

Molecular Markers for Selecting Low Trypsin Inhibitor Soybean

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Overview

Introduction

Goal & Objectives

Materials & Methods

Results & Discussion

Future Plans



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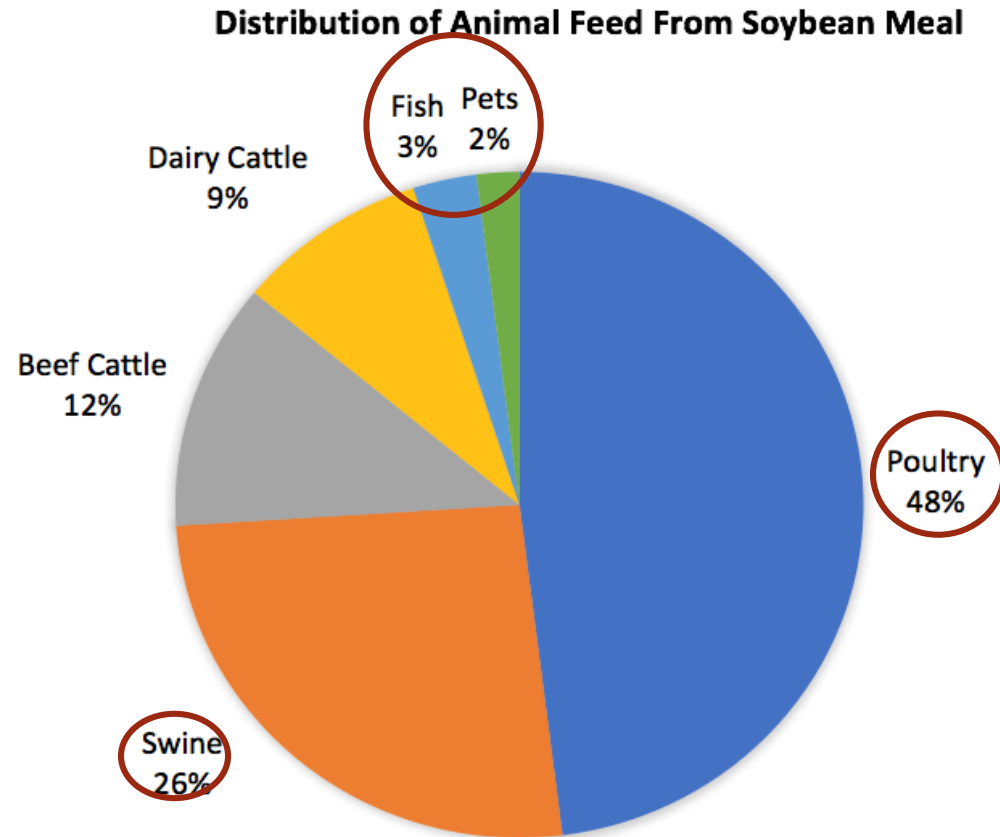
Results & Discussion

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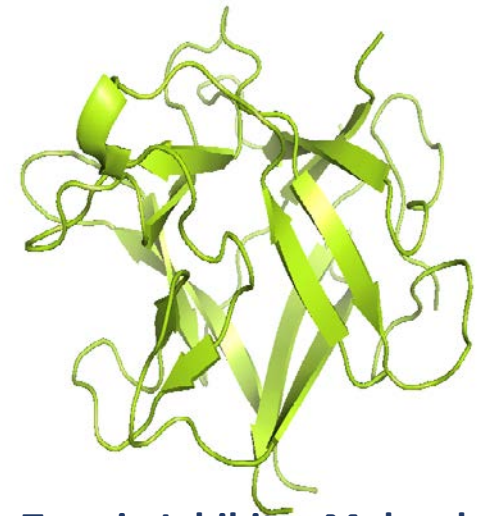
Importance of Soybean

- Agronomic crop
- 98% used as animal feed
- 79% are monogastric animals



Trypsin Inhibitor (Ti)

- **Anti-nutritional factor**
- **Prevents function of trypsin enzyme**
 - Protein  amino acid
- **Inhibits animal growth**
- **Denatures at 194°F**
 - Added cost
 - Risk of lowering nutritional value



