Pepper anthracnose resistance: New opportunities emerging from host-pathogen studies

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Anthracnose by *Colletotrichum*

- Anthracnose caused by a diverse number of *Colletotrichum* species poses a great threat to pepper.
- *Colletotrichum* is a hemibiotrophic fungal pathogen.
- Cultural techniques (e.g. fungicide application, crop rotation, and elimination of alternate hosts) provide incomplete protection
- Genetic resistance to anthracnose is desirable.

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https://www.apsnet.org/publications/imageresources/Pages/fi0000256.aspx

10 Isolates for Sequencing

No.	Isolate	Location	Aggregate Group (Based on BlastN of ITS Sequence)	Tentative Species Based on Single Gene Trees
1	13NJRP5	NJ	Acutatum	scoviellei
2	14DFR2b	NJ	Acutatum	scoviellei
3	14MHG5	NJ	Acutatum	scoviellei
4	13RP264	MD	Acutatum	nymphaeae or may be a new species
5	14OP99	MD	Acutatum	nymphaeae or may be a new species
6	14RCR5	NJ	Acutatum	nymphaeae or may be a new species
7	14NJRT11	NJ	Between Destructivum & Dematium (Coccodes?)	nigrum
8	13RP180a	NJ	Between Destructivum & Dematium (Coccodes?)	nigrum
9	CO2	?	Between Destructivum & Dematium (Coccodes?)	nigrum
10	13NJRT1	NJ	Between Destructivum & Dematium (Coccodes?)	nigrum

