CALIFORNIA LEAFY GREENS
RESEARCH BOARD

Seed Central℠
Center of Seed Excellence
and Innovation

UCDAVIS
DEPARTMENT OF PLANT SCIENCES

and

USDA Agricultural Research Service
U.S. DEPARTMENT OF AGRICULTURE

welcome you to *a special event*

Researchers funded by the California Leafy Greens Research Board speak about their research projects related to leafy greens

9:00 to 12:00
Presentations, Q&A, discussion

- **Bill Wintermantel**, USDA-ARS
- **Ivan Simko**, USDA-ARS
- **Beiquan Mou**, USDA-ARS
- **Kelley Richardson**, USDA-ARS
- **Maria Truco** (for Richard Michelmore), UC Davis
- **Maeli Melotto**, UC Davis
- **Allen Van Deynze**, UC Davis
- **Marilyn Warburton/Alex Cornwall**, USDA-ARS

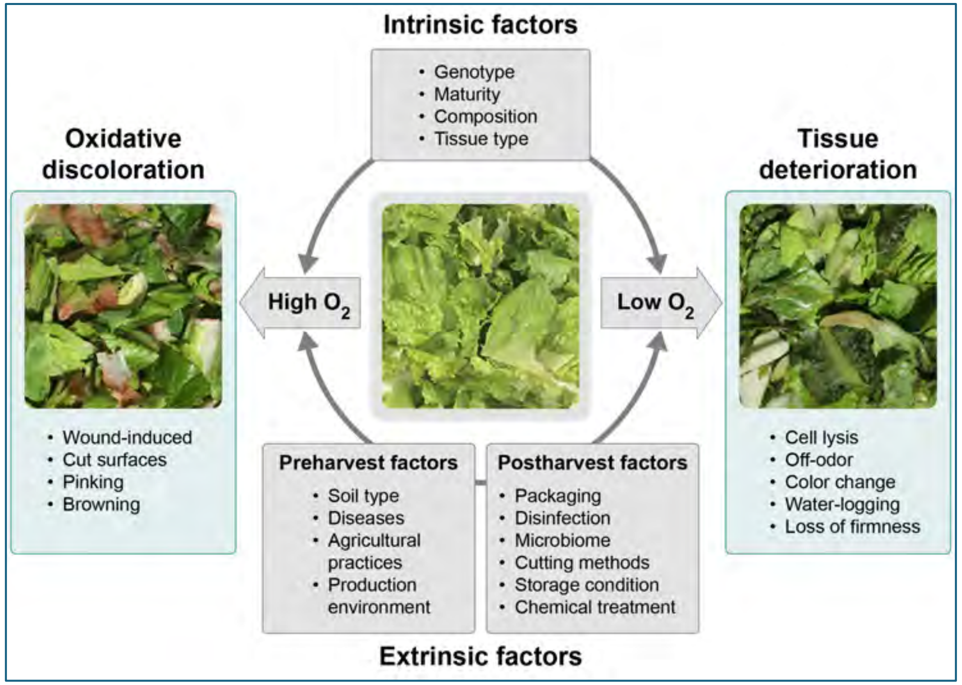
12:00 to 1:00
Networking lunch

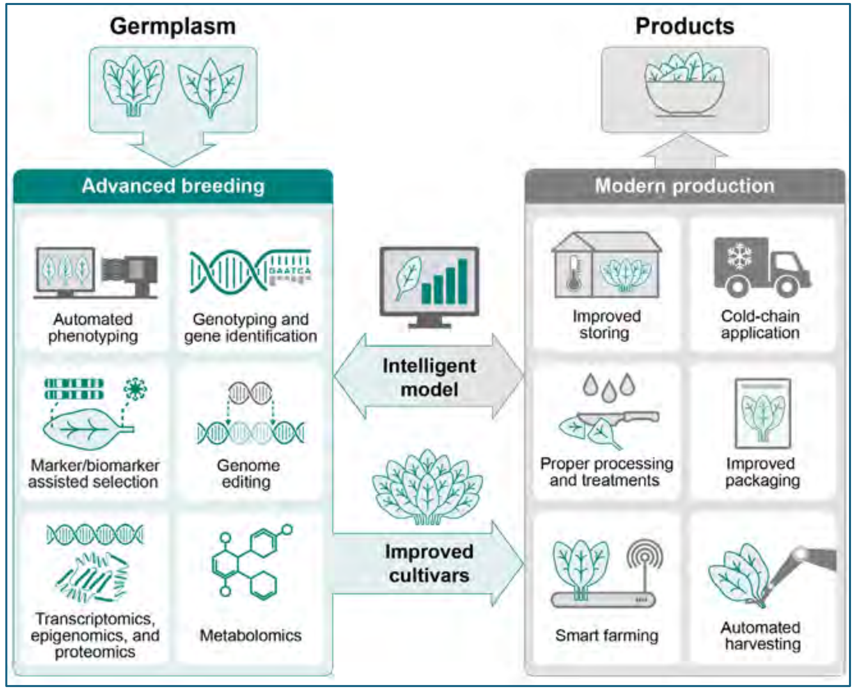
Breeding Lettuce for Biotic and Physiological Stress Resilience and Extended Shelf-Life

Ivan Simko

USDA-ARS, Salinas, California

Extending lettuce shelf life through integrated technologies





