plants by former 3-weeds was similar to hydroponic solution, but DIMBOA content in shoots was higher in pot than in hydroponic solution. With the increase in densities of *Digitaria sanguinalis* L., the DIMBOA content in wheat shoot increased greatly, which implied that the difference in weed density may be useful approach to artificially the improve crops resistance.

Key words: *Amaranthus retroflexus* L., *Avena fatua* L., Biological induction, *Capsella bursa-pastoris* L., *Digitaria sanguinalis*L., DIMBOA, hydroponic, *Lolium multiflorum* Lam., *Poa annua* L., pot culture, wheat

INTRODUCTION

Donor crops exhibits allelopathic effects on some weeds through the release of chemicals into agricultural systems (7). The live wheat seedlings, aqueous extracts of its shoots and residues cover, inhibit the seed germination, root length and stem length of *Digitaria sanguinalis* L. and *Amaranthus retroflexus* L. (8,10). Such effects vary with type and number of allelochemicals present in different cultivars (4). The question whether the synthesis of allelochemicals in the seedlings of some wheat cultivars can be induced due to the invasion of some weeds in the surrounding environment should be solved.

Plants are the natural treasure of biologically active chemicals, each having its special pathway. The chemical interactions between the plants-plants, plants-microbes and plants secondary metabolites and pests wounds are induced by secondary metabolites (2). The allelochemicals concentrations in crops/weeds may be increased by biotic and abiotic factors, particularly chemical and biotic stresses (1,3,5,9,11).

DIMBOA [2,4-dihydroxy-7-methoxy-(2H)-1,4-benzoxazin-3(4H)-one] a most important benzoxazinoids has been studied to understand the allelopathic phenomena in Gramineae crops [wheat (*Triticum aestivum* L.) and corn (*Zea mays* L.)] (6,7). The soil

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degradation dynamics of DIMBOA and its degradation products have been evaluated in wheat crop soil (7) and HMBOA [2-Hydroxy-7-methoxy-(2H)-1,4-benzoxazin-3(4H)-one was found as the degradation product. Wu *et al*. (12) reported that root exudates of wheat seedlings adversely affected the shoot and root growth of ryegrass. Wu *et al*. (11) evaluated the 453 wheat accessions from 50 countries and found that wheat accessions significantly differed in their allelopathic potential. Further research showed that wheat accessions varied significantly in the production of DIMBOA and some phenolic acids in the shoots and roots (13,14,15). Such significant variations in the production and exudation of DIMBOA among different wheat varieties suggest that wheat accessions may influence the DIMBOA content in wheat seedlings. However, Zheng *et al*. (18) found that some kinds of chemicals such as Methyl jasmonate (JA)，methyl salicylate (SA) and triadimefon can also affect the DIMBOA content in shoot in hydroponic solution of seedlings of 3 Chinese wheat varieties. We first studied the chemical induction effects of test chemicals on DIMBOA production from root exudates and aerial Parts of wheat seedlings. But weeds as inducing factor to increase the DIMBOA content were rarely reported in literature. This study aimed to describes the biological potential of 6-common weeds [*Digitaria sanguinalis* L.*, Avena fatua* L. and *Amaranthus retroflexus* L. weeds. While *Poa annua* L., *Lolium multiflorum* Lam. and *Capsella bursa-pastoris* L.] as an inducing factor and to determine the biological induction of DIMBOA in wheat seedlings.

MATERIALS AND METHODS

Test plants

The test wheat cultivars (Zhongfu9507, Zhongbeizhong39, Jing411) were obtained from the Institute of Crops, Chinese Academy of Agricultural Sciences, Beijing. While seeds of weeds (*Digitaria sanguinalis* L.*, Avena fatua* L., *Amaranthus retroflexus* L, *Poa annua* L., *Lolium multiflorum* Lam. and *Capsella bursa-pastoris* L.) with high germination ability were got from the Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing. Seeds were sterilised with 5% NaClO for 10 min and rinsed with distilled water, thrice before use in the experiments.