Green & Red pepper Red tomato — I

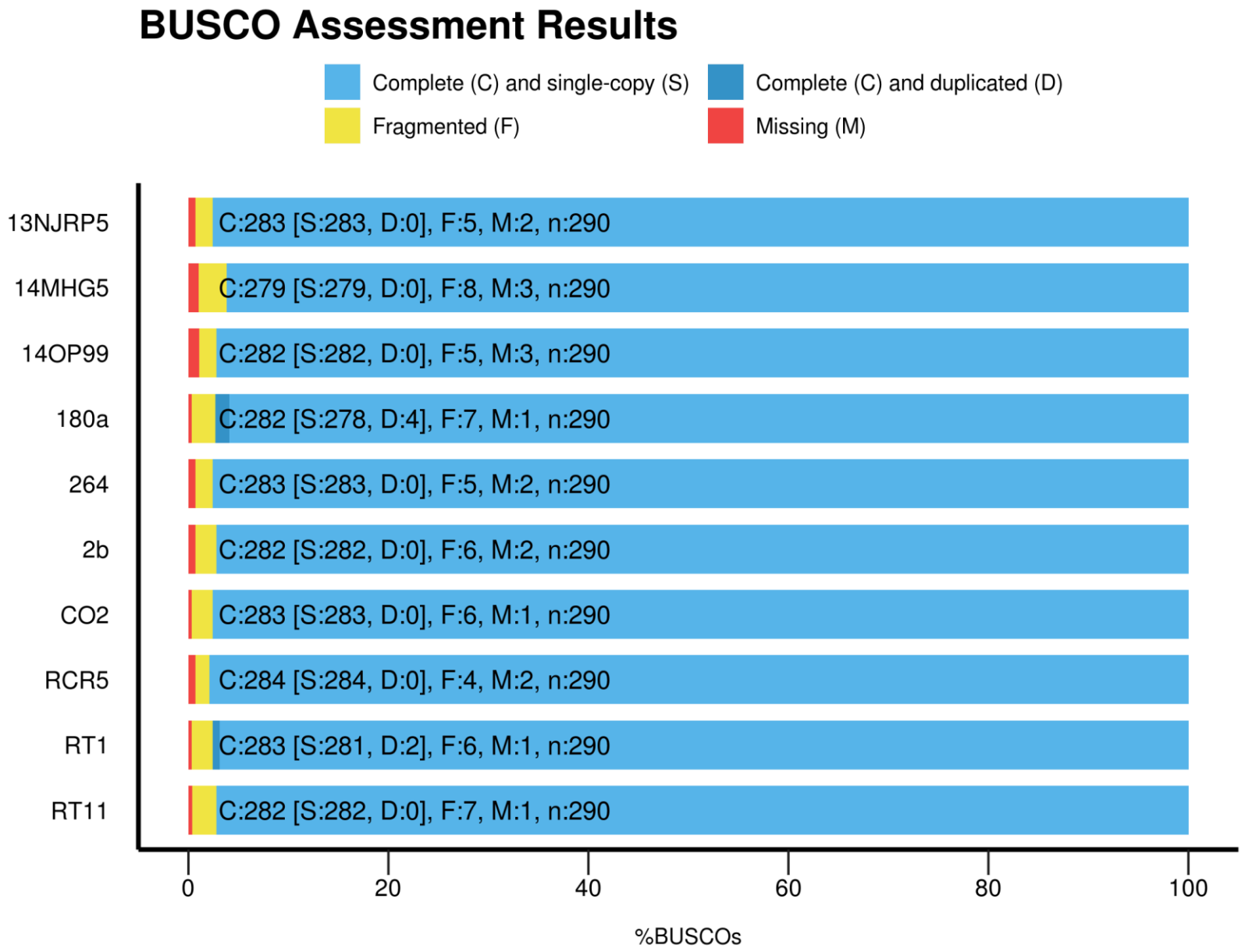
Red pepper & Red tomato — II

Red tomato — III

0.03

Isolates provided by Andrew Wyenandt
Rutgers Agricultural Research and Extension Center

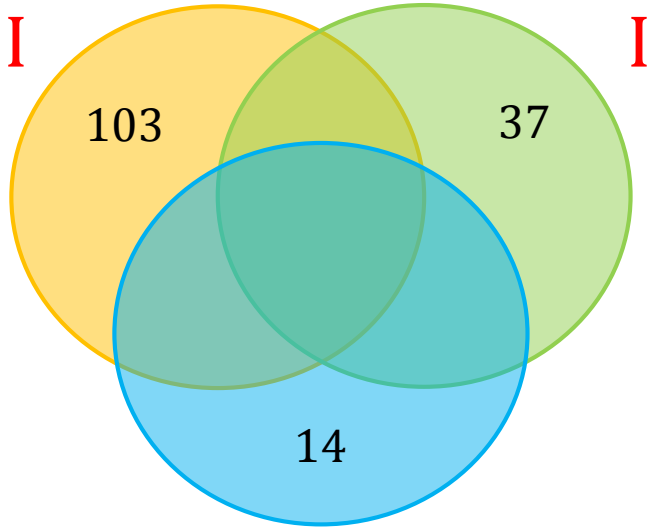
Whole Genome Sequencing & Gene Prediction

