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Scientia Horticulturae 116 (2008) 330–336
SCIENTIA HORTICULTURAE
www.elsevier.com/locate/scihorti

Allelopathic effect of ginger on seed germination and seedling growth of soybean and chive

Chun-Mei Han, Kai-Wen Pan *, Ning Wu, Jin-Chuang Wang, Wei Li

Chengdu Institute of Biology, Chinese Academy of Sciences, Chengdu 610041, China

Received 28 March 2007; received in revised form 28 December 2007; accepted 10 January 2008

Abstract

The rhizome, stem and leaf aqueous extracts of ginger were assayed at 10, 20, 40, and 80 g l^{-1} for their effects on seed germination and early seedling growth of soybean and chive. All aqueous extracts at all concentrations inhibited seed germination, seedling growth, water uptake and lipase activity of soybean and chive compared with the control, and the degree of inhibition increased with the incremental extracts concentration. The degree of toxicity of different ginger plant parts can be classified in order of decreasing inhibition as stem > leaf > rhizome. The results of this study suggest that rhizome, stem and leaf of ginger contain water-soluble allelochemicals which could inhibit seed germination and seedling growth of soybean and chive. The rhizome is the main harvested part of ginger. The residue (mainly stems and leaves) of the ginger plant should be removed from the field so as to diminish its inhibitory effect. Further work is needed to specify and verify the allelochemicals produced by this plant. The results of this study suggest that ginger allelochemicals are heterotoxic, and thus intercropping should not be practiced using ginger.

Keywords: Ginger; *Zingiber officinale* Rosc.; Soybean; *Glycine max* (L.) Merr.; Chive; *Allium schoenoprasum* L.; Germination; Seedling growth; Water uptake; Lipase activity; Allelochemicals

1. Introduction

Ginger (*Zingiber officinale* Rosc.) is an important horticultural crop in tropical Southeast Asia. It produces a pungent, aromatic rhizome that is valuable all over the world either as a spice or herbal medicine (Guo and Zhang, 2005). Many scholars have reported that ginger has the function of relieving the severity of nausea and vomiting during pregnancy (Vutyavanich et al., 2001; Portnoi et al., 2003). Additionally, it has been reported that crude extract of ginger rhizome can reduce rat paw and skin edema (Penna et al., 2003). However, ginger yields are low when this species is cultivated consecutively for years on the same land and rotated with other crops for at least 3 years. Under such regimes, emergence and early growth of ginger are inherently slow and considerable time elapses between sowing and the development of foliage cover (Lee et al., 1981). In addition, ginger normally propagates with a low proliferation rate (about 10–15 buds from one plant each year) by its rhizome, and is easily infected by soil-born pathogens such as bacterial wilt (*Pseudomonas solanacearum*), soft rot (*Pythium aphanidermatum*), and nematodes (*Meloidogyne* spp.), which cause heavy losses in yields (Guo and Zhang, 2005). Autotoxicity is a type of intraspecific allelopathy, where a plant species inhibits the growth of its own kind through the release of toxic chemicals into the environment (Singh et al., 1999). This phenomenon has been demonstrated in a number of crop plants such as annual crops like wheat, rice, maize, mungbean grown in monocultures, forage crops like alfalfa and clover, and oil crops such as sunflower and rapeseed, and others like asparagus, sugarbeet, cucumber, carrot, coriander, cumin and fennel. In most cases, it is related to the repeated sowing of monocultures leading to soil sickness (Singh et al., 1999). Autotoxicity may be one reason for lower production rates of successive crops of ginger. In China, the Shandong Province, Zhejiang Province, Guangdong Province and Sichuan Province are the main production areas of ginger. The use of other crops may alleviate the problem of autotoxicity caused by repeatedly planting ginger monocultures. Soybeans (*Glycine max* (L.) Merr.) and chives (*Allium schoenoprasum* L.) are two promising crops for intercropping with ginger.

Allelopathy is defined as any direct or indirect positive or negative effect of one plant on the other (including the

* Corresponding author. Tel.: +86 28 85248450.
E-mail addresses: hanchunmei@tom.com (C.-M. Han), pankw@cib.ac.cn (K.-W. Pan).

0304-4238/$ – see front matter
doi:10.1016/j.scienta.2008.01.005

microbes) through the release of chemicals into the environment (Rice, 1984). It plays a significant role in agro-ecosystems, and affects the growth, quality and quantity of the produce (Kohli et al., 1998; Singh et al., 2001). A number of plant species have been reported to have an allelopathic effect on other plant species (Mallik, 1987; Martin and Smith, 1994; Kato-Noguchi, 2003; Jefferson and Pennacchio, 2003; Oueslati, 2003; Djurdjevic et al., 2004). Allelochemicals produced by one crop species can influence the growth, productivity, and yield of other crops or the same crop (Batish et al., 2001). The avoidance of allelopathic effects between crops, or the exploitation of beneficial interactions in a rotation or a mixed cropping system may have direct bearing on the crop yield (Rizvi et al., 1992). In the present study, the allelopathic effects of ginger rhizome, stem and leaf aqueous extracts were examined to determine if inhibitory or stimulatory effects of ginger extracts influence seed germination and seedling growth of two crops (soybean and chive) that are commonly intercropped with ginger.

