CERCOSPORA BETICOLA MATING TYPES IN THE NORTH CENTRAL USA

Gary Secor[1], Viviana Rivera[1], Judith Rengifo[1] and John Weiland[2]
[1]Department of Plant Pathology, North Dakota State University and
[2]USDA-ARS, Northern Crop Science Laboratory, Fargo, ND 58105

Introduction and Objective

Cercospora beticola the cause of leaf spot of sugar beet became a serious disease in 1981 and is endemic in several sugar beet producing areas of the US, including the Red River Valley of ND and MN. The disease reduces both yield and sucrose content of sugar beets. Control is by crop rotation, resistant varieties and timely fungicide applications. The fungicides used include triphenyl tin hydroxide (Super Tin, Agritin), thiophanate methyl (Topsin), tetraconazole (Eminent), pyraclostrobin (Headline), and trifloxystrobin (Gem), and are usually 2-4 applications are made the last half of season. Fungicide sensitivity of *Cercospora beticola* is a major concern because all fungicides used for *C. beticola* control have a history of resistance in sugarbeets or other crops

C. beticola possesses variability in fungicide sensitivity, cercosporin production and culture morphology. This diversity suggests genomic recombination as a source of the variability, which in turn raises the possibility of cryptic sex in some Cercospora (Crous). Mating types have been found in many fungi both with and without teleomorphs (Alternaria, Fusarium), designated generically as mat1 and mat2. Mating types mat1 and mat2 have recently been reported for *C. beticola* in Europe The teleomorph has not been reported for any Cercospora species (*apii*, *beticola*, *zeae-maydis*, *zeina*). If the sexual stage exists, based on comparative Internal Transpositional Sequences, it would be expected to be in genus Mycosphaerella (Crous). The objective of this study is to determine if mat1 and mat2 are present in *C. beticola* in the north central sugar beet production area of the USA.

Methods and Materials

A mat2 primer was designed directly from the sequence reported by Groenewald et al. 2006. This primer pair only weakly amplified a band from putative mat2 isolates from the US. This band was cloned and sequenced and confirmed as the mat2 idiomorph (allele). This sequence was used to make a second mat2 primer pair with better amplification ability, and shown to have a 100% sequence homology with Groenenwald reported sequence of the German type isolate. The new mat2 primer pair produced abundant PCR product from mat2 isolates, and also amplified a product of a different size exclusive to the mat1 isolates thereby providing a single primer pair capable of distinguishing mat1 from mat2. Both the mat1 and mat2 bands from US isolates were cloned and sequenced, and the sequences matched 100% the gene sequences reported by Groenewald for mat1 and mat2 from a German isolate of C. *beticola*.

These new primer pairs were used to determine the frequency and distribution in the mating type genes in *C. beticola* isolates collected from naturally infected plants in ND and MN.

Eleven fields with *C. beticola* were sampled in ND and MN by collecting five leaves per field. A composite sample was made by collecting spores from five spots from each leaf (the composite sample represented 25 spots). Ten single spores from each field sample were grown on CV8 medium for 15 days at 25°C under fluorescent light. A total of 110 single spore isolates were tested for mating type using our mat2 primer pair.

A leaf with *C. beticola* symptoms was collected from eight fields, and ten single spores were collected separately per leaf spot; collections were made from five leaf spots per leaf. The single spore isolates were grown on CV8 medium for 15 days at 25C under fluorescent lights. A total of 400 isolates were tested to determine mating types using our mat2 primer pair.

DNA was extracted from 200 mg of fungal mycelium of each isolate using a FastDNA Kit (Bio101, Carlsbad, CA) according to the manufacturer's instructions. PCR was performed in a total volume of 25 ul containing 3 µl diluted DNA, 2.5 µl of 1 X PCR buffer, 0.5 µl dNTp, 0.125 µl AmpliTaq Gold (5 units/reaction), 2 µl MgCl2, 3.2 µl of each primer Cbmat2 and 10.5 µl H2O. Amplification reactions were done on a GeneAmpPTC 200 PCR system, and PCR products were separated by electrophoresis and stained with ethidium bromide.

Results and Conclusions

- A new primer pair was developed to detect mating types of *C. beticola* in a single PCR reaction
- mat1 and mat2 mating types are present in *C. beticola* in the north central US sugar beet production area of ND and MN
- The sequence of mat1 and mat2 genes found are identical to those reported in samples of *C. beticola* from Germany based on gene sequence
- The usage of the terms mating type 1 and mating type 2 nomenclature is only based on molecular data
- The presence of mat1 and mat2 genes is suggestive of mating, not proof
- Proof of mating will only be indicated by the discovery of the sexual stage
- Different mating types occur in the same field and in different *C. beticola* spots on the same leaf
- Only a single mating type is present within a single *C. beticola* spot
- The presence of *C. beticola* mating types may explain, at least in part, the variability observed in *C. beticola*