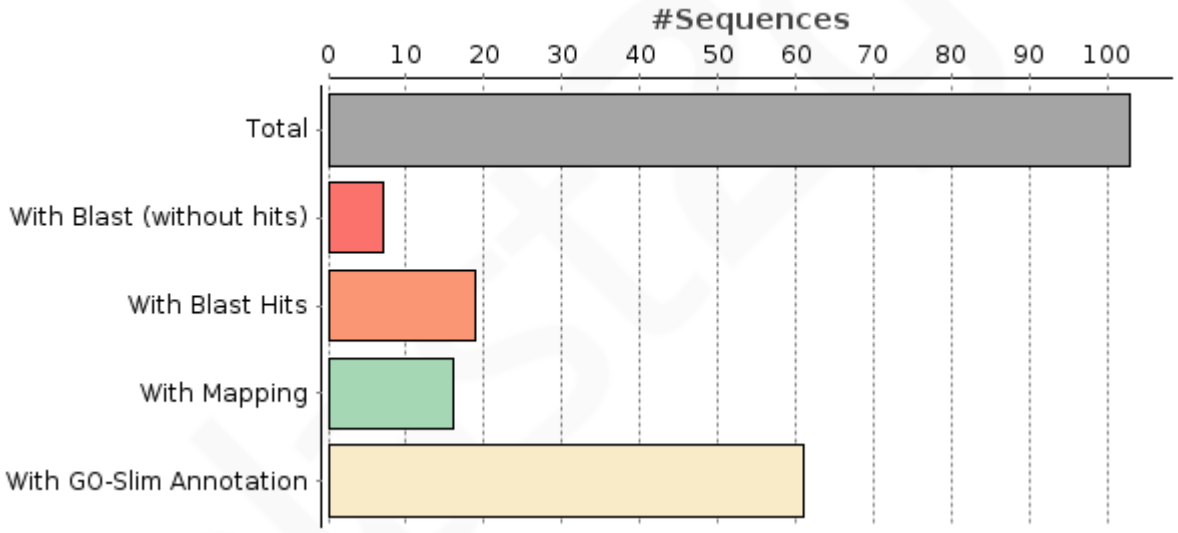


13NJRT1	13RP180a
44x	37x
342280	280649
12867	13126

Identify Orthogroups

- OrthoFinder was used to identify orthogroups among all isolates.
- Blast2Go was used for functional annotation.

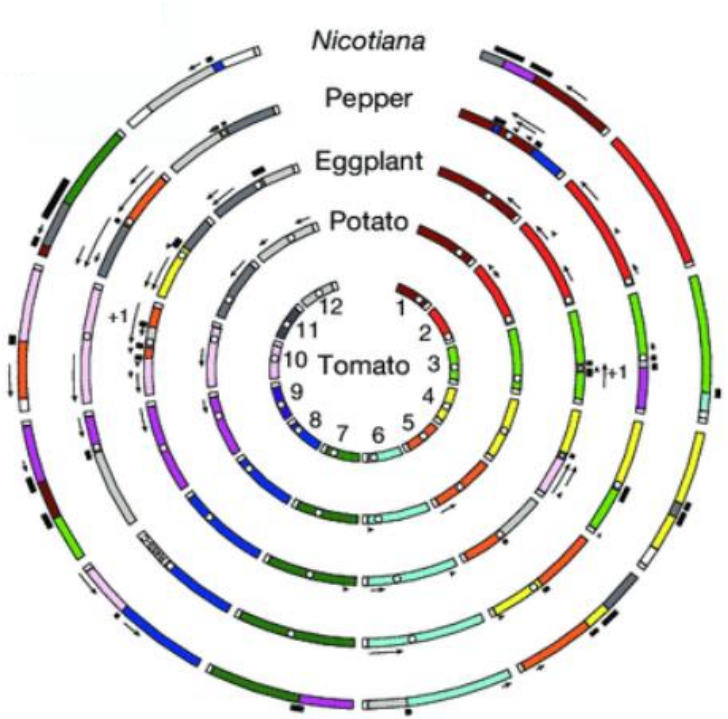




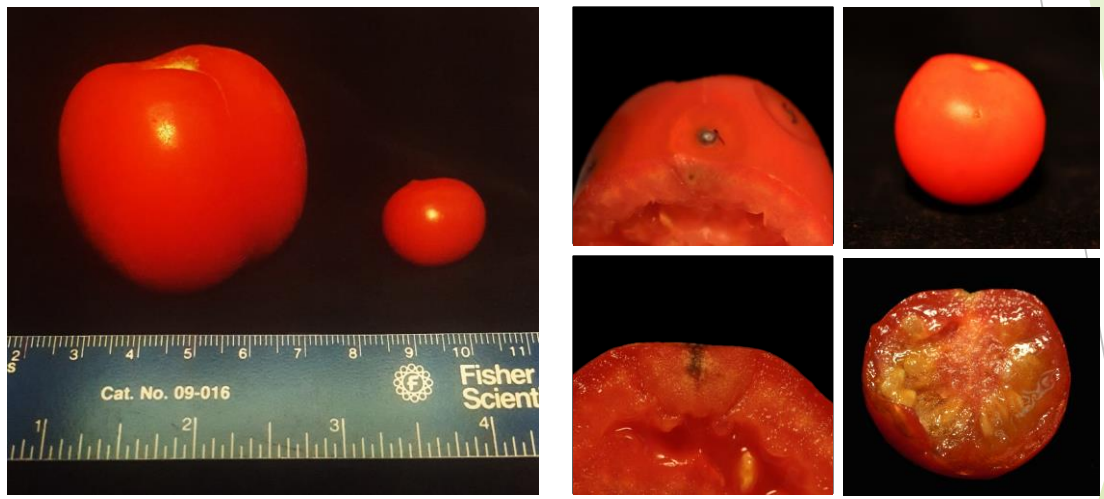
Catalytic activity 54%	1. Hydrolase 2. Transferase 3. Oxidoreductase 4. Lyase 5. Isomerase	Binding activity 35%	1. Ion binding 2. Organic cyclic compound binding 3. Heterocyclic compound binding

RNA-seq is undergoing to study the gene expression of all isolates.

