PHYTOTOXICITY OF INDOLE ALKALOIDS FROM CEREALS

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ABSTRACT

A test with the microalga *Chlorella vulgaris* was used to evaluate the allelopathic potential activity of indole alkaloids present in cereals. Gramine, the main indole alkaloid present in barley shows the highest toxicity. A model mechanism of action for auxin was used to analyze the structural effect on the observed toxicity.

Germination inhibition on seeds and shoot length inhibition activities of gramine on barley, rye, oat, wheat, lettuce cultivars and the weed *Lollium rigidum* were measured. Results are discussed in relation to the phytotoxic selectivity of gramine on the seeds germination. In addition, the toxicity of barley aqueous extracts on the germination of oat seed was also determined. Phytotoxicity of the extracts is in agreement with the phytotoxicity of pure gramine.

Key words: indole alkaloids, allelopathy, phytoactivity.

RESUMEN

El potencial alelopático de alcaloides indólicos presentes en ciertas especies de cereales fue evaluado contra la microalga *Chlorella vulgaris.* Gramina, el principal alcaloide indólico presente en cebada mostró la mayor actividad. Un modelo mecanístico de acción para auxinas fue utilizado para analizar el efecto estructural en la toxicidad observada.

Se midió la inhibición de la germinación de semillas y el desarrollo de plántulas de cebada, centeno, avena, trigo, lechuga y la maleza *Lollium rigidum* por la acción de Gramina.

Los resultados son discutidos en relación a la fitotoxicidad selectiva de Gramina en la germinación de semillas. Además, se determinó la toxicidad de extractos de cebada en la germinación de semillas de avena. La fitotoxicidad de los extractos está de acuerdo con la fitotoxicidad de la Gramina pura.

Palabras claves: alcaloides indólicos, alelopatía, fitotoxicidad.

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INTRODUCTION

Secondary metabolites with toxic properties are thought to protect plants against pest and pathogens. In cereals of great agricultural importance, hydroxamic acids derived from 1,4-benzoxazin-3-one **(6)** (Fig. 1) present in maize, wheat and rye, have been suggested to protect the plants against bacteria, fungi and insects (Sicker *et al*,. 2000; Sicker and Schulz, 2002; Copaja *et al.*, 2006; Bravo *et al.*, 2004). Indole Alkaloids such as 3-N,N-dimethylaminomethyl indole (gramine) **(1)**, Tryptamine **(2)**, 5-methoxy-tryptamine **(3)**, 5-methoxy-N,N-dimethyl tryptamine **(4)**, and N_W-methyltryptamine **(5)** (Fig. 1) are present in various species of gramineae, leguminosae and other plant families (Miyagawa *et al.*, 1994; Corcuera, 1993; Argandoña, 1987). They cause various deleterious effects on mammals, insects, fungi and bacteria (Corcuera, 1984; Matsuo *et al*,. 2001; Arnold and Hill, 1972; Pastuszewska *et al*,. 2001; Ishikawa and Kanke, 2000), which suggests a general role of these compounds against herbivores, pest and pathogens.

Other beneficial role of the secondary metabolites for the plants themselves arises from the phytotoxicity against competitive plants. Allelopathic interactions between individuals of different plant species or those of the same species are caused by plant-produced allelochemicals. Once released into the environment, they can influence germination, growth and development of neighboring plants either negatively or positively (Torres *et al.*, 1996). Research in allelopathic interactions have been focused between agricultural crops and weeds, as an option on the development of integrated weed management strategies, reducing environmental effect and cost of crop protection (Batisch *et al.*, 2001). For instance, allelopathic activity of decomposing straw of wheat and oat on some crop species has been reported (Dias, 1991). Allelopathic potential of rye (Putnam and De Frank, 1983; Sicker *et al.*, 2000; Burgos and Talbert, 2000) and rice (Chou, 1980; Ahn and Chung, 2000) has been extensively studied. Allelochemicals such as phenolic acids, coumarines, hydroxamic acids and alkaloids have been reported to exist in these cereals.