Hydroponics

The test wheat seeds were sown in the pots in moist vermiculite. Then the pots were kept in the illumination box [28$\pm$1^{o}C and the 12 h light per day]. After 5-days, well grown wheat seedlings were selected. Weed seeds were sown uniformly in the seedbed and then covered by 1-2 cm vermiculite and irrigated daily with water. The whole seedbeds were kept in the illumination box under the conditions of [28$\pm$1 ^{o}C and the 12 h light per day] till the seedlings were 5 cm long.

Three clusters of plants (15 plants per cluster) were wrapped with sponge and inserted into 3 holes of a hydroponic glass with 5 holes, then two clusters of weed seedings (15 weed seedings per cluster) were wrapped with sponge and inserted into remaining two holes in the hydroponic glass (Figure 1) and replicated thrice. Wheat seedlings were grown for 6 days.

Figure 1. Photograph of hydroponics culture

Pot culture

The 30 pre-germinated wheat seedlings of uniform size were planted in pots containing the vermiculite with 15 weeds seedlings. The treatments were replicated thrice. The DIMBOA content in the aerial parts of wheat seedlings were determined after 8 days.

Preparation of sample: Hydroponic solution

The constant volume of hydroponic solution was maintained at 200 ml by adding water. The hydroponic solution was shaken vigorously and its 20 ml samples were taken and dried with *Christ* rotational vacuum concentrator at 40^{o}C. The samples were re-dissolved in 0.8 ml methanol and 0.2 ml water for HPLC analysis.

Preparation of samples : Wheat seedlings roots and shoots

An aliquot of wheat seedlings (10 g) was weighed and extracted with 100 ml water for 2 min using Ultra-Turrax T25 mixer at 9500 rpm. Then the pH was adjusted to 3.0 with 0.1N phosphate buffer solution. After filteration, the filtrate was transferred to 500 ml separating funnel and ether extracted three times. The ether phases were dried over anhydrous Na_2SO_4 and concentrated to dryness with a rotary evaporator. The mixture obtained was finally filtered with a 0.45 µm nylon syringe filter for HPLC analysis.

Instruments

DIMBOA was determined in a Agilent HPLC systems fitted with UV-detector and a 250mm×4.6mm (id) stainless column (ODS HYPWEAIL BOSC18, USA). *Sigma* centrifugal (Germany), *Christ* rotational- vacuum- concentrator (Germany), tissue ultra-turrax (Germany), rotary evaporator (Switzerland), pipette (1ml, 10ml), PVDF membrane syringe filter units (Φ0.45 µm), Scout^{TM} *Pro* electronic balance (Japan) were employed.

Characterization and quantification of samples by HPLC

The samples were analyzed with HPLC on a HP model chromatograph coupled with a UV detector. HPLC analytical conditions were as follows: Column: ODS C-18 reverse phase column, 250 mm by 4.6 mm (id); eluted using methanol: water (50:50, v/v), 1 ml/min flowing rate; injection volume: 20 µl; identified at 263 nm using external standards.

Statistical analysis

The data on induction effects were analysed with EXCEL software and SPSS 13.0 and the treatments means were treated for least significant difference (LSD) at a 5% level of probability.

RESULTS AND DISCUSSION

Validation Method

DIMBOA was found linear when comparing the response to peak area. The calibration was done in triplicate with 5-concentrations (0.5, 1, 2.5, 5, 10.0 mg ml^{-1}) by external matrix-matched standards (Y=9.9605X+0.3632, R^2=0.9999).

