

Development and Characterization of Microsatellite Markers in *Sclerotinia trifoliorum*

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Outline

- Introduction
- Objectives
 - Development of microsatellite markers specific to *S. trifoliorum*
 - Characterization of microsatellite markers
- Summary

Introduction

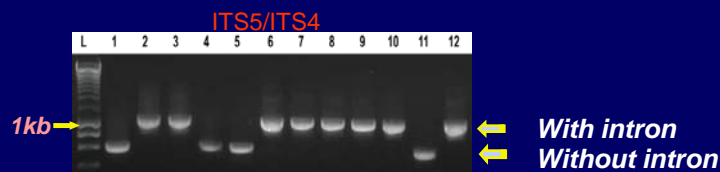
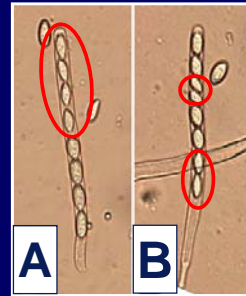
Stem & crown rot of chickpea



Picture courtesy of W. Chen

Characterization of *S. trifoliorum*

- Ascospore size dimorphism
- ITS sequence (Holst-Jensen *et al.*, 1998)
- Presence of group I introns (Powers *et al.*, 2001)



- Intra-specific differences observed among isolate

(Njambere *et al.*, 2008, Plant Dis. 92:917-922).

Studies on diversity and population structure of *S. trifoliorum*

- rDNA intron haplotypes
- Mycelial compatibility groups
- Microsatellite markers

Objective

Develop and characterize microsatellite markers specific to *S. trifoliorum*

Why microsatellite markers?

- Tandem repeats 2-6bp abundant throughout the genome
- Neutral markers and highly variable within various populations
- Discrete alleles, allelic ladders simplify interpretation
- Rapid processing is attainable

Characterization of microsatellite markers

Locus	Primer sequence (5'-3')	Repeat motif	T(°C)	Size range (bp)	H _E
ST2-2	F:CGTAATTGAGGGGAAGAGG R: GCCTAGCTAGCAGAATCAACC	(AAG) ₁₄	58	156-174	0.38
ST4-1	F:GATATTCCATGCCAAACAGG R: GGTGACTCCATAACCATCTCG	(CATA) ₁₁	55	243-263	0.49
ST4-4	F:CGGAGTAGGTAGTTCATTACGG R: GCTGCCTCTACTTCTGTCACAT	(GTAT) ₁₄	55	160-184	0.65
ST4-6	F:AGTGTGAGGCTGGTTGGTG R: ACCAACAATCCAATGACAGC	(TG) ₃₆	58	149-179	0.44
ST2-13	F:AGTCGTTGGCTCCTCCTTTC R: TGGATGGAATTCTGGGGTAG	(TC) ₈ CT(TC) ₂ GT(TC) ₈₉	58	136-158	0.80
ST3-6	F:GGTTTTGGTTTTGGCTTTGT R: CGAAGCGTACATTCATTTCAAT	(AGTG) ₁₁	58	96-120	0.658

Characterization of the 33 microsatellite markers

Characteristics	Range	Mean
Allele number per loci	2-8	4.4
Expected heterozygosity	0.38-0.80	0.63

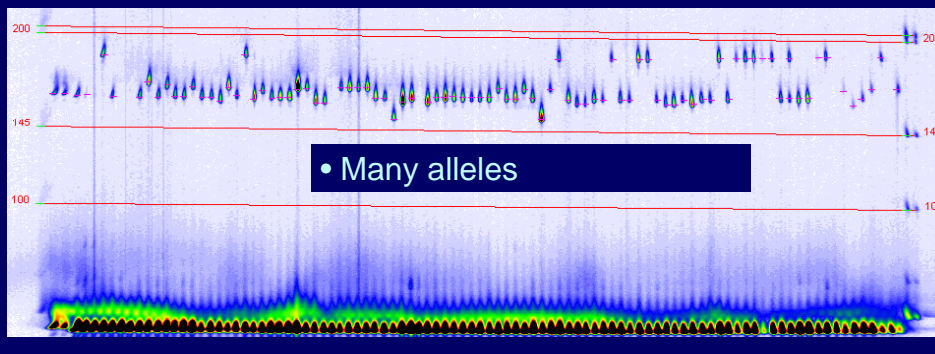
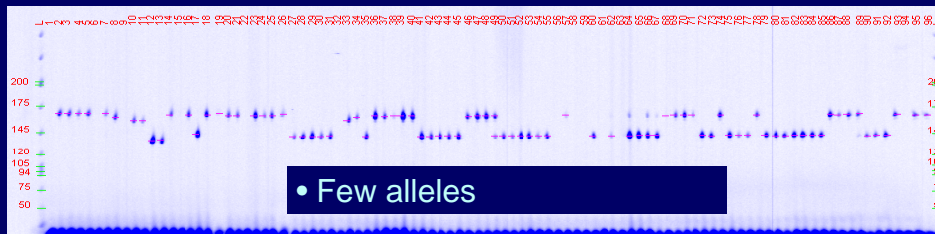
- Some loci had significant (p=0.05) linkage disequilibrium
- Most markers could cross- amplify *S. sclerotiorum* and were polymorphic

Transferability of the microsatellite loci

	<i>S. sclerotiorum</i> (4 isolates)	<i>S. minor</i> (5 isolates)	<i>S. homeocarpa</i> (6 isolates)
Number of loci tested	56	33	33
Amplifiable	30	9	1
Polymorphic	28	1	0



Population level polymorphism



Summary

- Thirty-three polymorphic microsatellite markers were developed for *S. trifoliorum* and characterized
- Most markers cross amplify *S. sclerotiorum* and *S. minor*, and many of them were polymorphic on *S. sclerotiorum*
- These markers are appropriate for population and ecological studies in *S. trifoliorum* and potentially in other *Sclerotinia* species

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Questions ?