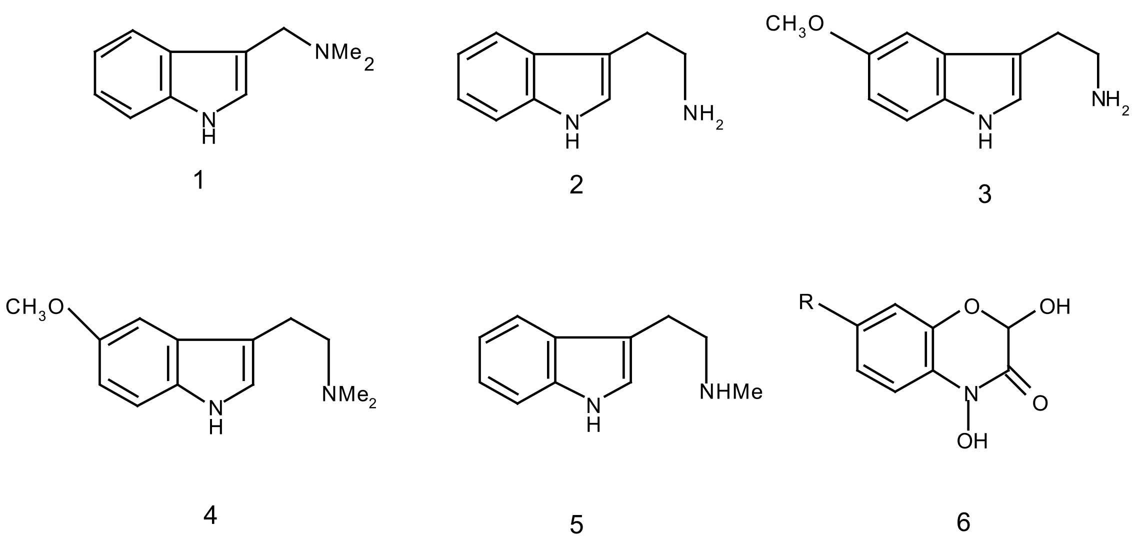

Figure 1. Indole alkaloids (**1-5**) and hydroxamic acids structures (**6**)

Barley (*Hordeum vulgare L.*) is a smother crop, which can suppress the growth of weeds by competition with environmental resources (Overland, 1966). Competitiveness may arise from different allelochemicals present in barley (Baghestani *et al.*, 1999).

Allelopathic potential of natural occurring indole alkaloids is not completely known. Therefore, to gain a deeper understanding of the phytotoxicity of these metabolites, we report in this work the antialgal activity of indole alkaloids against the alga *Chlorella vulgaris*, one of the most common used species in microalga toxicity test (Rioboo *et al.* 2002). Phytotoxicity of gramine was measured on the germination and shoot growth of a weed and competitive crop seeds. In addition, phytotoxicity of aqueous extracts of barley on oat seeds was evaluated.

MATERIAL AND METHODS

Chemical: Gramine, tryptamine, N_w-methyl tryptamine and 5-methoxy-N, N-Dimethyltryptamine were purchased from Aldrich Chemical Co.

Antialgal test: Test compounds were dissolved in nutrient growth media (Gibco) with the aid of either ultrasound or gentle heating. *Chlorella vulgaris* from Laboratory of Microbiology, Faculty of Science, University of Chile, Santiago, Chile, was ground in nutrient growth medium. In vitro serial dilutions were prepared in the concentration range from 30 to 1000 μg mL^{-1}, with increments of 50 μg mL^{-1}. This increment decreased to values from 10 to 20 μg mL^{-1} in the region close to the I_{50} values. Samples were incubated at 25°C for 10 days in test tubes containing 4.0 x 10^4 colony forming units (CFU) with continuous cold white fluorescent light with an intensity of 200 ft-c. The growth of *C. vulgaris* was assessed by turbidity measured spectrophotometrically at 600 nm.

Approximate I_{50} values were obtained from the percentage inhibition according to: $I\% = 100\,(T_s - T_c)/(100 - T_c)$ were T_s is the sample transmittance and T_c the control transmittance.

Germination assays: 20 barley (*Hordeum vulgare L.*), rye (*Secale cereale L., c.v. tetra*), wheat (*Triticum durum*), oat (*Avena sativa*), *Lollium rigidum* and 45 lettuce (*Lactuca sativa*) seeds were uniformly placed on Petri dishes covered with cotton (five Petri dishes by each specie). In order to maintain individual gramine concentration, each plate was watered with 8 mL of an aqueous solution of 0.57and 1.4 mM of gramine. Then, the plates were sealed and incubated at 25± 2°C in an 8h: 16h light:dark cycle for six day. Controls were incubated only with water. Each assay was performed three times. After 6d sowing, germination inhibition of seeds and growth inhibition of shoots were expressed as percentage of the control.