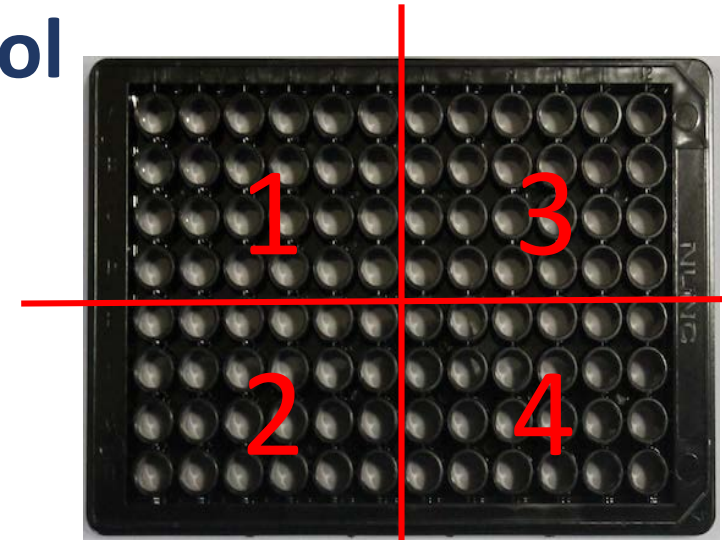
Trypsin Inhibitor Molecule

Solution – Low Ti Varieties

- **Reliable measuring protocol**

- **Colorimetric Bioassay**

- Time consuming
- Time sensitive reaction
- Expensive
- Non-repeatable



- **Quick way to identify low trypsin inhibitor**

- **Molecular marker**

- New markers
- Reliable?

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Goal and Objectives

- **Goal – Develop low Ti soybean varieties**
- **Objectives**
 - To establish a quick and reliable methodology to quantify Ti composition
 - To identify new or confirm published genetic markers associated with Ti composition

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Method - Ti Composition

- High performance liquid chromatography (HPLC)
- High throughput
- Reliable
- Repeatable



Materials- Marker Identification

- **Genotypic**

- **F₂ population**

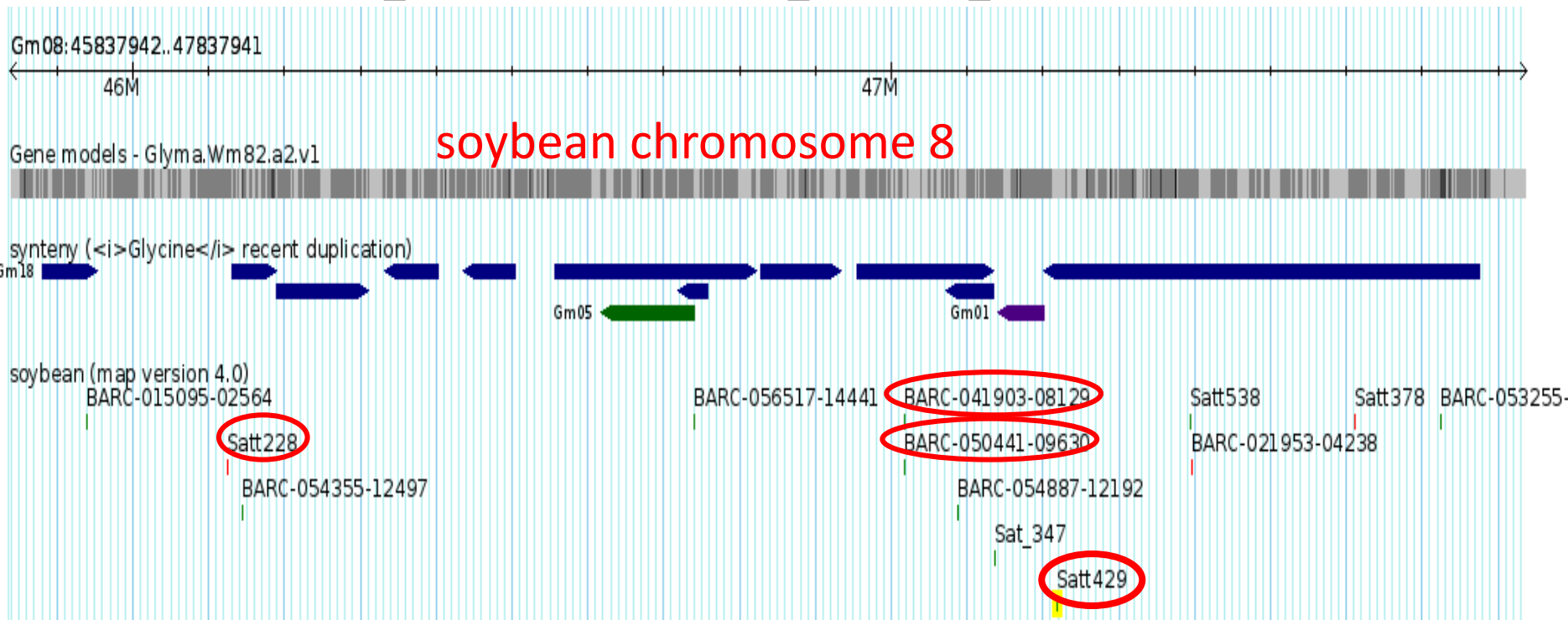
- Glenn (normal Ti) x PI 547656 (low Ti)
- DNA extraction from leaf tissue
- 5 polymorphic markers between parents