Synteny among Solanaceous Species



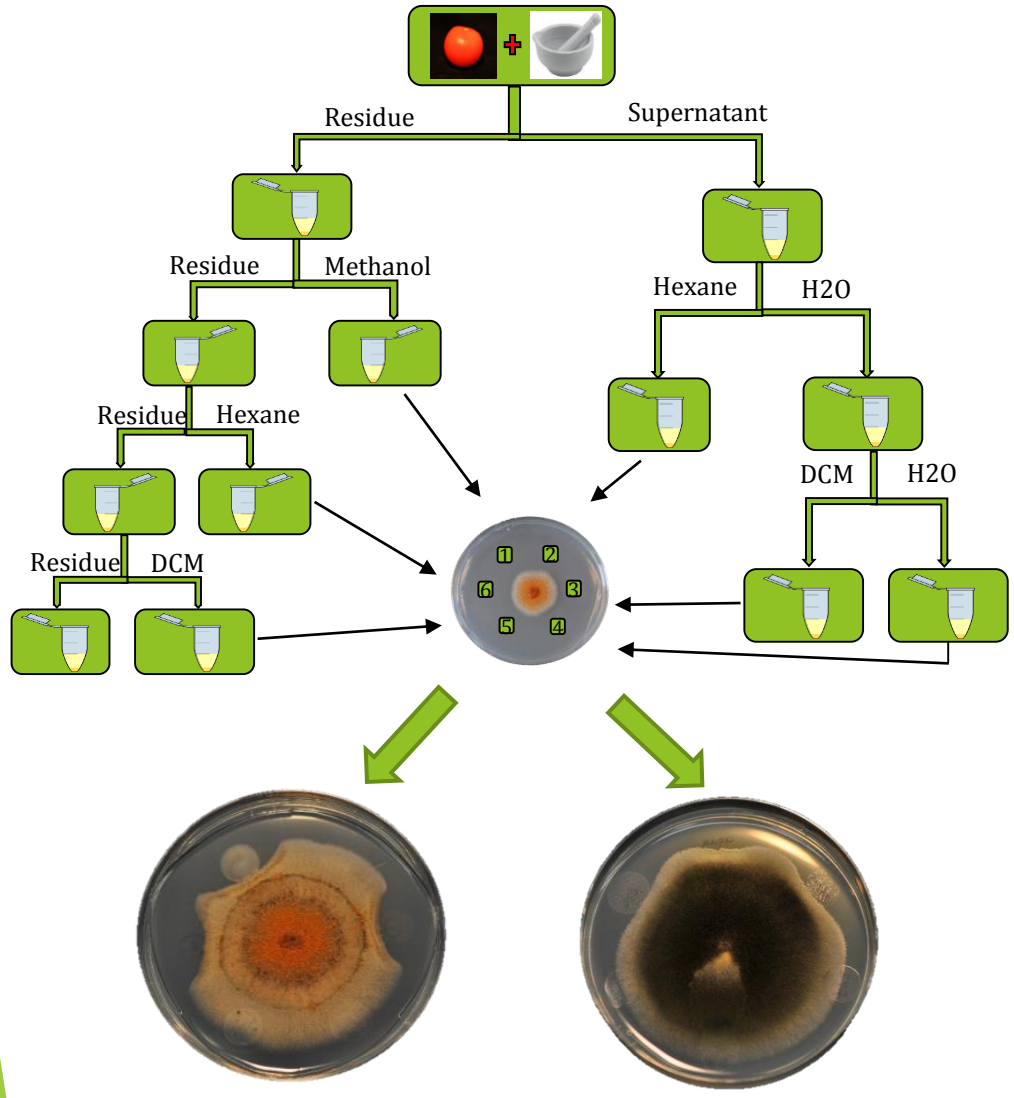
Rio Grande (susceptible) 95L 368 (resistant) Rio Grande (susceptible) 95L 368 (resistant)



95L 368 was selected from the unadapted tomato (*Solanum lycopersicum)* accession PI272636*.* It shows small fruit size and strong resistance to different *Colletotrichum* sp.