Extract from barley and bioassay

Barley seeds were planted in plastic pots containing sterile soil and were cultivated at 25 ± 2°C under continuous cold white fluorescent light with an intensity of 200 ft-c for 12 d. 38 g of grown barley shoots tissue were macerated and diluted in 100 mL of ethanol and allowed to stand for 24 h at room temperature. The macerated was filtered with cheesecloth and evaporated to dryness under reduced pressure. The remaining residue was dissolved in 83 mL of distilled water. Aqueous solution was centrifuged at 4000 g for 25 min. Serial aqueous solutions were prepared in the range of 0.55 to 0.14 mM of gramine from supernatant. Concentration of gramine was determined by HPLC method as previously described (Matsuo *et al.*, 2001). Germination of oat seeds in Petri dishes treated with aqueous solutions from supernatant were developed under the same conditions described as before. After 6d sowing germination inhibition of seeds were expressed as percentage of the control.

RESULTS AND DISCUSSION

Microalgae respond rapidly to environmental changes owing to their short generation time. Green microalgae such as *Chlorella* are taxonomically classified as plants bearing some similarity to higher plants. For this reason, microalgae tests may be used to evaluate the herbicidal activity against higher plants. Phytotoxicity of indole alkaloids were measured against the fresh water green alga *Chlorella vulgaris*. I_{50} values are shown in Table I.

Table 1. Antialgal activity of indole alkaloids against *C. vulgaris*

Indole alkaloids	I_{50} (μg/mL)
Gramine	65 (0.35)
Nw-methyltryptamine	100 (0.57)
5-methoxy, N, N, dimethyl tryptamine	110 (5.4)
Tryptamine	500 (3.1)

Values in parenthesis show mM concentration. Each value corresponds to the mean of three samples; replicate values show errors below 6% in all cases.

In the concentration range studied (30-1000 μg mL^{-1}), all the tested compounds displayed toxic effects against *C. vulgaris*. Gramine displays the highest activity and tryptamine is the less active alkaloid. Hansch *et al.* (1963) proposed a theory to rationalize relations between chemical structure and biological activity of auxins. Their hypothesis assumes that auxins with an aromatic ring and a side chain react with a plant substrate via two points, one on the side chain and the other one on the aromatic ring. Studies on the antialgal activity of indole alkaloids agree with this model. I_{50} values suggest that, part of phytotoxicity should arise from changes on the length of the side chain and changes on the structure of the amino group. Tryptamine, the less active alkaloid, has two carbon atoms on the side chain without a methyl group in the amino moiety. Gramine, the most active compound, has one carbon atom and a dimethylated amino group. Tryptamine derivatives (mono or dimethylated amino groups) show intermediate activity. Furthermore, more derivatives will be needed to be synthesized in other to clarify the role of the side chain in the phytotoxicity of indole alkaloids.

Phytotoxicity depends on the dose and target species. By the other hand, abiotic and biotic factors can trigger the allelopathic potential of a plant. The effectiveness of an allelochemical is therefore, considered as highly dynamic. Gramine shows the highest antialgal activity; for this reason it was selected to evaluate the phytotoxicity on wheat (*T. durum*), Rye (*S. secale L.*), barley (*H. vulgare L.*), oat (*A. Sativa*) lettuce (*L. sativa*) and the weed *Lollium rigidum*.

Phytotoxicity was measured from seeds germination and shoot length indicators. In Table 2 the effect of aqueous solution with two different concentrations of gramine on the germination and shoot length for five cultivars and one weed are showed. Germination inhibition indicator (%) displayed more diverse values on the competitive cultivars and allowed a better evaluation of the selective phytotoxicity of gramine. Germination of barley and rye seeds were not inhibited by the two concentrations of gramine studied. Gramine is the main indole alkaloid present on barley that can be released into the environment (Argandoña *et al.*, 1987). Then, this result suggests that barley has not autotoxicity from gramine and this cereal does not interfere germination of competitive rye cultivar. Germination of wheat and oat seeds was the most inhibited at the two gramine concentrations. Oat was the less tolerant toward gramine.

