FEED THE FUTURE
The U.S. Government's Global Hunger & Food Security Initiative

RUST RESISTANCE IN THE GUATEMALAN CLIMBING BEAN GERMPLASM COLLECTION

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ICTA

Legume Innovation Lab

Feed the Future Innovation Lab for Collaborative Research on Grain Legumes

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FROM THE AMERICAN PEOPLE

Introduction

Climbing Beans

- *P. vulgaris*, *P. coccineus,* and *P. dumosus*.
- High nutritional value
- Potential of higher yield “up to 5 ton/ ha”
- Planted on marginal lands (Central and South America, and Africa)
- Breeding effort vs. bush type.

Introduction

Relevance of Climbing Beans in Guatemala

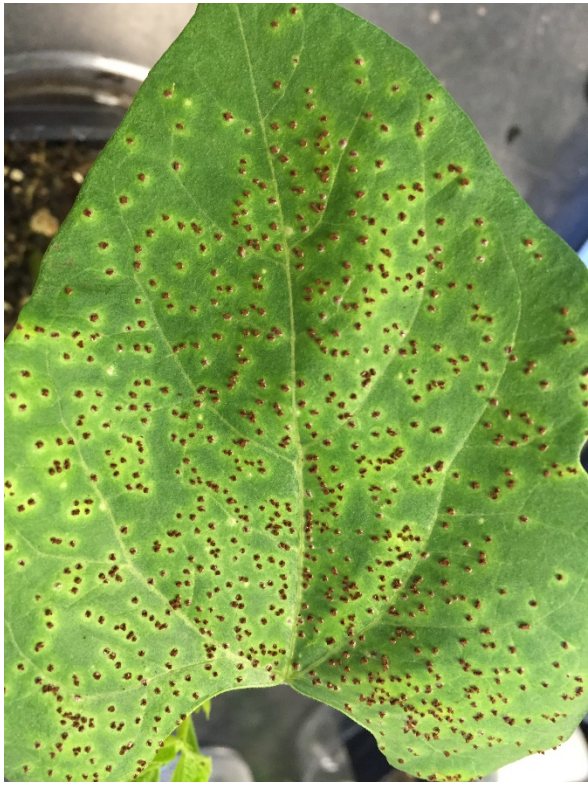
- Top producer region: Latin America and Caribbean
- 43% under five are malnourished
- 66% beans planted by smallholders on hillsides
- **Milpa system**

Main source of protein and calories

Introduction

Uromyces appendiculatus – Bean Rust

- Critical in tropical and subtropical areas (Guatemala's weather)
- Up to 100% yield losses (defoliation)
- High genetic variability
- > 20 *Ur* named genes
- Limited information in Guatemala



Objectives

- 1) To assess the race diversity of bean rust from the Western Highlands in Guatemala.

- 2) To evaluate disease reaction of the Guatemalan climbing bean germplasm to rust races from Guatemala and North Dakota.

- 3) To identify genomic regions associated with rust resistance via GWAS.

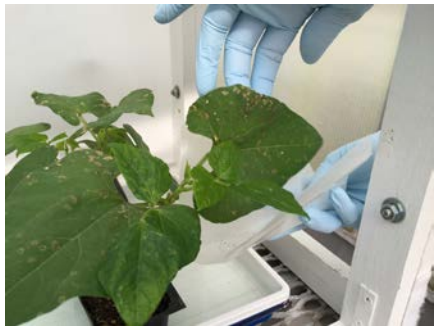
Pathogen Characterization

2015: 23 samples
across 11 locations

UNL
Pinto UI 114

21 ± 1°C
2 ½ hours on 1 ½ off
16 h

Spore increasing
14 days



Characterization
12 differential cultivars

Single Ureidinium
Isolate

1-6 scale

Pathogen Characterization

Description of infection types of *U. appendiculatus* isolates on 1-6, based on pustule diameter.

Description	Infection type	Genotype
No visible symptoms	I	Resistant
Necrotic flecks or spots without sporulation	2+	Resistant
Uredinia ≤ 300 µm in diameter	3	Resistant
Uredinia 300 – 499 µm in diameter	4	Susceptible
Uredinia 500 – 799 µm in diameter	5	Susceptible
Uredinia ≥800 µm in diameter	6	Susceptible

Stavely, 1984; Mmbaga et al., 1996

Pathogen Characterization Results

Disease reaction of *P. vulgaris* rust resistance genes to bean rust race 63-1 and 31-1.