Picture from Tomato Genome Consortium, 2012. *Nature*

Tissue Extracts From 95L 368 Inhibit Fungal Growth



Colletotrichum scoviellei

Alternaria alternata

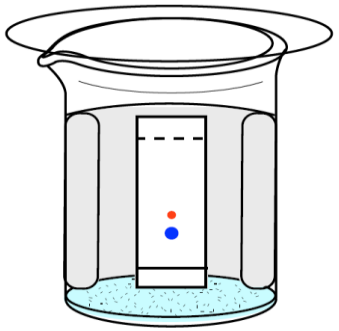
Sample	Heat treatment (98°C)	pH neutralization	Inhibition
Rio Grande	-	-	✕
	+	-	✕
	-	+	✕
95L 368	-	-	✓
	+	-	✓
	-	+	✓

Solvent	Polarity	Inhibition
Methanol/H2O	↑ Increasing Polarity	✓
Dichloromethane (DCM)		✕
Hexane		✕

Size exclusion column	Inhibition
10K	✓
30K	✓
100K	✓

The resistance against fungal pathogens in 95L 368 is from a **PRE-FORMED** compound.
The compound is a **HEAT-STABLE, pH INSENSITIVE, SAMLLER THAN 10K** and **POLAR** compound soluble in water and methanol.

