

# DA Microsatellites reveal genetic diversity in Rotylenchulus reniformis populations



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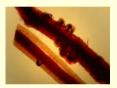
**ABSTRACT** 

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Rotylenchulus reniformis is the predominant parasitic nematode of cotton in the Mid South area of the United States. Variable levels of infection and morphological differences have been reported for this nematode, but this is the first report on the use of simple sequence repeats (SSR) that document the genetic variability of R. reniformis. We developed 192 microsatellites or SSR markers for R reniformis. These markers were tested on six R. reniformis cultures from four states, TX, LA, MS and GA, in the USA. Based on performance we selected 156 SSR markers for R. reniformis from which 88 GA, in the USA. Based on performance we selected 156 SSR markers for *R. reniforms* from which 88 were polymorphic across the six reniform nematode populations. The polymorphic information content of the markers ranged from 0.00 to 0.82, and the percentage of heterozygous loci of the isolates was between 40.9 and 45.1%. Genetic variability was detected among the three MS isolates, for which 22 SSR markers were polymorphic. We also tested the level of infection of four of these isolates on six cotton genotypes, where significant differences were found between the Texas and Georgia isolates. Coincidentally, 62 polymorphic markers were able to distinguish these two populations. Further studies will be necessary to establish possible relationships, if any, between the markers and the aggressiveness of the nematode. The SSR markers developed here will be useful in the assessment of the genetic diversity of this nematode, can be used in <u>breeding</u> programs for crop resistance, and can help in detecting the origin and spread of this nematode in the United States.

### Rotylenchulus reniformis and root infection





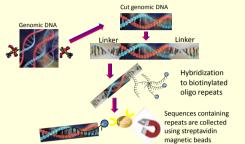
### **SAMPLES**

Isolate	Geographic origin	Population developed	Original
		from	host
MSRR01	Mississippi (Elizabeth farm)	combination of 300 egg masses	cotton
MSRR03	Mississippi (Elizabeth farm)	one egg mass	cotton
MSRR04	Mississippi (Elizabeth farm)	one egg mass	cotton
LA		many individuals	cotton
TX		many individuals	unknown crop
GA		many individuals	cotton

### **PATHOGENICITY**

Treatment	Level	Females per g fresh root <sup>a</sup>
Cotton genotype	Gossypium hirsutum (T 1347)	37 a
	Gossypium hirsutum (T 19)	32 a
	Gossypium hirsutum (FiberMax 960 BGRR)	32 a
	Gossypium hirsutum (T 1348)	26 a
	Gossypium barbadense (TEX 110)	6 b
	Gossypium arboreum (A2-190)	5 b
	F	28.34
	P>F	<0.0001
Nematode	GA	23 a
population		
	LA	18 ab
	MS (RR04)	17 ab
	TX	13 b
	F	2.51
	P>F	0.0606
Cotton x Nematode	F	0.47
	P>F	0.9523

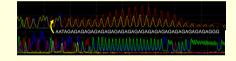
# **SSR-Enriched Libraries** Cut genomic DNA

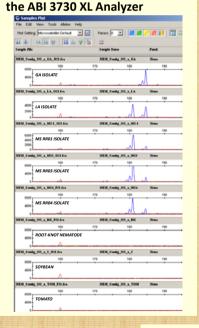


**Optimized Method to Generate** 

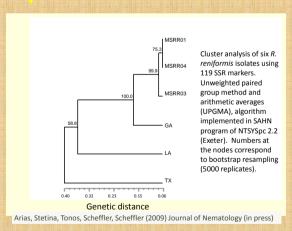
Techen, Arias, Scheffler (2009) Molecular Ecology Resources (in press)

## Example of simple sequence repeats (SSR) after sequencing





Example of markers after run on



### **CONCLUSIONS**

Maximum genetic distance (0.35) was observed for isolates from GA vs. TX and 62 markers were polymorphic between these two isolates. The GA population caused higher levels of infection than the TX population. The markers did not result in amplicons when DNA from the plant hosts (cotton, soybean, tomato) and root-knot nematode were used (see example of electropherogram). These 156 SSR markers will be available for genetic studies of reniform nematode that aid in breeding programs or to detect genetic variation within and between geographical areas.

#### References:

- 1) Arias, Stetina, Tonos, Scheffler, Scheffler (2009) Journal of Nematology (in press)
- 2) Techen, Arias, Scheffler (2009) Molecular Ecology Resources (in press)