Factors contributing to enzymatic discoloration and rapid tissue deterioration of fresh-cut lettuce. Though both physiological processes can occur at a range of O_2 levels, enzymatic discoloration starts earlier and is more intense at relatively high O_2 levels, while rapid tissue deterioration is more noticeable at relatively low O_2 levels. It is conceivable that other compounds existing in the headspace atmosphere (e.g. CO_2) play a part in the initiation or progress of these processes.

Diagram depicting multiple steps involved in the development and the production of high-quality lettuce products. A diverse germplasm is used to develop new cultivars with a desirable performance, including low enzymatic discoloration and a slow rate of tissue deterioration after processing.

Peng and Simko, 2023

Current Opinions in Biotechnology

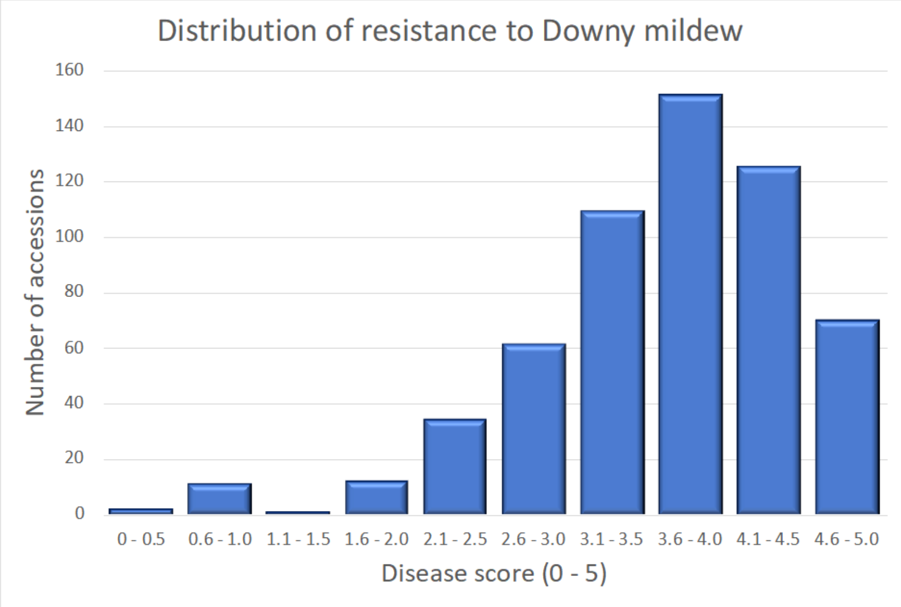
Shelf life gene (*qSL4*) functional studies

Rapid
deterioration
check cv.
La Brillante

Slow
deterioration
check cv.
Salinas

Resistance to downy mildew (*Bremia lactucae*)

Downy mildew
(Bremia lactucae)