Thin Layer Chromatography Bio-assay



Developing

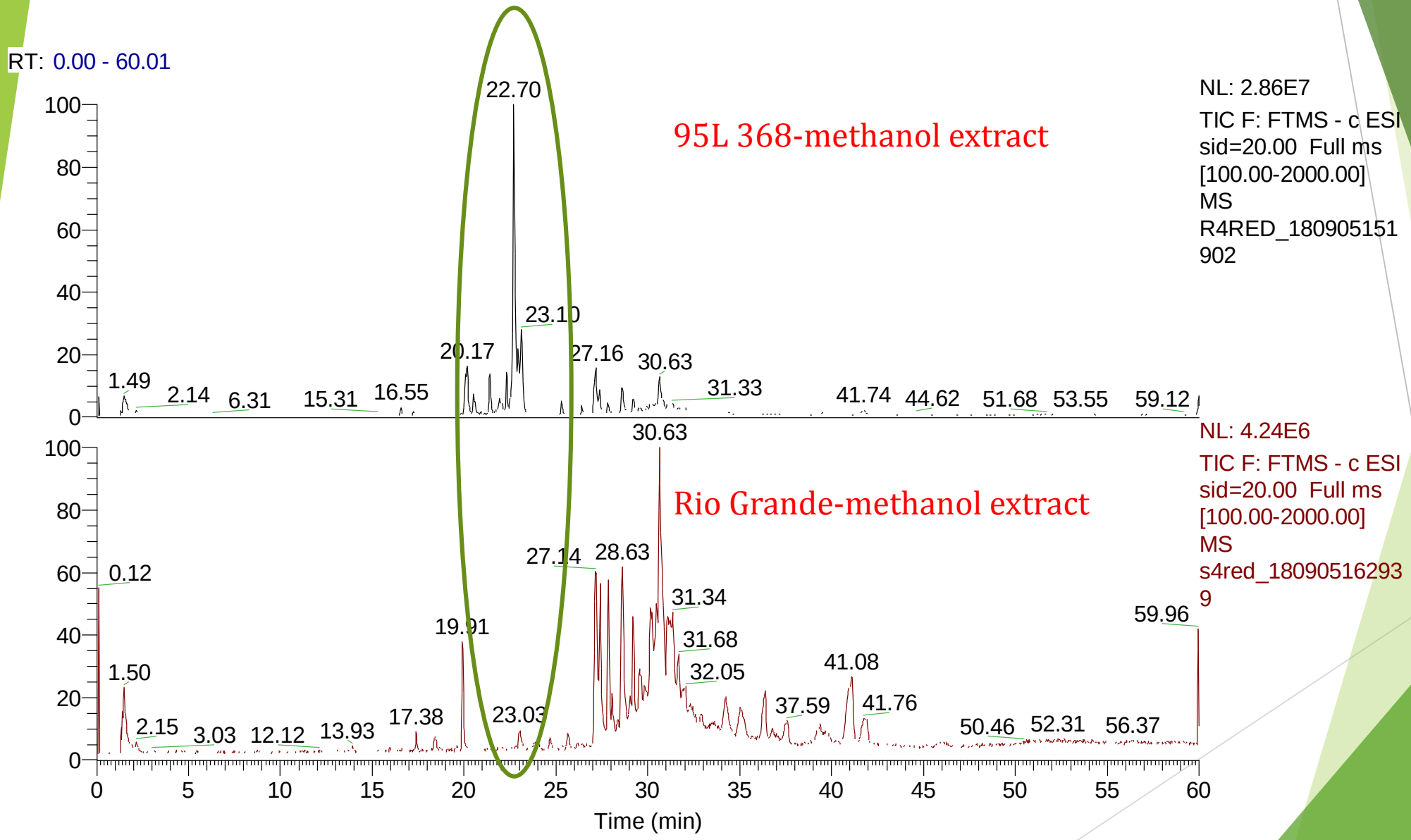
1 2

1. 95L 368
2. Rio Grande

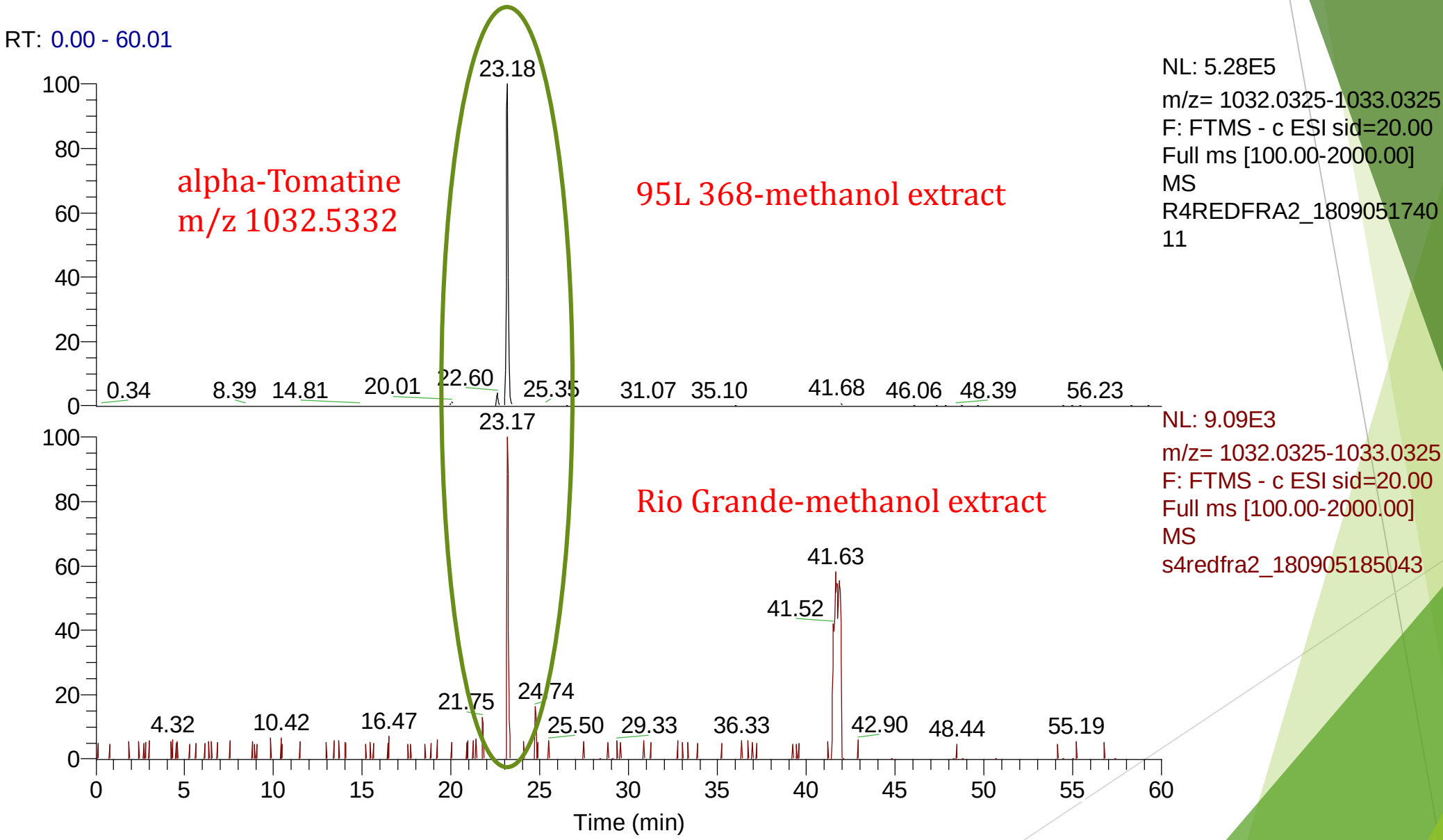
1 2

LC-MS

Total Ion Chromatogram



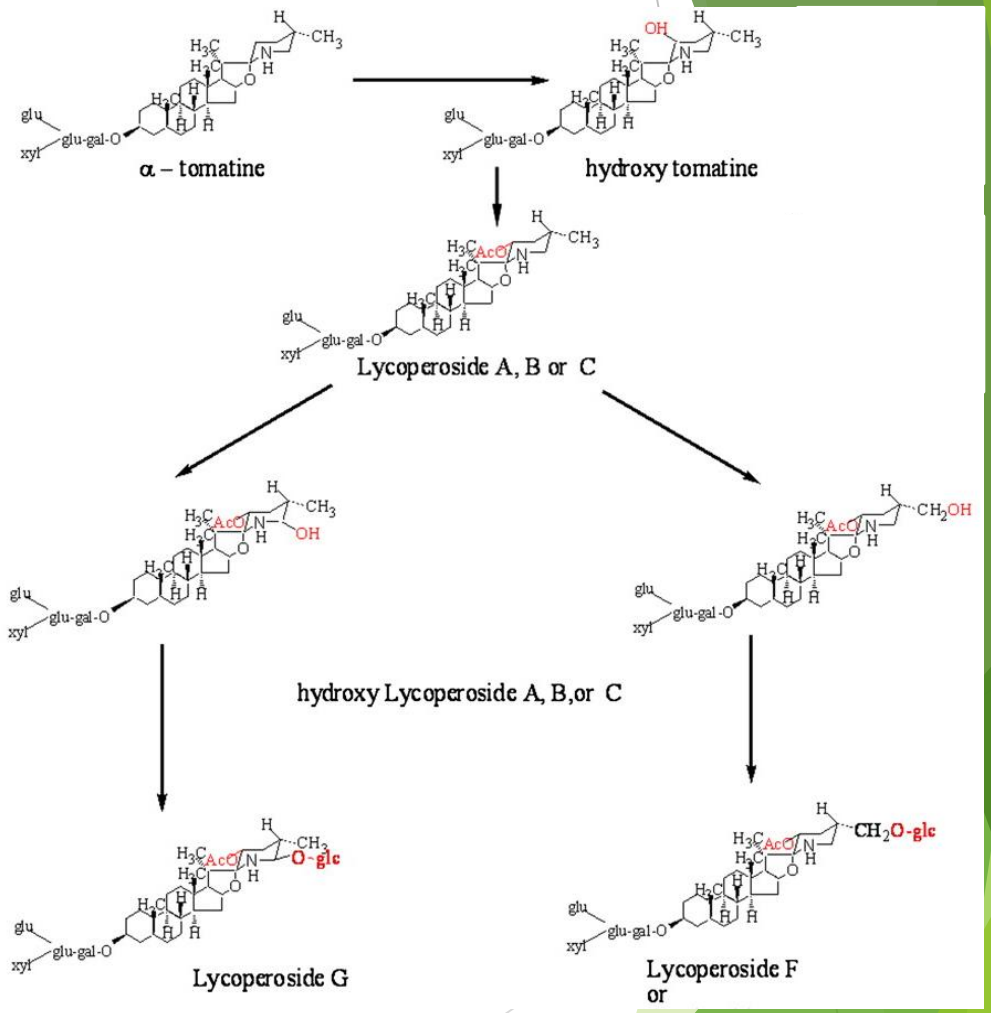
Extracted Ion Chromatogram



Steroidal Saponins Levels Elevated Drastically in 95L 368

Compound	Ion intensity ratio (95L 368/Rio Grande)