				Disease Reaction	
Differential Cultivar	Rust Resistance Gene	Gene Pool	Binary Value	63-1	31-1
Early Gallatin	***Ur-4***	Andean	1	Virulent	Virulent
Redlands Pioneer	***Ur-13***	Andean	2	Virulent	Virulent
Montcalm	***Ur-Unknown***	Andean	4	Virulent	Virulent
PC 50	***Ur-9, Ur-12***	Andean	8	Virulent	Virulent
Golden Gate Wax	***Ur-6***	Andean	16	Virulent	Virulent
PI 260418	***Ur-Unknown***	Andean	32	Virulent	Avirulent
GN 1140	***Ur-7***	Mesoamerica	1	Virulent	Virulent
Aurora	*Ur-3*	Mesoamerica	2	Avirulent	Avirulent
Mex 309	*Ur-5*	Mesoamerica	4	Avirulent	Avirulent
Mex 235	*Ur-3+*	Mesoamerica	8	Avirulent	Avirulent
CNC	*Ur-Unknown*	Mesoamerica	16	Avirulent	Avirulent
PI 181996	*Ur-11*	Mesoamerica	32	Avirulent	Avirulent

Stavely, 1984; Mmbaga et al., 1996

Steadman et al.

Sample ID	Town	Department	Bean Variety	Race
G1501	La Esperanza S1 [a]	Quetzaltenango	Landrace	63-1
G1502	ICTA Labor Ovalle S1	Quetzaltenango	V. 1026	63-1
G1503	ICTA Labor Ovalle S2	Quetzaltenango	Labor Ovalle	31-1
G1504	ICTA Labor Ovalle S3	Quetzaltenango	Texel	31-1
G1505	ICTA Labor Ovalle S4	Quetzaltenango	Labor Ovalle	31-1
G1506	ICTA Labor Ovalle S5	Quetzaltenango	296-Guate1074	31-1
G1507	ICTA Labor Ovalle S6	Quetzaltenango	Landrace	63-1
G1508	ICTA Labor Ovalle S7	Quetzaltenango	Guate-257	31-1
G1509	Parramos S1	Chimaltenango	Landrace	63-1
G1510	Parramos S2	Chimaltenango	Landrace	63-1
G1511	Parramos S3	Chimaltenango	Landrace	31-1
G1512	Patzicia S1	Chimaltenango	Landrace	63-1
G1513	Patzicia S2	Chimaltenango	Landrace	No results[b]
G1514	Tecpan S1	Chimaltenango	Landrace	No results
G1515	Patzicia S3	Chimaltenango	Landrace	63-1
G1516	Patzicia S4	Chimaltenango	Landrace	No results
G1517	San Pedro Sacatepequez	San Marcos	Loma Linda	63-1
G1518	Las Barranquitas S1	San Marcos	Landrace	63-1
G1519	Las Barranquitas S2	San Marcos	Landrace	63-1
G1520	San Pedro Sacatepequez	San Marcos	Landrace	No results[b]
G1521	Aldea Champollap	San Marcos	Landrace	No results
G1522	Loma Linda	San Marcos	Landrace	31-1
G1523	La Esperanza S2	Quetzaltenango	Landrace	No results

U. appendiculatus samples, and races identified in the 17 Guatemalan isolates based on virulence reaction of the 12 bean rust differential set.