- **Phenotypic**

- 600 F₃ individuals, Blacksburg in 2015
- 200 F_{3:4} rows, Blacksburg and Orange in 2016

Methods – Marker Identification

- SSR – Satt429, and Satt228
- SNP – BARC_041903-08129, BARC_0504414_09630, and Kti SNP



- Satt 429 was reported in Kumar et al, 2015
- Kti SNP was developed based off the Ti sequence

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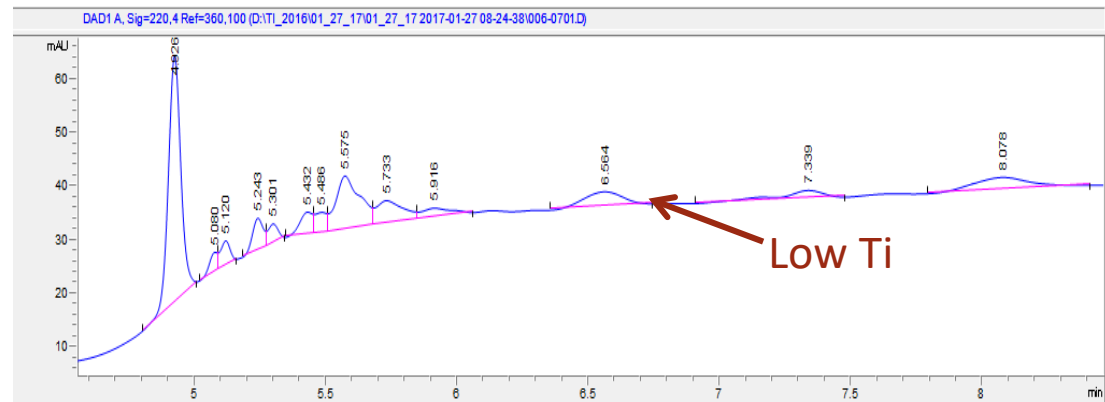
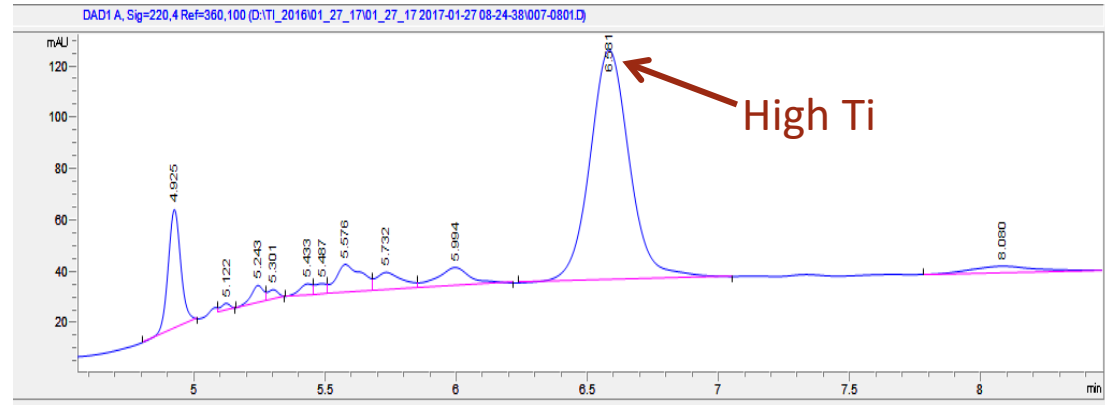
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Result - Ti HPLC Protocol