α-Tomatine	58.5
Hydroxy Lycoperoside A, B or C	284.4
Lycoperoside A, B or C	390.1
Dehydro-Lycoperoside A, B or C	978.2
Lycoperoside H and its isomer	1051.7

Over Supply or
Limited Degradation???



Mintz-Oron et al, Plant Physiology, 2008

Future Directions

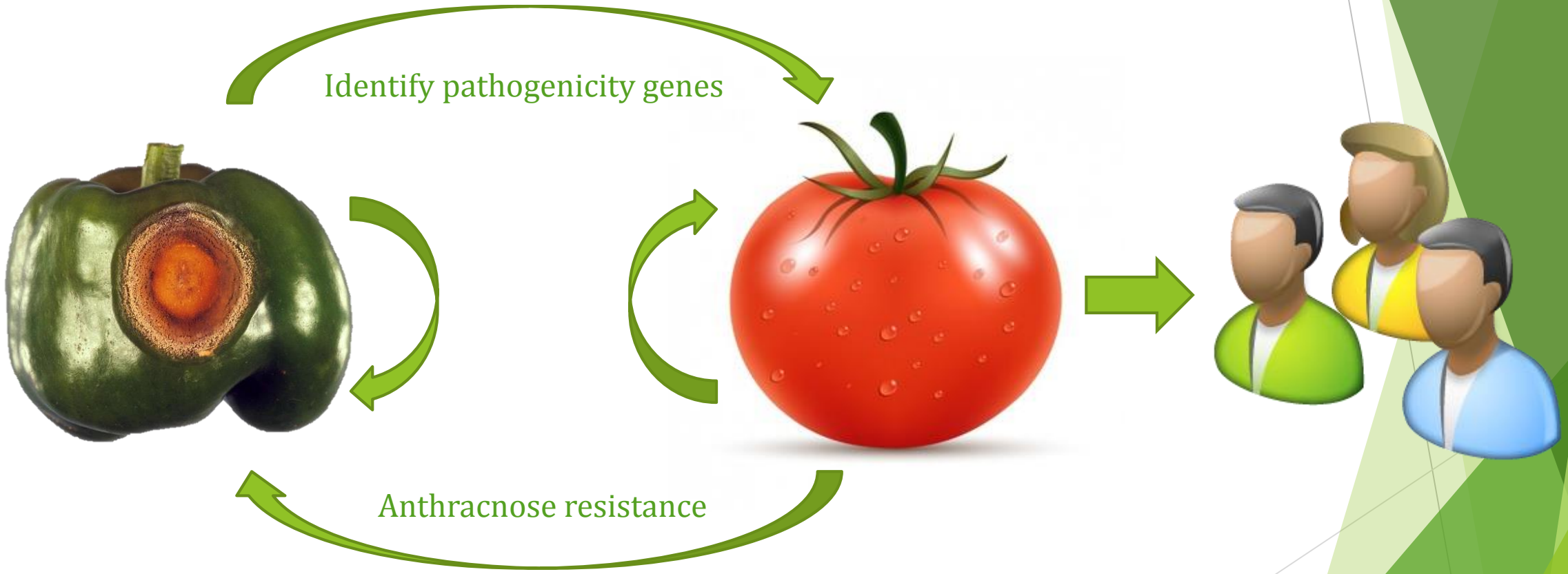
- ***Colletotrichum* isolates**

1. RNA-seq is undergoing to study the gene expression of all isolates.
2. Functional study of specific genes.

- **Tomato**

1. RNA-seq of both resistant and susceptible tomatoes.
2. Using RNAi to downregulate α-tomatine biosynthesis in resistant tomato.
3. QTL mapping to identify locus responsible for resistance.

Final Goals



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