- Focusing on polygenic resistance
- Combining multiple resistance genes into a single line
- Released breeding lines of leaf and iceberg-like types
- Most resistant lines show almost no disease when tested across multiple years and several races of the pathogen

Simko *et al*. 2023: Theoretical and Applied Genetics

Resistance to lettuce dieback (*TBSV/LNSV/MPV*)

Lettuce dieback
(TBSV/LNV/MPV)

- Soilborne viral disease
- Vector not yet identified
- No known methods to prevent the disease
- *Tvr1* – single dominant gene that provides durable resistance
- Released first romaine breeding lines (SM09A, SM09B, SM13-R2, and SM13-R3) with combined resistance to dieback and long shelf life
- Resistance gene originates from primitive accessions PI 491214 and PI 491224
- Other resistance haplotypes of *Tvr1* identified in iceberg-type accessions (e.g., cv. Salinas) and wild species *Lactuca serriola*, *L. saligna*, and *L. virosa*
- Developed PCR-based molecular marker assay to identify *Tvr1*
- New breeding lines are tested with the molecular marker assay to determine presence of resistance loci

Resistance to lettuce Verticillium wilt race 1

Verticillium wilt

(Verticillium dahliae)

- Soilborne fungal disease
- *Vr1* – single dominant gene that provides resistance against race 1
- Resistance locus (*LsVe1L*) identified in cv. La Brillante, but found in several other cultivars of all types (e.g., cv. Annapolis, Defender, Eruption, Little Gem, Lolla Rossa, Merlot, Pavane, Sentry)
- Developed PCR-based molecular marker assay to identify *Vr1*
- New breeding lines are tested with the molecular marker assay to determine presence of resistance loci
- Released iceberg breeding lines with combined resistance to Verticillium wilt race 1 and corky root

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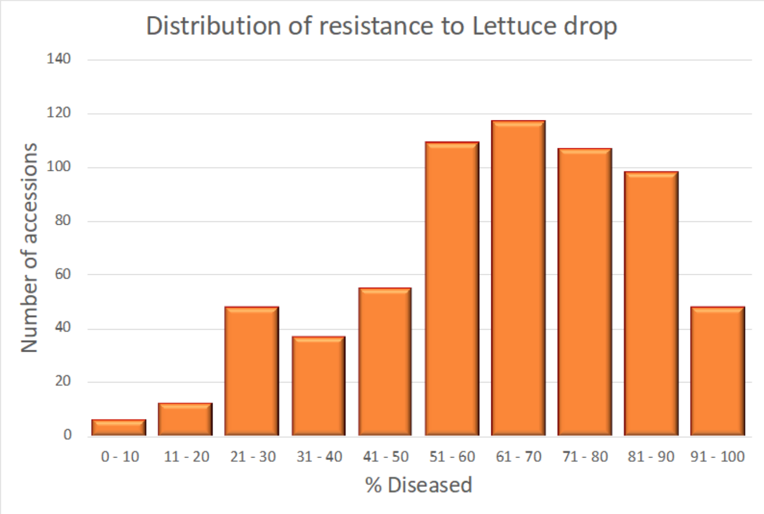
- Performed tests for resistance to race 3 of Verticillium wilt
- Partial resistance found in cv. Sentry
- Cv. Sentry is resistant against both races 1 and 3 of the pathogen, cv. La Brillante is resistant to race 1 but susceptible to race 3

Resistance to lettuce drop (*Sclerotinia minor*)

Lettuce drop
(Sclerotinia minor)