2. Materials and methods

2.1. Location

Ginger plants were collected from fields (29°15′N, 103°47′E, 510 m altitude) in Sichuan Province, Leshan City, China, in August 2005. The experiment was carried out at the ecological center, Chengdu Institute of Biology, Chinese Academies of Sciences, from July to October 2006.

2.2. Preparation of extracts

Fresh ginger plants were separated into leaves, stems and rhizome. The stems and leaves were chopped into 1 cm long pieces and rhizomes were chopped into 0.5 cm thick slices. The components were then oven dried at 60 °C for 5 days. Eighty grams of dried rhizomes, stems and leaves were respectively extracted by soaking in 1 l deionized water at 25 °C for 24 h in a shaker to give a concentration of 80 g dry tissue l^{-1} (g l^{-1}). The extracts were respectively filtered through four layers of cheesecloth to remove the fiber debris, and centrifuged at 3000 rpm for 4 h (Chon et al., 2002). The supernatant was filtered again using a 0.2-mm filterware unit. Fresh stock extracts were kept in a refrigerator at 2 °C until used.

2.3. Seed bioassay

Stock extracts (rhizome, stem and leaf) were diluted with sterile distilled water to give final concentrations of 0, 10, 20, 40 and 80 g l^{-1}. Seed germination tests were conducted for each extract as follows: 30 soybean and chive seeds were surface sterilized with 5.25% (w/v) sodium hypochlorite solution for 15 min, rinsed three times with distilled water and were evenly placed on two-layer filter paper in sterilized 9-cm Petri dishes. Due to differences in the size of soybean and chive seed, 15 and 5 ml of extract solution were added to Petri dish containing soybean and chive seeds, respectively. Distilled water was used as a control treatment. All Petri dishes were placed in a dark room at 25 °C. Treatments were arranged in a completely randomized design with three replications. Germination was determined by counting the number of germinated seeds at 24-h intervals over a 6-d (soybean) and 17-d (chive) period and expressed as total percent germination. Germination was deemed to occur only after the radicle had protruded beyond the seed coat by at least 1 mm. Radicle and hypocotyl lengths of soybean and chive seedlings were measured 6 d and 17 d after germination, respectively. After measuring the radicle and hypocotyl lengths, the dry weights of seedlings were determined by drying the plant material in an oven at 60 °C for 24 h prior to weighing. The inhibitory or stimulatory percent was calculated using the following equation given by Chung et al. (2001):

$$\text{Inhibition}(-)\ \text{or stimulation}(+)\ \text{percentage}\ (\%) = \left(\frac{\text{extracts} - \text{control}}{\text{control}}\right) \times 100.$$

2.4. Water uptake

Approximately 1-g samples (W_1, the original seed weight) of soybean and chive seeds were separately soaked for 4, 8, 12, 16 and 20 h in the aqueous extracts. Distilled water was used as the control treatment. At 4-h intervals, seeds were taken from the solution, blotted for 3 h between two folds of filter paper, and weighed (W_2, the final seed weight). This is based on the method given by Turk and Tawaha (2003). Water uptake percentage is expressed as follows:

$$\text{Water uptake}\ (\%) = \frac{W_2 - W_1}{W_1} \times 100.$$

2.5. Assay of lipase activity of soybean and chive seed

After measuring water uptake, the seeds were shelled, grounded and extracted for 1 h with 0.1 M Tris–HCl buffer (pH 7.0) at 25 °C. After centrifugation, the soluble proteins in the supernatant were fractionated and precipitated with 50% (v/v) and 80% (v/v) ethanol at 4 °C. The precipitates obtained were dissolved in 0.1 M Tris–HCl buffer (pH 7.0). To prepare an acetone powder, the germinated seeds were ground in ice-cold acetone and then washed several times with the cold acetone (Ncube et al., 1995). Lipase (glycerol ester hydrolase, E.C. 3.1.1.3) activity was assayed using a modification of the titrimetric method of Khor et al. (1986). The assay mixture contained 5 g of substrate, 2.5 ml of hexane to solubilize the oil, and 0.5 g of the crude enzyme. The mixture was incubated at 30 °C for a period of 1 h with continuous stirring, using a magnetic stirrer. At the end of the incubation, 25 ml of acetone–ethanol (1:1, v/v) were added to stop the reaction and to extract the free fatty acids (FFAs) liberated. The FFAs in the mixture were then estimated by direct titration with 0.01 M NaOH using phenolphthalein as an indicator of lipase activity. Lipase activity was expressed as the percent FFAs liberated after 1 h