On the other side, gramine shows a moderate and similar toxicity on lettuce and *Lollium rigidum*, two small-seeded spe-

Table 2. Inhibitory effect (%) of aqueous solution of gramine, on germination seeds and shoot length on barley, rye, *Lollium rigidum*, lettuce, wheat and oat.

Cultivars	Germination Inhibition (%)		Shoot length Inhibition (%)	
	0.57 mM	1.4 mM	0.57 mM	1.4 mM
Barley (*H. vulgare* L.)	0.0	0.0	2.8	14.9
Rye (*S. secale* L.)	0.0	0.0	9.6	29.2
Lollium rigidum	10.0	18.0	17.2	22.0
Lettuce (*L. sativa*)	10.3	15.8	3.5	17.5
Wheat (*T. durum*)	22.9	23.4	6.6	21.6
Oat (*A. sativa*)	52.6	63.1	7.8	10.9

Each value corresponds to the mean of three samples; replicate values show errors below 5% in all cases.

cies. These results could indicate a more diverse phytotoxic selectivity of gramine on the large-seeded crops. Shoot elongation indicator displayed a moderate and less diverse value on all species. *Lollium rigidum* showed the higher shoot length inhibition (17.2%) at the lower gramine concentration. Rye displays the higher shoot length inhibition effect (29.2%) at the higher gramine concentration studied.

These results may reflect the allelopathic potential of natural occurring indole alkaloids, although the concentrations used are probably greater than in the field. But, the observed effects occur within the range of concentration in which gramine is found in plants (0.2 to 1.6 mmol/Kg Fr wt.) (Matsuo *et al.*, 2001; Argandoña *et al.*, 1987). Part of phytotoxicity in the field can be arising when the allelochemicals are released during the decomposition of competitive cultivars. Therefore, testing the effects of extracts of plant materials should be reasonably adequate assay to compare pure and natural phytotoxic chemical. Gramine is the main indole alkaloid from barley and our results showed that the higher gramine toxicity is produced on oat seed germination, therefore, phytotoxic activity of barley extracts were measured on oat seeds germination. Table 3 shows the germination inhibition (I%) of a series of aqueous solutions in the concentration range of 0.55 to 0.14 mM of natural gramine. Toxic effect decreased in same proportion of a decrease in gramine concentrations. The higher concentration of natural gramine solution (0.55 mM) is similar at the lower concentration of pure gramine solution (0.57 mM) used in the above experiment (Table 2). Both closely displayed toxic effect on the seeds germination (57.0% and 52.6% respectively). According to these results the phytotoxicity of gramine from barley extracts is preserved.

Table 3. Inhibitory effect (I%) of aqueous extracts from barley (*H. vulgare* L.) shoots with different concentrations of gramine on the germination of oat seeds.

[Gramine] mM	Germination Inhibition (I%)
0.55	57.0
0.27	50.0
0.18	31.3
0.14	25.0

Each value corresponds to the mean of three samples; replicate values show errors below 5% in all

Vainillic and *o*-coumaric acids along with scopoletin have been suggested that maybe responsible for the allelopathic effects of *H. vulgare* (Baghestani *et al.*, 1999), our results strongly suggest that part of the allelopathy of barley on competitive cultivars could be also related to the content of gramine in the plant.

CONCLUSION

Indole alkaloids in cereal species showed toxic activity against microalgae *C. vulgaris.* Gramine, the main indole alkaloid in barley displayed the highest activity in the studied series. Phytotoxicity measured from seeds germination and shoot length indicators allowed conclude that barley has not autotoxicity from gramine. Moreover gramine does not inhibited germination of rye cultivar. Gramine displayed the highest activity in the germination of wheat and oat seeds. Also gramine displayed a moderate and similar toxicity in the germination of the small-seeded species lettuce and *Lollium rigidum.* Shoot elongation indicator is moderate and not significant in all species. On the basis of theses results, an allelopathic potential of the natural occurring indole alkaloids is inferred. This is supported from the toxic effect observed of aqueous extract of barley fresh shoot in the germination of oat seeds. Phytotoxic activity is in agreement with the content of gramine in the extracts.

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