Improved Method for Breeding Soybean for More Durable Resistance to SCN

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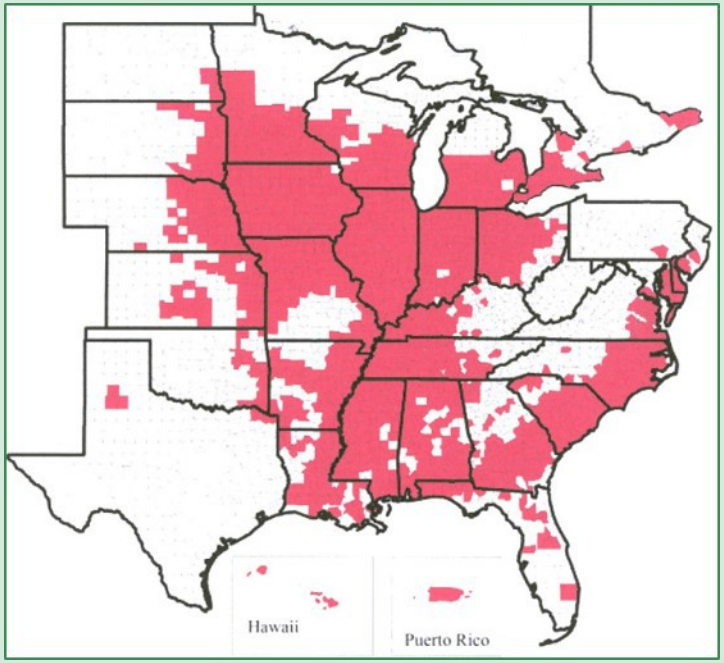
Economic Impact

- Soybean is a major oil seed crop produced and consumed worldwide.
- Soybean cyst nematode (SCN) is a pervasive pest on soybean in the USA and other soybean growing countries.
- SCN reduces yields by nearly 114 kg/ha in the USA, causing annual losses estimated at $1 billion (Koenning & Wrather, 2010).

A Brief History of SCN



- SCN is an obligate root parasite of soybean.
- Reports indicate SCN was first observed in North Carolina in 1954 (Winstead et al., 1955).
- SCN soon spread to all soybean growing states in the USA.
- Sources of resistance were identified, and breeding was initiated.

Breeding Strategy

Primarily, bi-parental crosses are used.

Segregating populations are advanced through:

- Single plant selections and/or
- Modif ied bulk selections

Plant rows from 2014 single plant selections grown in Jackson, TN in 2015.

Breeding Strategy

Resistant progenies are identified in greenhouse bioassays with curated nematode populations derived from field populations:

- Primarily, F5 or F6 populations are used for our bioassays.
- Bioassays are labor intensive and time consuming.
- We use only greenhouse cultured nematode populations for bioassays.

Cysts are blasted from roots of increases – plantings of susceptible lines

Hot water circulates under the benches to provide heat

Cysts are collected in a f ine mesh sieve and used to inoculate tests

Cooling pads on west wall

Fans on east wall

For inoculation of soybean plants, cysts must be ruptured to release their eggs

A modified drill press is used for cyst grinding

For calculation of Female Index, cysts are placed in a gridded petri dish and counted using a stereomicroscope

A single cyst, greatly enlarged

Marker Assisted Selection

- MAS may be a more efficient, faster, and more reliable confirmation technique for identifying soybean progenies with SCN resistance.
- We use MAS in conjunction with greenhouse bioassays.
- Breeders may confirm lines rapidly for resistance based on alleles of genetic markers linked to SCN resistance genes in soybean lines.
- MAS is not a labor intensive process.