- Ground into fine powder
- 10 mg of seed powder
- 1mL NaOAc
- Shaker
- Centrifuged
- Filtered into vials
- Ran on HPLC machine

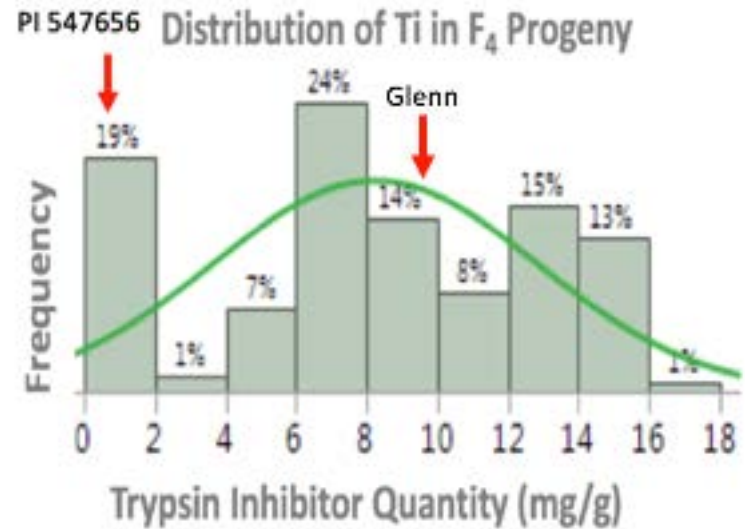
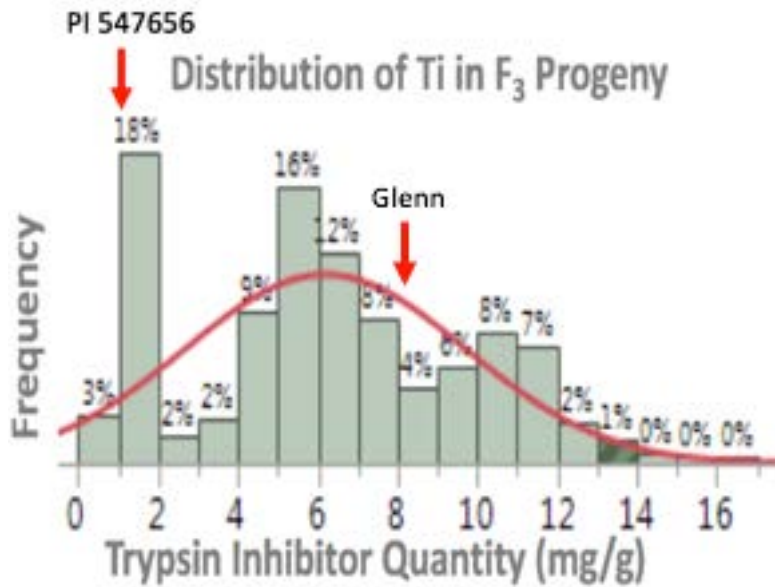


Retention Time – 6.5 minutes

Discussion- Ti Procedures

HPLC	Colorimetric Bioassay
<ul style="list-style-type: none">• Accurate quantification• Repeatable• High throughput• Less Expensive	<ul style="list-style-type: none">• Not accurate• Repeatability issues• Time consuming• Expensive

Result - Phenotypic distribution



Several Major Genes

Result - Marker & Trait Association

Marker	2015			2016		
	P Value	LOD	R ²	P Value	LOD	R ²
Satt 429	0.30	1.45	0.01	0.68	0.83	0.02
KtiSNP	0.20	0.29	0.002	-	-	-
BARC_041903-08129	0.18	0.25	0.002	-	-	-
Satt 428	-	-	-	-	-	-
BARC_0504414_09630	-	-	-	-	-	-

Discussion - Markers

- **Satt 429 not linked to Ti gene**
 - Different genetic background
 - The phenotypic data in Kumar et al, 2015 obtained using colorimetric bioassay
- **The new, tested markers not linked to Ti gene**
 - Need run the rest markers
 - Not in functional region of Ti gene
 - Multiple genes involved

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Future Plans

- Screen more markers at different regions of the Ti gene
- Map QTLs associated with Ti composition



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Thank You!