CERCOSPORA BETICOLA INTERACTIONS WITH AXENIC SUGAR BEET SHOOT CULTURES

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Introduction

Much of our current knowledge of the pathology and genetics of *Cercospora* and sugar beet interactions was derived from a number of pioneering studies reported in the 1970s (Ruppell, 1972; Smith and Ruppell, 1974; Ruppell and Scott, 1974; and Lewellen and Whitney, 1976). Recently, our laboratory successfully produced transgenic sugar beet plants carrying introduced genes specifying antimicrobial peptides (Snyder et al., in press). In this study, experiments were performed in an attempt to determine whether these novel genotypes could inhibit the growth of *Cercospora beticola*, the causal organism responsible for leafspot disease in sugar beet. A series of replicated *in vitro* analyses designed to detect and quantify growth inhibition, if any, gave inconsistent results. Initial results indicated the inhibition of *Cercospora* by certain of the new sugar beet genotypes, but further tests established the fact that sugar beet leaf pieces stimulated the growth of *C. beticola* on chemically defined medium. Evidently axenic shoot segments supply this phytopathogenic fungus with growth factors.

Methods and Materials

Sugarbeet genotypes

Axenic sugar beet shoots were maintained on chemically defined tissue culture medium containing Murashige-Skoog (1962) basal salts with Gamborg's B5 vitamins (1970), 1.0 mg/l pantothenate, 0.01 mg/l biotin, 0.5 g/l MES, 30.0 g/l sucrose, and 5.0 g/l agargel at pH 5.8 medium with 0.25 mg/l BAP. Novel transgenic genotypes were produced by Synder et al. (in press). The parental genotype, Rel-1, was originally obtained from Dr. Joe Saunders at MSU, East Lansing, MI.

Cercospora *strains*

Cercospora beticola strains C1, C2, F573 and H1-12 were obtained from Earl Ruppel at Fort Collins, CO, USA . Potato dextrose agar (PDA) was used for maintenance.

Co-Cultivation Tests

Incubations were at room temperature with a diurnal regime of light and dark, with light supplied by fluorescent lights. Tests for *in vitro* inhibition were conducted by aseptically transferring fungal mycelia with and without freshly excised, axenic leaf segments to deep Petri dishes containing 25ml freshly prepared sterile tissue culture medium. Approximately square leaf segments were cut out of axenic tissue culture shoots using a sterile blade. This procedure seemed desirable since many of the transgenic constructs being examined had introduced genes that are under the control of a wound-inducible promoter, such as the one for the osmotin gene.

In some experiments, fungi were placed in or near the center of the dish with and without sugar beet leaf segments present. Plant leaf segments were placed at various distances from the fungal pathogen. In some tests two large leaf segments were placed on the agar medium and one

was inoculated with *Cercospora* and the other was not inoculated.

Results and Discussion

Our first experiment, which did not employ sugar beet tissue cultures, involved the transfer of pure cultures of the fungal pathogen onto two very different culture media for growth comparison. All four *Cercospora beticola* isolates grew more rapidly and extensively on nutrient-rich potato dextrose agar (PDA) than on the chemically defined tissue culture medium (TCM) (Table 1). These results clearly indicate that, unlike PDA, TCM does not contain all of the nutrients needed to support good growth of *Cercospora*. *Cercospora* is known as a relatively slow-growing genus of phytopathogenic fungi, and one with a requirement for a number of nutrients. It is not unusual for a plant pathogen to require a variety of nutritional or growth factors. Indeed, Norman et al. (1981), when formulating a chemically defined growth medium for *Cercospora rosicola*, determined that a large number of the amino acids and vitamins found naturally in potato were needed to support good growth of the fungus.

Table 1: Colony Diameter in Centimeters of Pure Cultures of Four *Cercospora beticola* Strains After 14 days Incubation on Two Very Different Media*

Cercospora Strain	PDA	TCM
C1	3.8	1.0
C2	4.4	1.5
H1-12	4.4	1.7
F 573	4.1	1.2

* Values are the means of four replicates.

A series of *in vitro* pathogen/ sugar beet interaction studies were done next. Co-cultivation of *Cercospora beticola* with *Beta vulgaris* genotypes initially gave some interesting results which suggested some variable growth inhibition of *Cercospora* by selected novel genotypes. Apparent inhibition was observed when the distance from fungal pathogen to shoot piece was less than 1.0 cm. Further tests revealed clear evidence of the stimulation of the growth of *Cercospora* by the axenic sugar beet leaf pieces as a factor complicating the analysis of growth inhibition. For example, it was found that four 3 x 7 mm leaf segments at an equal distance of about 3 or 4 cm from the point of *Cercospora beticola* inoculation stimulated the fungus to grow to a diameter of about 3.9 cm in 14 days, a large increase over the colony size of approximately 1.8 cm on the control plate with *Cercospora* inoculation but without the presence of any axenic sugar beet leaf segments. From these studies we conclude that axenic, excised sugar beet leaf segments release, into the medium, diffusible substances that dramatically stimulate *Cercospora* growth. This seriously complicated our attempts to test transgenic sugar beets, which carried introduced genes specifying the production of antimicrobial peptides, for their ability to inhibit *Cercospora*.

Perhaps the production of antimicrobial peptides could be at least partially masked by the release of stimulatory amino acids. Since one observes inhibition, if any, only at a close distance ($\leq$ 1cm) from shoot segment to fungus, but at greater distance only clear growth stimulation is obtained, it is clear that close proximity of the shoot to fungus is required. Why? Perhaps virulence genes in the pathogen must first be induced by slowly diffusing compounds coming from the plant shoot.

Results obtained with plates on which two axenic shoot segments were placed, one directly inoculated with *Cercospora* and one uninoculated, showed that the *Cercospora* fungi grew rapidly and, within 7 days of incubation, covered the entire inoculated shoot segment. Interestingly, one novel sugar beet genotype, namely the Osm-*osm* transgenic, was evidently a very favorable substrate for the growth of *Cercospora* since leaf segments of this genotype were covered entirely by white fungal mycelia within five days of incubation compared with a 7-day incubation period required for similar fungal growth on leaf squares from axenic shoot cultures of the other transgenic sugar beet genotypes or from those of the parental genotype.

Summary

In conclusion, we discovered that axenic sugar beet shoot segments can serve as an excellent growth substrate for the *in vitro* growth of *Cercospora beticola*. We would like to study how virulence genes in the pathogen may be turned on by signal molecules from the sugar beet host plant. Greenhouse tests with intact plants are planned in order to continue testing transgenic sugar beets for their interaction with *Cercospora* pathogens.

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