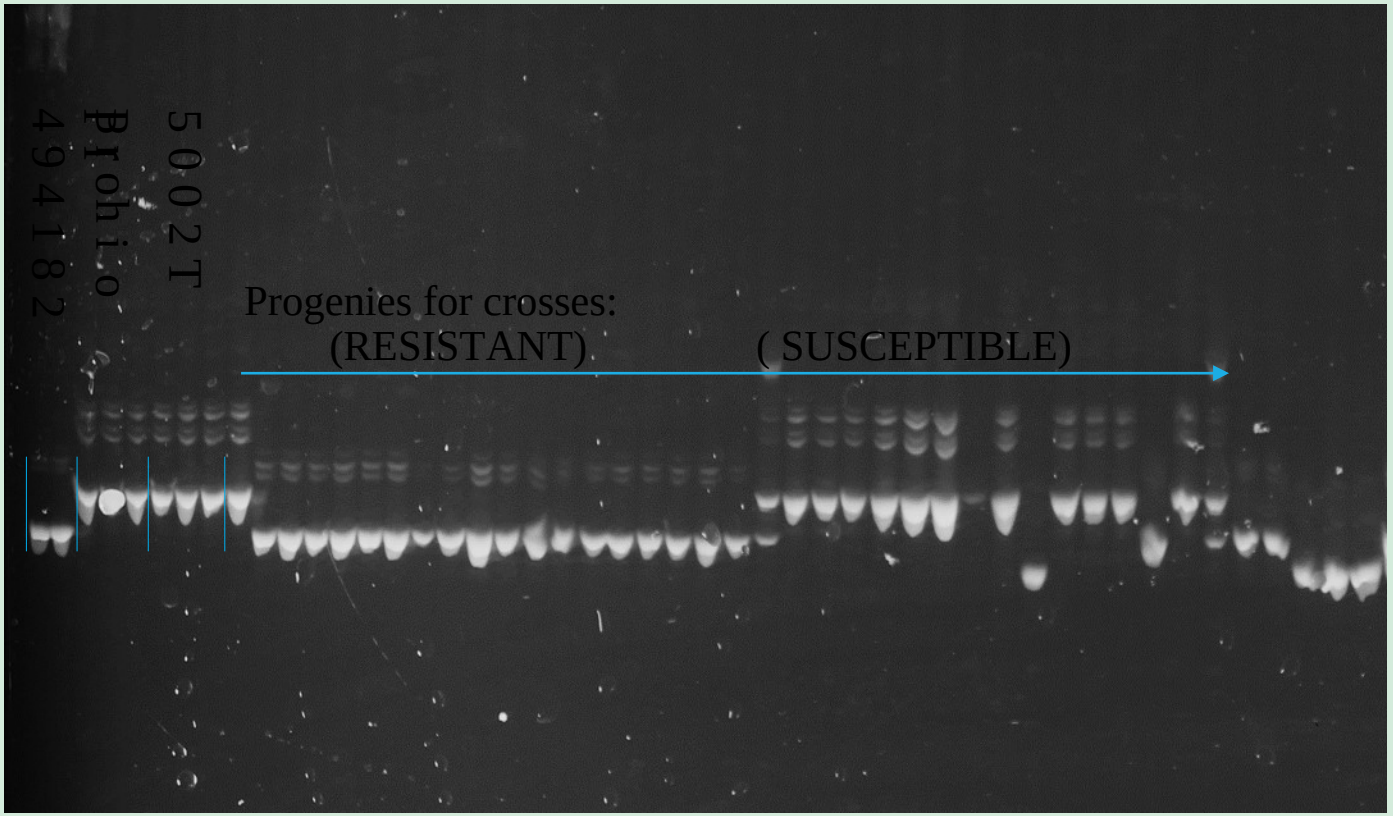
MAS Methods

1. DNA samples are collected in the field or greenhouse using Whatman FTA cards (GE HealthCare).
2. A 2 mm disc is removed from the card, placed in a reaction tube, and purified.
3. *Taq* polymerase, SSR primer pairs, and other reagents are added to the disc for polymerase chain reaction (PCR).
4. PCR products are loaded into 6% vertical polyacrylamide gels and dyed with EZ-Vision One loading dye (Amresco).
5. Gels are visualized and documented using 365 nm UV light.
6. Progenies are scored according to whether they have a reaction consistent in size with that of the resistant parent or of a susceptible check.