Bloguitravel.com

Collaborative Research on Grain Legumes

Germplasm greenhouse evaluation

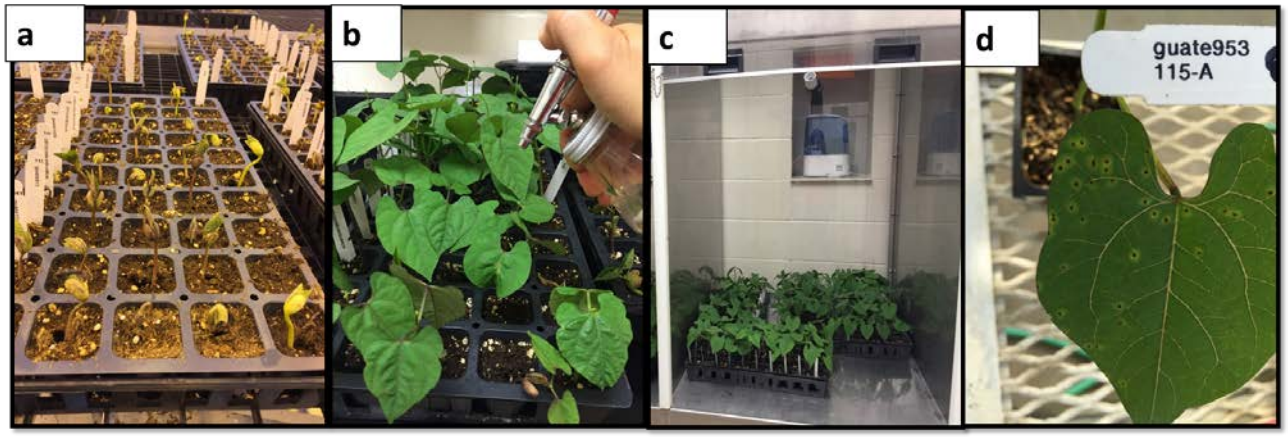
372 climbing beans (*P. vulgaris*) (ICTA)

Rust inoculum: 63-1 & 31-1 (UNL), and 20-3 (NDSU)

RCBD, 3 reps

Reps= blocks

Susceptible control: Pinto UI 114.



Procedure for germplasm evaluation in greenhouse. a) Planting, **b)** Inoculation, **c)** Incubation in mist chamber, and **d)** Disease reaction scored 10-14 days after inoculation.

Germplasm Greenhouse Evaluation Results

Analysis of Variance with fixed effects for rust diseases reactions in 372 climbing bean accessions.

Rust race	Effect	Num DF	Den DF	F Value	Pr > F
20-3	Genotype	371	741	9.06	<.0001
63-1	Genotype	371	740	3.32	<.0001
31-1	Genotype	371	741	3.16	<.0001

Reaction of the climbing bean germplasm collection from Guatemala to bean rust races under greenhouse conditions.

			LSM - Quantitative score (1.1-6.1)	
Rust Race	**Resistant (%)**	**Susceptible (%)**	**Max**	**Min**
20-3	85	15	5.3	1.1
63-1	75	25	6.0	1.0
31-1	81	19	5.4	1.0

Genome-Wide Associations Studies GWAS

- 364 genotypes - GBS sequencing (~150,000 SNPs)
- 78,754 SNPs <0.05 MAF

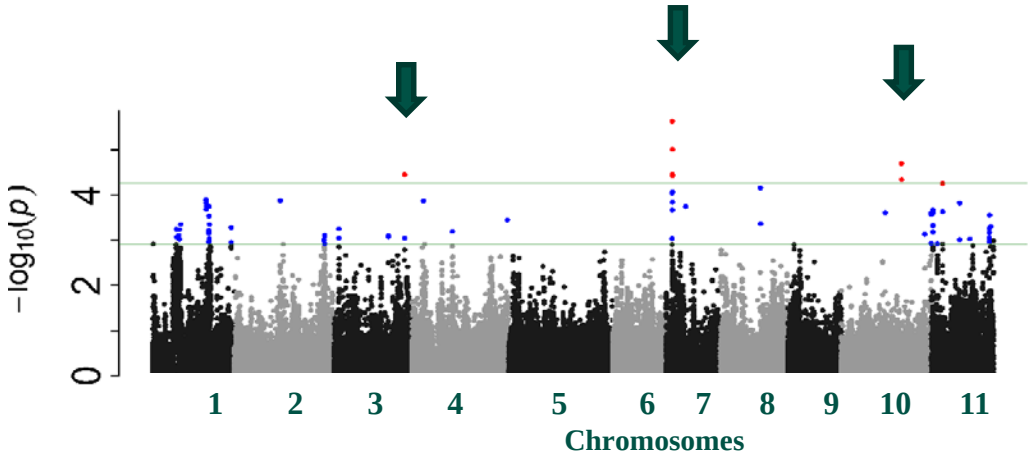
- Binary data: 0= 1 -2, 1= 4 - 6
- **Eliminated: Intermedium resistant and segregating accessions**
- GenABEL V. 1.8 (Aulchenko et al., 2007).
- PC, EMMA and Mixed model