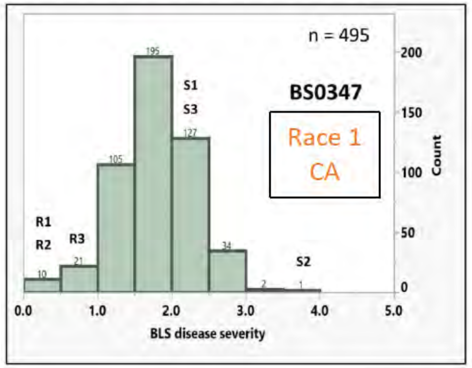


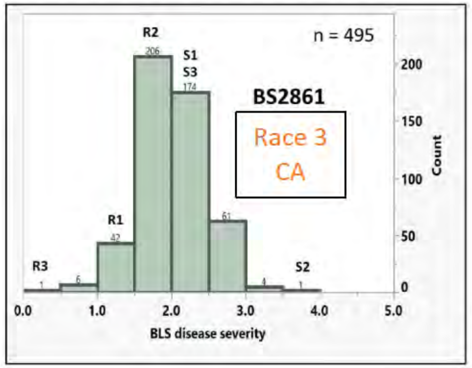
- No accession complete resistant to the disease
- Stem degradation influenced by localized microenvironment around the stem base
- Plant stem composition (lignin and hemicellulose) affects stem degradation by the pathogen
- Released romaine breeding lines (crosses with Latin cv. Eruption)
- Better resistance than in any commercially grown, green-colored romaine cultivar

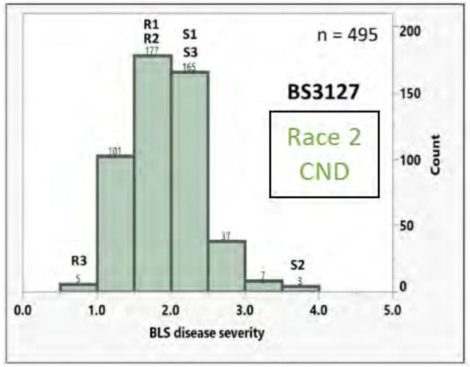
Simko *et al*. 2023: HortScience

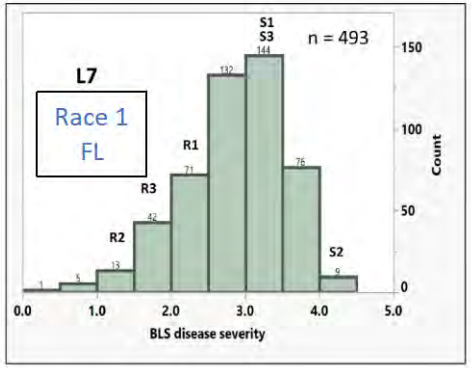
Resistance to Bacterial leaf spot (*Xanthomons hortorum* pathovar *vitians*)

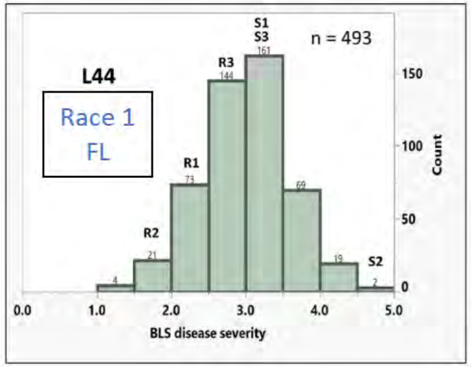
Bacterial leaf spot
(Xanthomonas hortorum pv. vitians)