Hydroponic solution: Induction effects in shoots of wheat seedlings

The analytical results of LSD showed that significant difference in induction were detected by *Digitaria sanguinalis* L., *Avena fatua* L. and *Amaranthus retroflexus* L. as compared with control (Figs 2,3) and they showed some biological induction effects. In hydroponic solution, the *Digitaria sanguinalis* L. weed increased the DIMBOA contents in the roots of test wheat cultivars of Zhongfu9507, Zhongbeizhong39 and Jing411 by 0.442, 0.341, 0.876 times and in their shots by 0.535, 0.557, 0.268 times, respectively. This also showed induction effects on DIMBOA in roots exudates and shoots of 3 test wheat cultivars. These results were consistent with Zheng *et al.* (18) that DIMBOA concentration could be induced by several chemicals and the inducement effect on DIMBOA production in shoot of wheat seedlings were higher than in root. Other weeds (*Poa annua* L., *Lolium multiflorum* Lam. and *Capsella bursa-pastoris* L.) showed weak induction effects, due to less DIMBOA accumulation in shoots and roots of wheat seedlings.

The induction of DIMBOA in shoots of wheat seedlings showed that the weeds with strong inducing ability have strong adaptive ability, fast speed of growth, short growing cycle, high germination ratio, strong drought endurance ability and growing force and vice versa.

Pot culture: Induction of DIMBOA in shoots of wheat seedlings

The induction effects of test weeds shoots on potted wheat seedlings are presented in Figure 4. The biological induction experiments done in pot culture gave consistent results similar to hydroponic experiments. *Digitaria sanguinalis* L., *Avena fatua* L. and *Amaranthus retroflexus* L. showed greater induction effects than *Poa annua* L., *Lolium multiflorum* Lam. and *Capsella bursa-pastoris* L. These results showed the biological effects of hydroponic experiments on root exudates and shoots. The DIMBOA contents were increased more in pot culture than in hydroponic studies, perhaps because the pot environment was more favourable for the synthesis of DIMBOA in wheat seedlings. Besides, the DIMBOA content in the aerial parts of hydroponic solution and potted Zhongbeizhong39 and Zhongfu9507 were easily induced by weeds than in Jing 411. The inducement effects of weeds were different than chemicals (methyl jasmonate, methyl salicylate and triadimefon) (18).

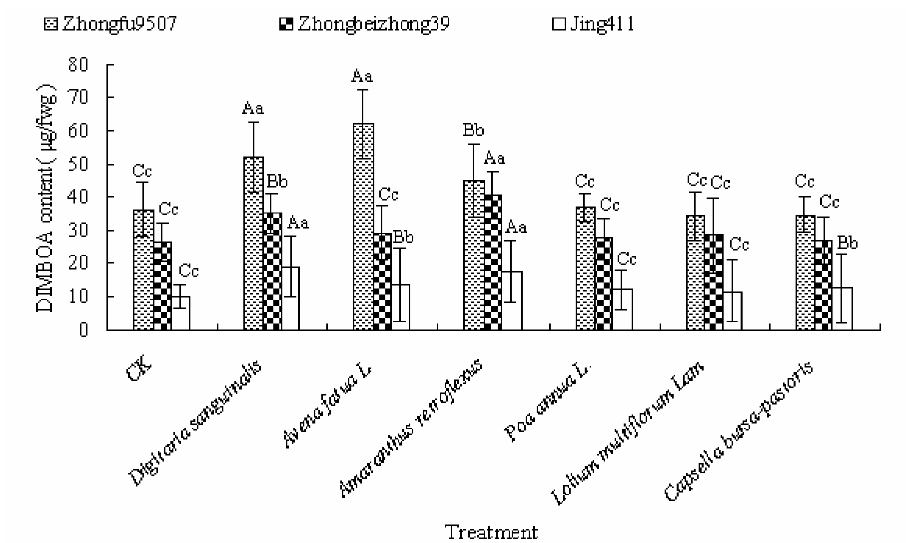

Figure 2. Induction effects of test weeds on DIMBOA production in hydroponic solution of wheat seedlings. Capital letters and small letters denote the significance of difference at the level of $P_{0.05}$ and $P_{0.01}$ by LSD. Different letters indicate significant difference and same letters indicate non significance.