- GWAS by each race

Mean Square Deviation (MSD) of each GWAS Statistical Model

Data	Rust Race	7PC	EMMA	7PC+ EMMA
Binary	20-3	0.15	0.16	0.20
Binary	63-1	0.15	0.17	0.23
Binary	31-1	0.16	0.17	0.20

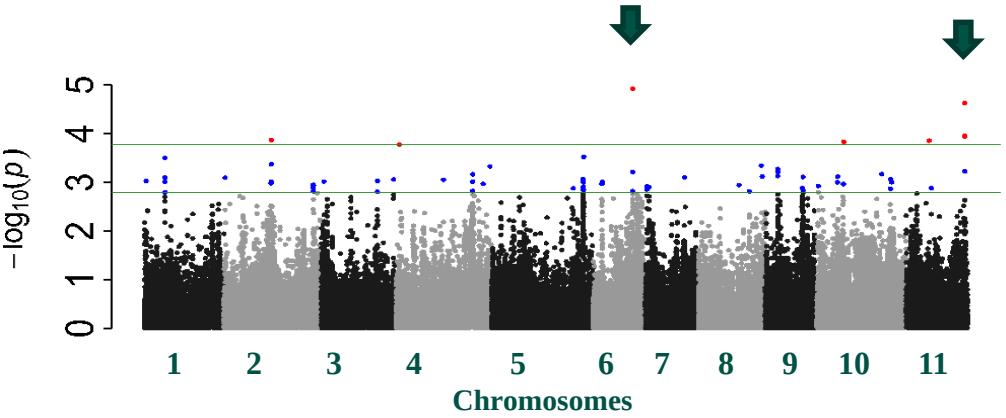
Genome-Wide Associations Studies Rust Race 20-3 (ND)



Manhattan plot for bean rust resistance to race 20-3 by using EMMA model, and b) q-q plot of EMMA model. The green line is the cutoff value to identify a significant peak. SNPs that pass the 0.01 percentile are red colored, and those falling from 0.01 to 0.1 percentile are blue colored.

- **Pv07:** within Phvul.007G046600 and Phvul.007G046700 - Unknown function
- *Ur-12*
- **Pv10:** Unknown function
- **Pv03:** Phvul.003G226600 - a novel plant snare 11

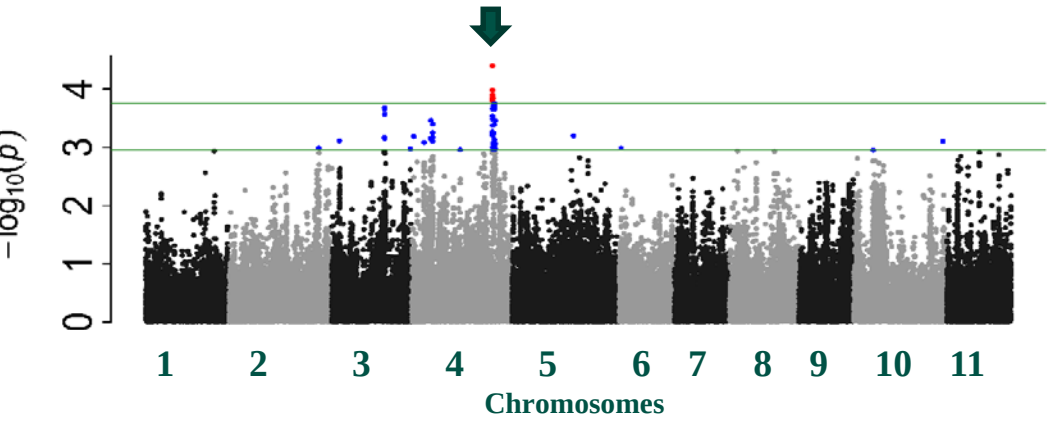
Genome-Wide Associations Studies Rust Race 63-1 (Guatemala)