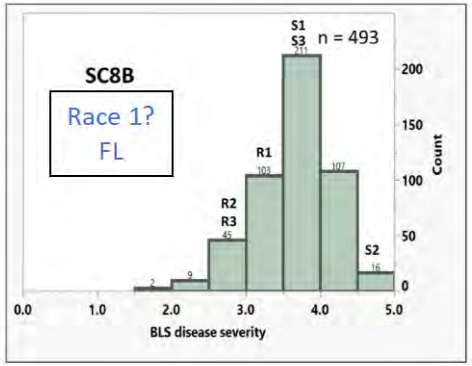












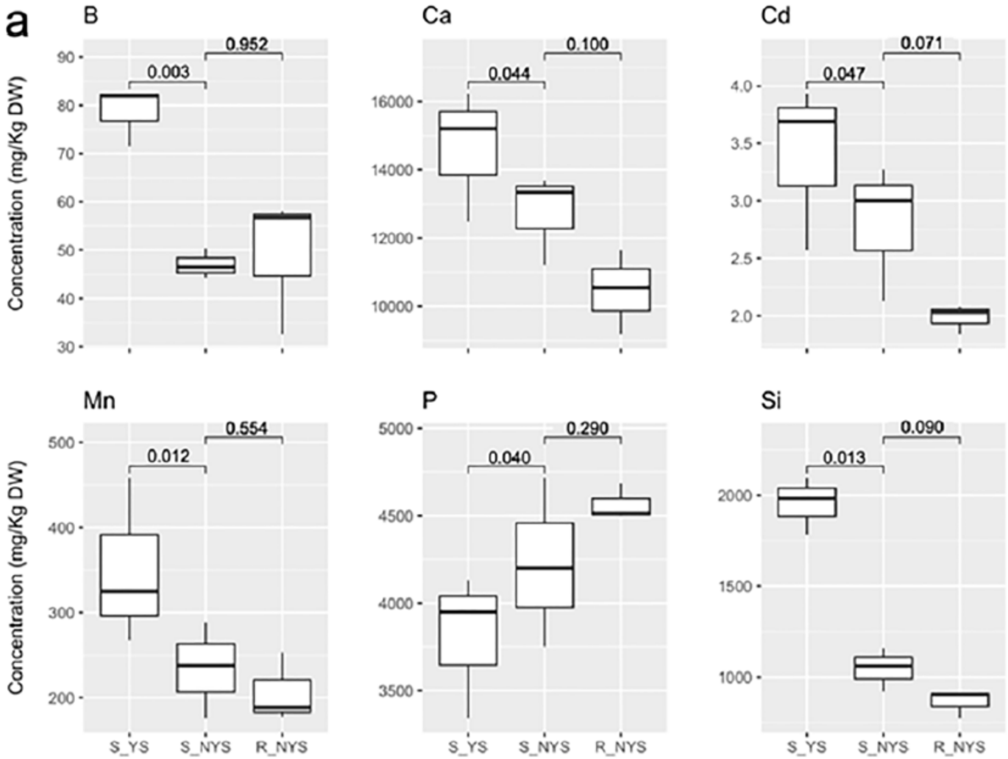
Accessions with an overall highest resistance:

- Bunte Forellen, PI 226514, La Brillante, ARM09-161-10-1-4, Grenadier, Bella, PI 491210, Delight, Romana Verde del Mercado

Sthapit Kandel *et al*. 2022: Plant Disease

Resistance to yellow spot malady (YSM)

Yellow spot malady



Yellow area vs. green area of affected plant:

- boron, calcium, cadmium, manganese, phosphorus, silicon
- Most resistant accessions: Auburn, Grand Rapids, Romabella, PI 226514, and PI 249536

Peng *et al*. 2022: Scientia Horticulturae

Resistance to INSV (*Impatiens Necrotic Spot Virus*)

Impatiens necrotic spot virus
(INSV)

- Virus transmitted by western flower thrips (*Frankliniella occidentalis*)
- No accession completely resistant to the disease
- Accessions with lower disease incidence generally associated with:
  - Later plant maturity
  - Dark red color (high anthocyanin content)
  - Fewer thrips (evaluated as feeding damage)
  - Light green color (lower chlorophyll content)
- Most resistant accessions:

  Outredgeous Selection, Red Splash Cos, Infantry, Sweet Valentine, Annapolis, Velvet, Rubens Red, Eruption, Bunte Forellen, Romaserra

Simko *et al*. 2023: Frontiers in Plant Science

Resistance to INSV (*Impatiens Necrotic Spot Virus*)

Tested

- ~500 lettuce accessions
- 23 field experiments

Inducing phenotypic diversity in cultivar Eruption

Robust resistance to:

- INSV
- Lettuce drop (*Sclerotinia minor*)
- Verticillium wilt race 1 (*LsVe1L*)

Susceptibility to:

- Bacterial leaf spot
- Dieback (TBSV/LNSV)
- Fusarium wilt (?)
- Tipburn

Acceptable traits:

- Resistance to downy mildew
- Postharvest tissue deterioration
- Bolting

Limitations:

- Small head size
- Red color
- Leaf shape
- Enzymatic discoloration

Inducing phenotypic diversity in cultivar Eruption

Photo: Rebecca Zhao

Inducing phenotypic diversity in cultivar Eruption

Photo: Rebecca Zhao

INSV resistance in Eruption mutant lines

Greenhouse INSV evaluation

- Kelley Richardson’s group
- 26 mutant lines
- Plus Eruption and Defender

Breeding for Leafminer Resistance in Lettuce

Beiquan Mou
Research Geneticist
Sam Farr United States Crop Improvement and Protection Research Center
USDA-ARS
Salinas, California

Leafminer

Leafminers

- Predominant species in central California is *Liriomyza langei*.
- Wide host range including broccoli, cauliflower, celery, lettuce, melons, spinach, tomato, and many weeds.
- Chemical control is not long-lasting, and leafminers can develop a high degree of resistance to insecticides.

Question #1

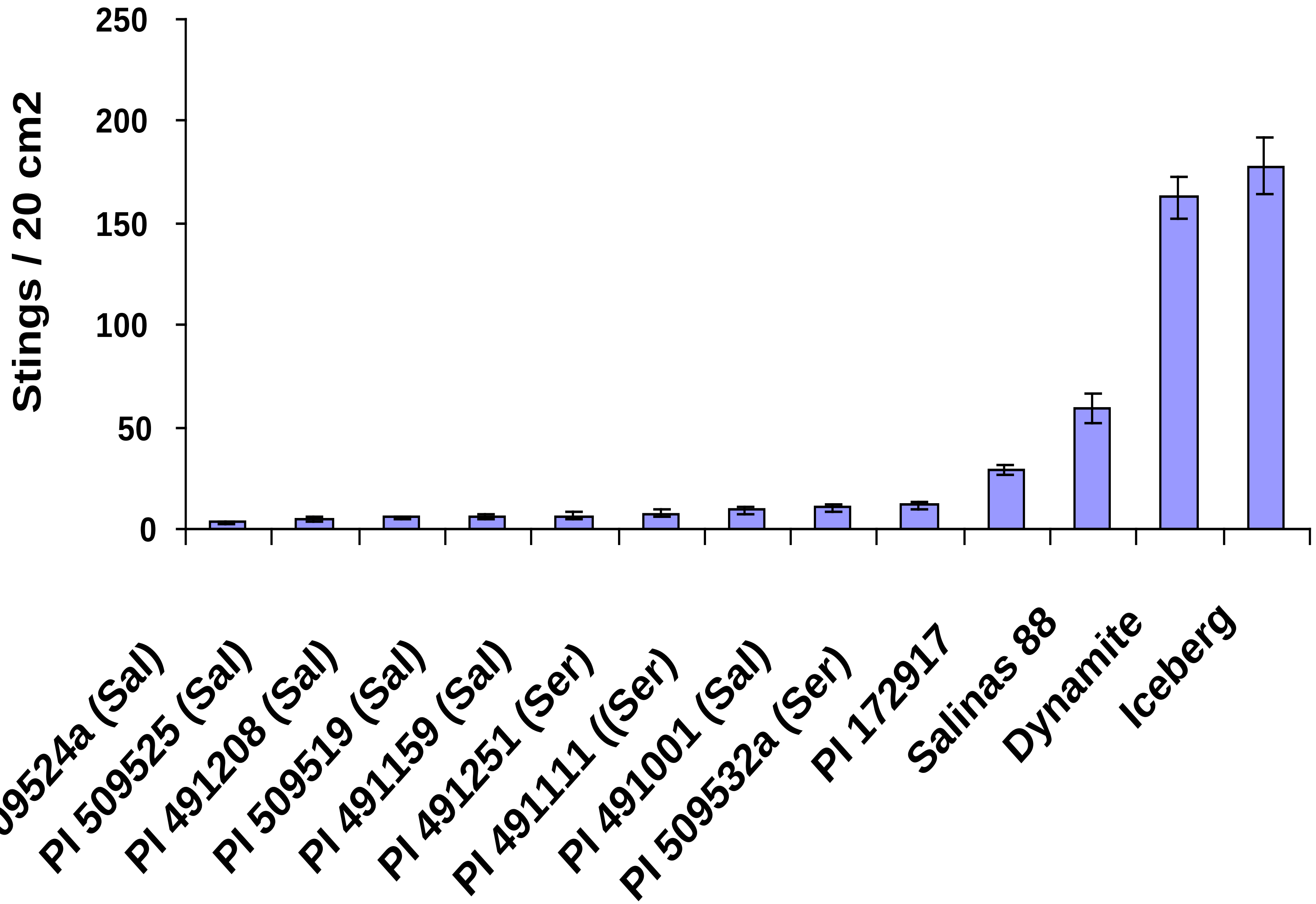
Are there any differences in leafminer resistance among lettuce genotypes ?