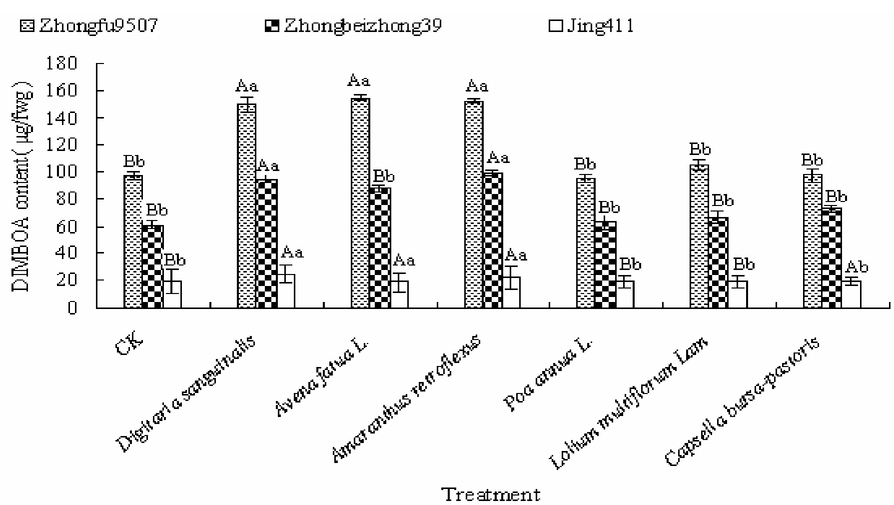

Figure 3. Induction effects of test weeds on DIMBOA production in shoots of wheat seedlings in hydroponic solution. Capital letters and small letters denote the significance of difference at the level of $P_{0.05}$ and $P_{0.01}$ by LSD. Different letters indicate significant difference and same letters indicate non significance.

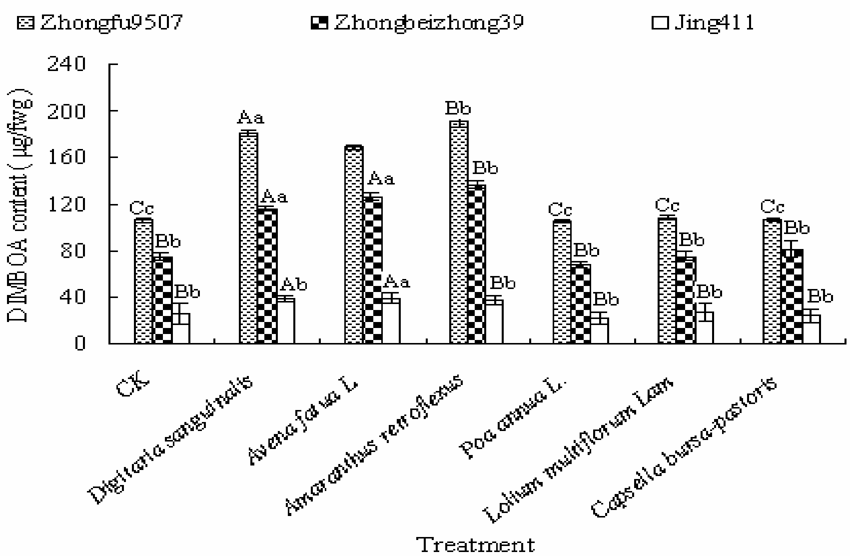

Figure 4. Induction effects of test weeds on DIMBOA in shoots of wheat in pot culture. Capital letters and small letters denote the significance of difference at the level of $P_{0.05}$ and $P_{0.01}$ by LSD. Different letters indicate significant difference and same letters indicate non significance.

