Induction of *Cercospora* Leafspot Disease on Parental and Selected Transgenic Lines Carrying Antimicrobials: *New Sources of Anti-Cercospora Genes will now be used for Bioengineering Disease Resistance.*

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Abstract

The relative susceptibility of selected *Beta vulgaris* genotypes to *Cercospora* leafspot was tested under controlled humidity and temperature in a plant-growth chamber. Replicate plants of two new transgenic clones designated OOT and OsmPrS-2 and their REL-1 parental line were infected with *Cercospora beticola* and disease progression was followed. *Cercospora* leafspot symptoms in terms of the number of lesions per leaf in an infection cycle were significantly greater in the two transgenics by a factor of about 4- to 5-fold relative to the nontransformed genotype. When the area of the necrotic lesions is considered this difference is magnified by more than 200x with OOT. These results demonstrate that the presence of the introduced chimeric genes encoding small antimicrobial peptides diminishes rather than enhances *Cercospora* resistance in these particular transgenic sugar beets.

Species of fungicide-producing *Pseudomonas* will now be tested to determine whether they can serve as a new source of genes for the bioengineering of disease resistance in sugar beets. The introduction of the *Cercospora cfp* gene, for toxin export gene, into sugar beet will soon be tested as a potential effective control of leafspot. The *cfp* gene is currently being transformed into sugar beet in my lab using *Rhizobium-* (formerly *Agrobacterium-*) mediated transformation which produces only single genomic insertions.

Introduction

Plant molecular biologists have long envisioned the control of plant/microbial interactions which are diseases using bioengineered plants. Progress in some crop plants has been made but sugar beets are difficult to transform. Despite this difficulty, candidate genes, which could theoretically increase disease resistance and suitable for fusing with plant promoters, have been introduced into sugar beets. Thus far these genes include those specifying either cecropins (from moths), thionins or pathogenesis-related proteins (both from other plants). Snyder et al., (1999) reported transformation of chimeric constructs of these genes into sugar beet genotype Rel-1, obtained from Dr. Joe Saunders, USDA/ARS at Michigan State University. Rel-1 is a "biotechnology clone" Joe registered (1998) as forming highly regenerative callous tissue on B1 medium at 30^0 C in the dark. Hall et al., 1997, first reported that stomatal guard cells are evidently totipotent. Drs. Saunders, Ann Smigocki and I have been trying to devise a simple single-step procedure useful for obtaining efficient regeneration following DNA uptake, for example. A paper on that work appears elswhere in this volume (Saunders et al., 2001).

Two years ago, after spending some months trying to determine whether shoot cultures of the sugar beet transgenics already available in the lab had any anti-*Cercospora* activity *in vitro* (Kuykendall, 1999, Kuykendall and Smigocki, 1999), I decided to test plants in growth chambers for leafspot susceptibility from spores. The two genotypes evaluated were OOT and *OsmPrS2*.

Materials and Methods

Shoot cultures were first multiplied on 0.3mg/L BAP medium (MS) and then roots were generated on the shoots using NAA medium without cytokinin. At least 20 small regenerated plants were produced for each clone that we wished to examine. Reduced vigor in these lines and subsequent losses makes large numbers necessary to come out in the end with at least 10 plants of about equal vigor for each genotype. Genotypes OOT and *OsmPrS*-2 were transgenics not previously reported; in the former, a dual construct, a barley thionin gene was controlled by the osmotin promoter and an intact osmotin gene was controlled by its own promoter. *OsmPrS*-2 had the gene encoding pathogenesis-related protein "S" under the control of the osmotin promoter. Both genotypes produced relatively small plants relative to Rel1, their parent. The Rel1 plants were also developed from tissue culture shoots.

Relatively vigorous plants of both lines were moved from the greenhouse to the growth chamber prior to the inoculation study. Lee Panella supplied the leafspot-infected leaves that were the source of the spore suspensions used to inoculate the leaves by use of a small paint brush from our local hardware store. The enclosed chamber with no ventilation was used in order to maintain high humidity. Several inches of water were circulated at the bottom of the chamber using small, commercially available, submersible water pumps. For a day before and several days to a week after inoculation, small ultrasonic fog-generating devices, of the type used to simulate a natural environment in aqua-terrariums, were used to increase the humidity. It was noted that these devices also produced heat and that some of the "fog" generated was actually smoke. Day temperatures between 30-35^{o} C and night temperatures between 25-30^{0} C were maintained on a 14:10hr cycle.

Results and Discussion

Given the data available (Ingersoll et al., 1996) that the osmotin promoter is expressed in sugar beets at several times the level of the S35 promoter, I was expecting that the two transgenics would show no symptoms but that the Rel1 parent would have leafspot lesions present. But a totally unexpected result was obtained since Rel1 had only a few leafspots whereas the plants of the two particular transgenics evaluated had at least 4- to 5-fold more leafspots (Table 1), and moreover this difference was magnified by 200x when the area of the necrotic lesions was compared (Table 1). OOT was particularly vulnerable to *Cercospora*, and *OsmPrS*2, while less susceptible than OOT, was nevertheless clearly not as resistant was the parental Rel1. While these particular clones were more disease susceptible, other transgenics with antimicrobial genes may be found with greater resistance, given the investment of more research effort. The bioassays for *Cercospora* leafspot worked.

Table 1: Leafspot Symptoms on Parental and Transgenic Genotypes under Controlled Conditions (visual scoring was performed at 3 weeks post infection)

Sugar Beet Genotype	Number of lesions/leaf	Area of Necrosis or Decay
Rel1 (parent)	7 A	0.02cm^2
*OsmPrS*2	30 B	0.59cm^2
OOT	38 B	19.09cm^2

Means followed by a different letter are significantly different (P<.05).

It is important to add that valuable information was reported by Ruppel and Gaskill,1971, Smith and Ruppel, 1974, Smith and Martin, 1978, Steinkam, Martin, Hoefert, and Ruppel, 1981, and Owens and Roberts, 1992.

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