Markers Used for MAS

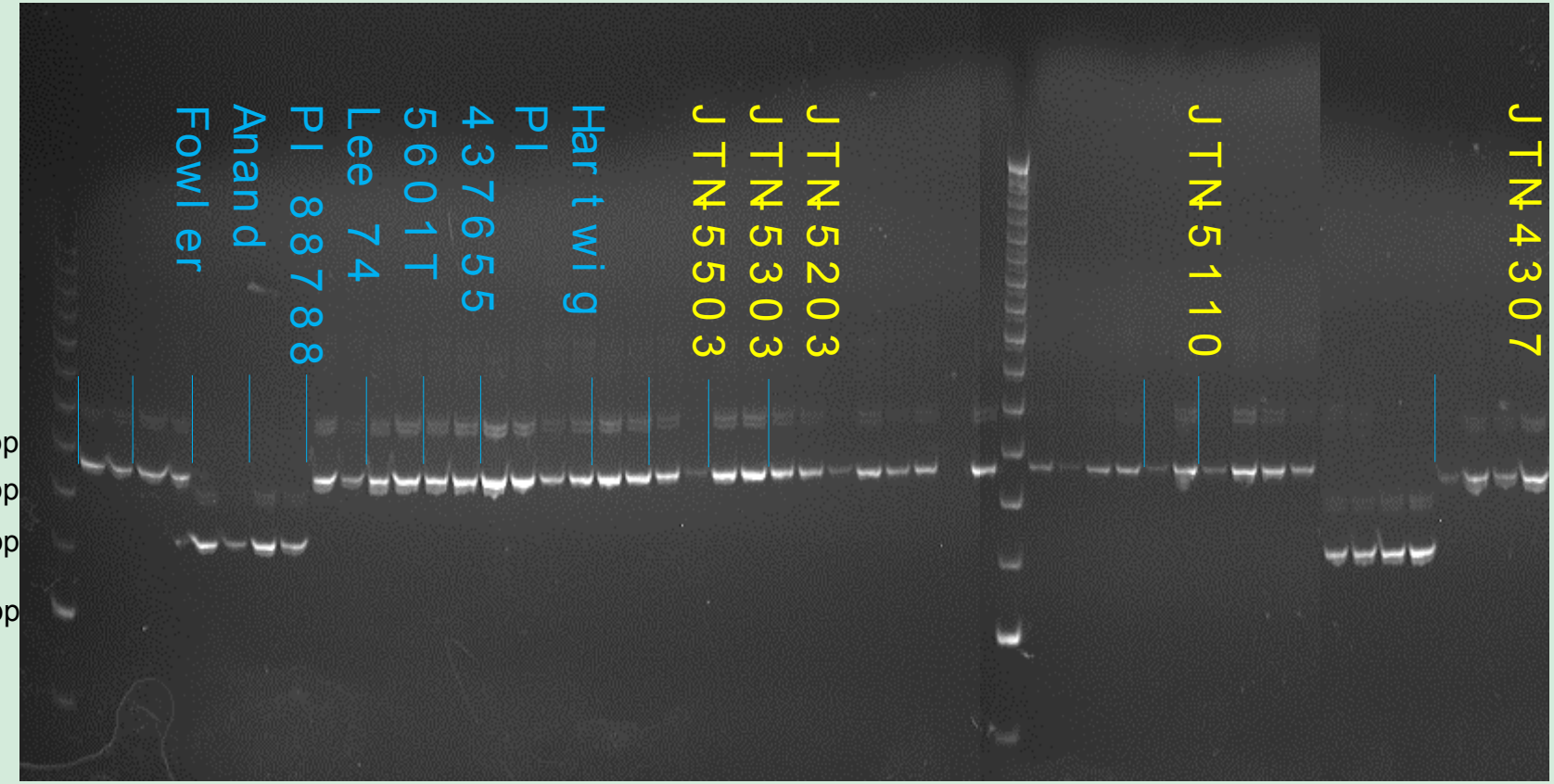
- We routinely use SSR markers to conf ırm resistance and to develop soybean germplasm lines for SCN resistance.
- These markers include:
  - **Satt309** & **Sat_168** (LG G)
  - **Sat_162** & **Satt632** (LG A2)
  - **Satt574** (LG D2)
  - **Satt592** & **Satt331** (LG O)
  - Others

Comparison of Progeny Lines with Satt309 on LG G



6% polyacrylamide gel run 1/22/2015 (EZ Vision One)

Comparison of Soybean Lines with Satt574 on LG D2



6% polyacrylamide gel run 1/25/2011 (ethidium bromide)

How Can Durable Resistance Be Improved Further?

- By utilizing new and diverse sources of resistance.
- By using improved methods, such as Marker Assisted Selection (MAS).

New Sources of Resistance used in our Breeding Program.

Resistance Source	PI 494182	PI 437655	AR8SCN (Sel.) (PI 88788 x Columbia)
Seed			
Progeny rows in Field - 2015	 Prohio x PI 494182	 5601T x PI 437655	 LG01-5822 x AR8SCN(Sel,)

Summary

A breeding program for SCN resistance should include:

- Use of curated/cultured nematode populations developed from field populations for greenhouse bioassays. Avoid using field populations directly in bioassays.
- Use of MAS as a confirmation test for SCN resistance in progenies.
- Utilization of new sources of resistance. These may provide broad resistance to slow down nematode shifts in the soybean fields.

Collaborators

- Silvia Cianzio, Iowa State University
- Grover Shannon, University of Missouri
- Brian Diers, University of Illinois
- Dechun Wang, Michigan State University
- Rouf Mian, USDA-ARS
- Zenglu Li, University of Georgia
- David Lightfoot & Khalid Meksem, Southern Illinois University

Thank you all!