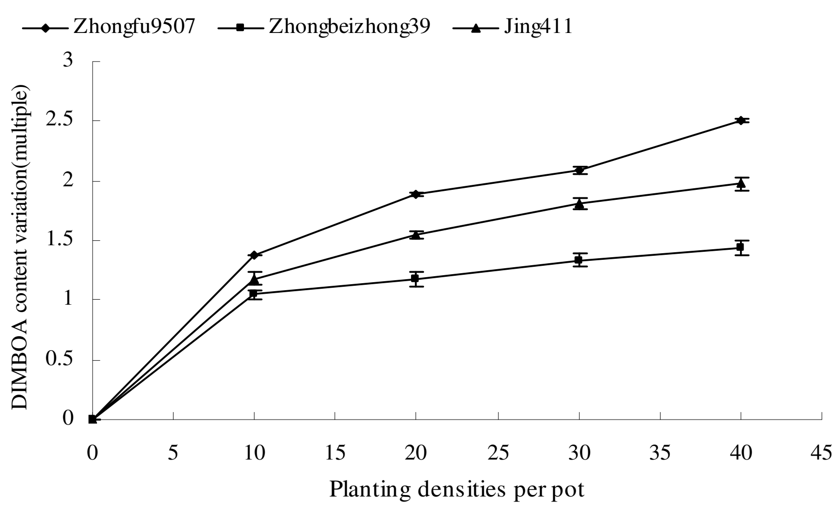

Figure 5. Induction effects of *Digitaria sanguinalis* on DIMBOA in wheat seedling at different densities in mixed-planting in pot culture.

Table 1. The correlation between the density of large crabgrass and DIMBOA content and increased multiple of DIMBOA content

Regressive model / Wheat cultivars	Correlation between density and DIMBOA content		Correlation between density and increased multiple	
	Linear equation	Logarithmic equation	Linear equation	Logarithmic equation
Zhongfu9507	y=4.053x+121.620 r=0.975	y=86.731lnx-45.665 r=0.985	y=0.036x+0.072 r=0.988	y=86.731lnx-45.665 r=0.985
Zhongbeizhong39	y=1.043x+71.165 r=0.996	y=22.073lnx+28.886 r=0.981	y=0.011x-0.081 r=0.990	y=0.237lnx-0.528 r=0.957
Jing411	y=0.688x+25.361 r=0.985	y=14.979lnx+25.361 r=0.985	y=0.026x-0.030 r=0.985	y=0.573lnx-1.146 r=0.999

Although there was a lack of effective supply of exogenous nutrients, but the DIMBOA content of aerial parts in pot culture experiments was higher than in hydroponic experiments. Compared the two matrixes, the gas-permeating ability in vermiculite was higher and bring more active oxygen. The DIMBOA existed mostly in the seedlings of wheat, thus the effective supply of active oxygen may produce more secondary metabolite DIMBOA in wheat seedlings. Thus the gas-permeating characteristics of cultivating matrix may be an important factor to influence the total amount of DIMBOA produced.

Densities: Induction effects of *Digitaria sanguinalis*

All mixed density levels of *Digitaria sanguinalis* L. showed good induction effects, when planted with 3 wheat cultivars. The induction effects of *Digitaria sanguinalis* L. on aerial parts of potted wheat seedlings are shown in Figure 5. In certain densities, good linear and logarithmic correlation was found between the planting density of *Digitaria sanguinalis* L. and the DIMBOA content and increased in multiples (the regression model and related coefficient, Table 1). *Digitaria sanguinalis* L. have inducting effects on the synthesis of DIMBOA in wheat seedlings thus changes in the density of plants with inducing activity, may develop crops resistance to pathogens.

CONCLUSIONS

The hydroponic and pot culture experiments showed that DIMBOA synthesis in both exudates and aerial parts of wheat seedlings was significantly induced by *Digitaria sanguinalis* L.. *Avena fatua* L. and *Amaranthus retroflexus* L.. DIMBOA content in shoots increased with the increase in planting density of *D. sanguinalis,* which was further verified by the induction effects of 3 weeds on DIMBOA synthesis. Weeds may not be able to induce fast and specially the expression of wheat as common chemical inducers, and should need a longer delay period to reach obvious effects. However, the role of weeds to produce DIMBOA as a kind of adversity factor can not be denied. These conclusions provided the theoretical and technical support to breed resistant wheat cultivars to control weeds.

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