- **Pv06:** Phvul.006G098100 that encode for glyoxal oxidase-related protein
- Not *Ur-4*
- **Pv11:** Phvul.011G202966 that encodes for NB-ARC
- *Ur-3*, *Ur-11* – Not *Ur-6* & *Ur-7*
- NBR cluster

a) Manhattan plot of Mixed model (EMMA+7PC) for bean rust resistance to race 31-1 and b) q-q plot of Mixed model. The green line is the cutoff value to identify a significant peak. SNPs that pass the 0.01 percentile are red colored, and those falling from 0.01 to 0.1 percentile are blue colored.

Genome-Wide Associations Studies Rust Race 31-1 (Guatemala)



- **Pv04:** nearby candidate genes Phvul.004G115700 and Phvul.004G139700 that encode for a Nucleotide Binding-Leucine Rich Repeat (NB-LRR) proteins for disease resistance.
- *Ur-5* and *Ur-14* at the opposite arm of the chromosome
- *Ur- unknown* (PI 260418)

a) Manhattan plot of Mixed model (EMMA+7PC) for bean rust resistance to race 31-1 and b) q-q plot of Mixed model. The green line is the cutoff value to identify a significant peak. SNPs that pass the 0.01 percentile are red colored, and those falling from 0.01 to 0.1 percentile are blue colored.

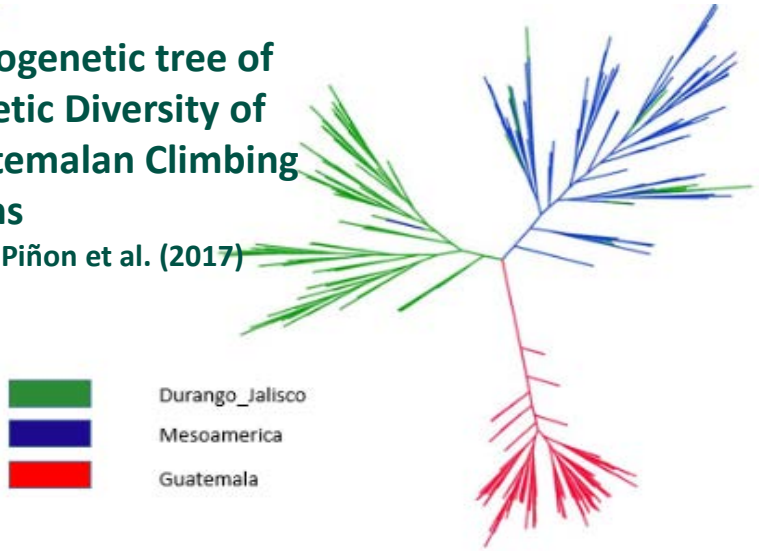
Conclusions

- Rust races 63-1 and 31-1 were identified across three Departments at the Western highlands of Guatemala.
- Race 63-1 has not been previously reported.
- Those races affected most of the resistance genes of Andean Origin.

Conclusions

- Some of those reported genomic regions associated with rust resistance might have not been reported before: Pv10 (27.54 Mb) and Pv03(45.76 Mb); Pv06 (20.86 Mb)

Phylogenetic tree of Genetic Diversity of Guatemalan Climbing beans
Tobar Piñon et al. (2017)



Further studies.

- Further marker analysis to confirm genomic regions conferring resistance on climbing beans
- 20-3: Pv07 (3.72 Mb-3.79Mb)– *Ur12*?
- 63-1: Pv11(51.87 Mb) – *Ur-3* or *Ur-11?*
- 31-1: Pv04 (39.6 Mb-40.17Mb) – *Ur- Unknown ?*

Acknowledgment

- Dr. Juan M. Osorno
- Committee members: Dr. Elias, Dr. McClean and Dr. Markell
- Dr. Steadman (UNL)
- Dry bean breeding team NDSU
- Bean Genomic & Bio Info. Lab, NDSU
- USAID Legume Innovation Lab
- ICTA Guatemala
- Department of Plant Sciences, North Dakota State University

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