Screening for Leafminer Resistance in Lettuce

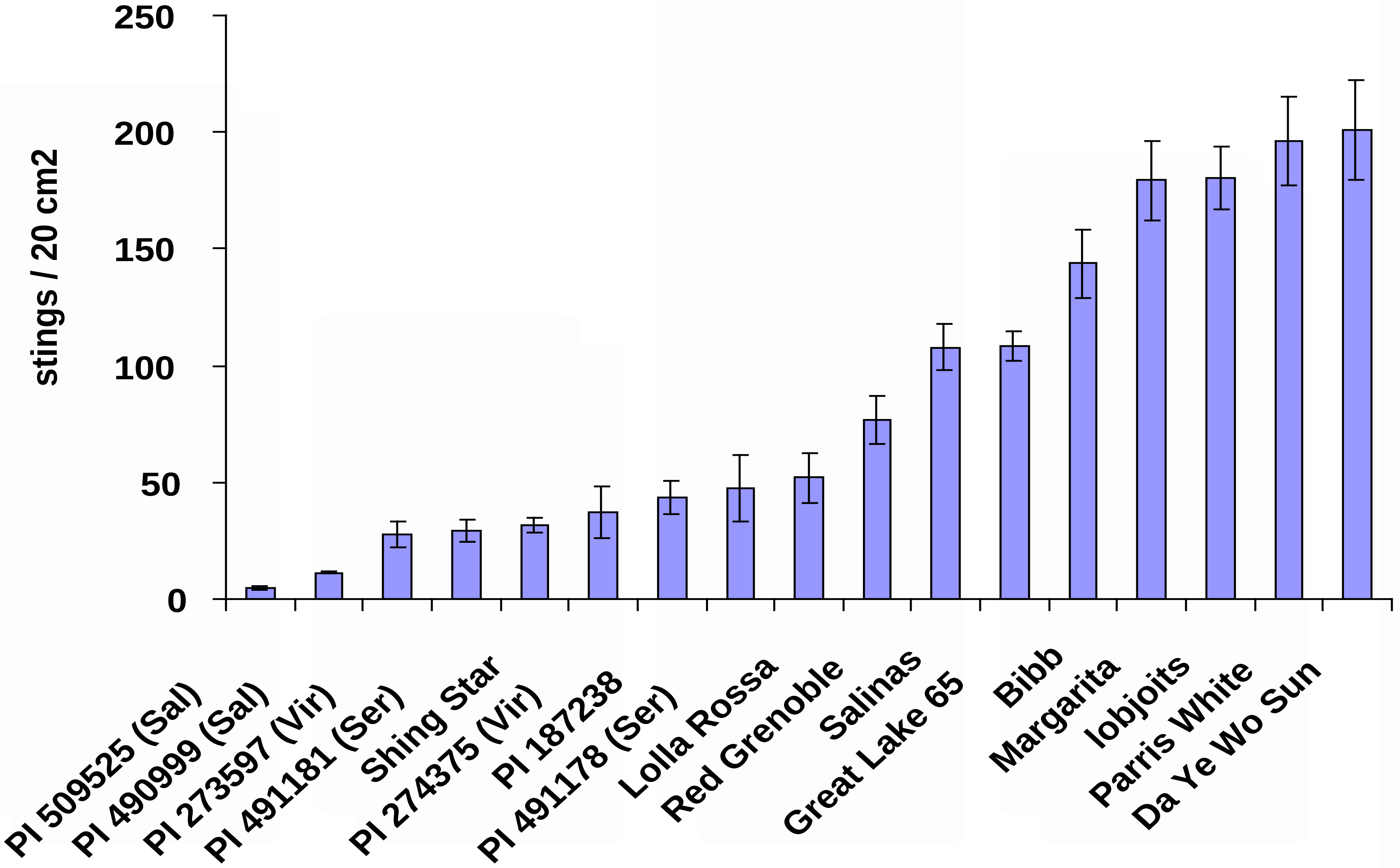
- Screened more than 200 lettuce varieties in replicated tests in insect cages, and the results were confirmed in field tests.

