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Commentary

Exogenous amino acids inhibit seed germination and tubercle formation by *Orobanche ramosa* (Broomrape): Potential application for management of parasitic weeds

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Abstract

Parasitic plants are among the most problematic weeds that are responsible for major losses to many crops. Early growth stages, such as seed germination stimulated by host root exudates and tubercle development, are key phases for the development of these parasites. Inhibition of these early phases by naturally occurring compounds could be a general strategic option for management of parasitic plants. In this study, we report that certain natural amino acids cause severe physiological disorders of germinating broomrape seeds. In particular, methionine was able to inhibit almost totally the germination of seeds of *Orobanche ramosa* when applied at a concentration of 2 mM. Applied to tomato roots, methionine strongly reduced the number of developing tubercles of the parasite. These findings suggest that: (1) appropriate amino acids applied exogenously to a root zone might result in control of parasitic plants such as *Orobanche* sp.; (2) amino acid excreting microbes introduced into the crop rhizosphere might control root parasitism; and (3) amino acid producing broomrape pathogens might be selected in order to enhance their virulence.

Keywords: Amino acids; Parasitic plant management; Biological control of weeds; *Orobanche ramosa*; Natural compounds; Amino acid excretion; Virulence enhancement; Plant pathogens; Mycoherbicides; Frenching disease; *Fusarium oxysporum*; Methionine

1. Introduction

Parasitic species of the genus *Orobanche*, commonly called broomrapes, are among the worst weeds (Holm et al., 1997). They are responsible for severe losses to vegetable, legume, and sunflower crops, by their interference with water and mineral intake and by affecting photosynthate partitioning. Difficulties in broomrape control are due to the production of a large number of seeds that can lay dormant in the soil for many years, only germinating if stimulated by host root exudates. Once a seed is stimulated, it produces a germ tube that grows in the direction of the host plant. The germ tube develops a haustorium when it comes in contact with the host plant, penetrates the root, and forms a tubercle. The underground tubercle formation and development is the most damaging phase during which the parasite withdraws water, nutrients, and photosynthates from the host. By the time the parasite emerges from the soil, most of the damage to the host plant has already occurred. Traditional control methods are rarely effective: selective herbicides often do not differentiate between the crop and the parasite, except on herbicide-tolerant transgenic crops (Joel et al., 1995; Surov et al., 1997); solarization is costly and limited to the upper soil layers; soil fumigation, despite its effectiveness, is rarely applicable because most of the efficacious fumigants have been banned due to negative effects on the environment (Foy et al., 1989); mechanical control is not feasible because of the physical connection between the weed and the host; and biological control by pathogens cannot cur-

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rently be used because of the lack of effective organisms ready for commercialization (Boari and Vurro, 2004). Considering that the early stages of parasite development are key to the parasitic plant's lifecycle, searching for natural compounds able to interfere with these early phases could be an attractive and environmentally friendly approach for management of parasitic weeds. This approach would be especially attractive if rhizosphere microbes could be selected to overproduce and excrete such natural compounds (Sands et al., 2003).

As an example of how this approach could be effective, consider the physiological disorder of tobacco, called "Frenching disease." It is caused by saprophytic bacteria growing on tobacco roots that overproduce isoleucine which inhibits the activity of acetolactate synthase (ALS) in the plant shutting down plant biosynthesis of valine and leucine (Steinberg, 1946; Steinberg et al., 1950). The macroscopic effect of this disrupted essential protein metabolism is leaf chlorosis, wilting, and reduced plant growth. Several modern chemical herbicides also inhibit single biosynthetic enzymes in plants (including ALS) (Kishore and Shah, 1988), similarly rendering treated plants incapable of producing a metabolite essential for plant growth.

Based on these observations, we have observed that relatively high amounts of essential amino acids cause disorders in the physiological processes that occur during the germination of broomrape seeds thereby inhibiting germination, germ tube elongation, or tubercle development. Considering that amino acids are not normally toxic to humans or to the environment, and are rapidly metabolized by soil microorganisms, they could represent safe agents for management of parasitic weeds. A "Frenching disease" strategy, where a soil microbe or an invasive plant pathogen is used for in situ overproduction of an amino acid, could thus have important application in control of parasitic weeds. This paper deals with preliminary data of this unusual, promising, and attractive strategy applied to seeds of *Orobanche ramosa* L.

2. Material and methods

2.1. Seed germination assay

Toxicity of amino acids was assessed by their ability to inhibit the germination of stimulated *O. ramosa* seeds, using a modified procedure previously described by Boari and Vurro (2004). *O. ramosa* seeds, collected from infested tomato and tobacco fields in Southern Italy, were surface sterilized (1% sodium hypochlorite for 10 min), rinsed with sterile tap water, and placed on wet glass microfiber filters (GF/A Whatman) cut to fit in large glass petri dishes (16 cm diameter). Seeds were kept at 25–26 °C in the dark for 3 weeks. Filters were then cut in small pieces, each containing approximately 100 seeds. Two pieces of the cut filters were placed in small petri dishes (6 cm diameter) containing another filter disk that had been moistened with 1 ml of the amino acid solution at the desired concentration. This filter disk also contained 5 μg/ml of the synthetic strigolactone analog stimulant GR24 (kindly supplied by Professor Jonathan Gressel, Weizmann Institute of Science, Israel) (Wigchert et al., 1999, and references cited therein). Plates were again kept at 25 °C in the dark. Three replications were used for each compound assayed. After 4 days, the number of germinated seeds was determined and compared with that of the control prepared with the same procedure as the treatments but without amino acids. Shape and length of germ tubes were also observed. In a preliminary screening, the L forms of 13 amino acids (at 2 mM) were tested in this assay: alanine, arginine, cysteine, glutamine, glycine, histidine, leucine, lysine, methionine, proline, serine, threonine, and valine. The data were analyzed by analysis of variance according to scheme of randomized blocks. Each mean was directly compared with the control, using the Bonferroni test, with $\alpha = 0.05$, using the NCSS software (Number Cruncher Statistical System, NCSS, UT, USA).

Three of the amino acids that were among the most efficient in the preliminary screening (arginine, lysine, and methionine) were then assayed at five concentrations ranging from 0.5 to 4 mM. These were selected not only because of their ability to inhibit the germination, but also considering other effects, such as modification of the shape and the length of the germinated seeds. For each experiment, three replications were prepared for each treatment. The control was prepared using the same procedure without exogenous amino acid. The data were analyzed by analysis of variance according to a two-way completely randomized design.

2.2. Effect of the timing of application

The effect on seed germination of the timing of the application of methionine with respect to that of application of the germination stimulant was evaluated with the procedure illustrated in Table 1. At time = 0, filter pieces containing the seeds were placed on filter disks containing methionine (at 2 mM), or the stimulant, or both, and left for different times of exposure (1, 2, or 3 days). After the designated exposure, the disks were carefully rinsed with distilled sterile water, to remove the compounds, and placed on new filter disks containing the stimulant, with or without methionine, according to the planned procedure. After 4 days, the extent of germination was recorded. For each treatment three plates were prepared. The data were analyzed by analysis of variance according to a completely randomized design and the means compared using the Duncan's multiple range test.

2.3. Combined application

The possibility of increasing the efficacy of amino acids or, on the contrary, suppressing their inhibitory effect, was investigated in preliminary tests using combinations of two