- Thirty-four resistant varieties, mostly wild species or primitive types, were identified. Leafminer sting density among cultivated lettuces: romaine > butterhead > crisphead > leaf type.

leafminer Stings in Cage



Leafminer Stings in the Field



Question #2

What is the mechanism of leafminer resistance ?


WARNING
AVISO

Genotype	Fly life day	Stings /20cm^2	Stings /plant	Mines /Plant	Pupae /plant
PI 187238	5.3	32.3	205	5.7	0.0
PI 273597 (vir.)	4.6	37.4	229	14.0	0.0
PI 491178 (ser.)	6.0	49.1	138	1.1	0.0
PI 509525 (sal.)	10.1	6.1	40	0.0	0.0
Red Grenoble	9.4	53.0	402	29.0	0.0
Salinas	8.9	385.0	2,923	5.7	1.9
Da Ye Wo Sun	11.7	860.7	5,959	20.1	5.4
$LSD_{0.05}$	2.1	146.4	1,268	4.6	1.0

Phenotypic Correlation Coefficients

	Leafminer stings/cm^2		Mines/plant
	30 DAP	65 DAP	30 DAP
Leafminer sting/cm^2 30 DAP	----	0.923**	0.603*
Aphids/100 g plant	- 0.080	- 0.018	- 0.074
G. Sucrose concentration	0.657**	0.730**	0.790**
F. Flavonoid concentration	- 0.467	- 0.560*	- 0.700**
F. Chlorogenic acid	- 0.484	- 0.598*	- 0.594*
F. Rutin	- 0.379	- 0.491	- 0.603*

* $P = 0.05$

** $P = 0.01$

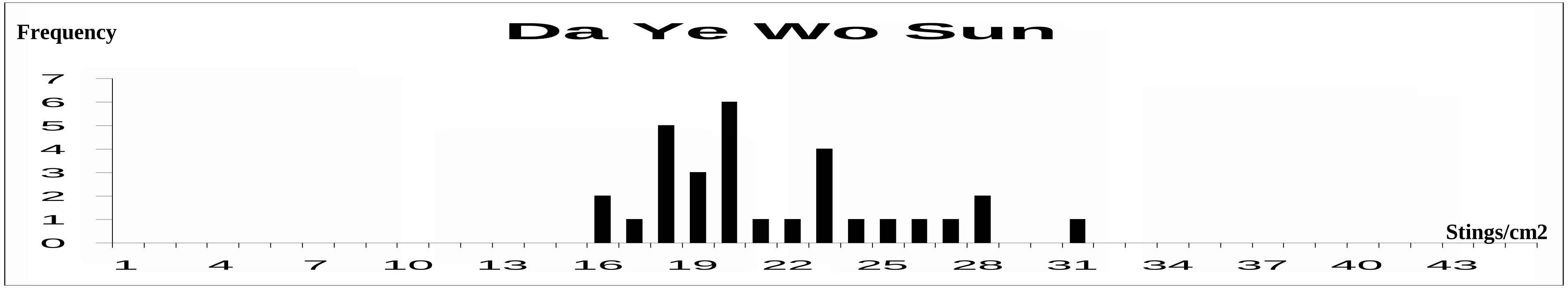
Question #3

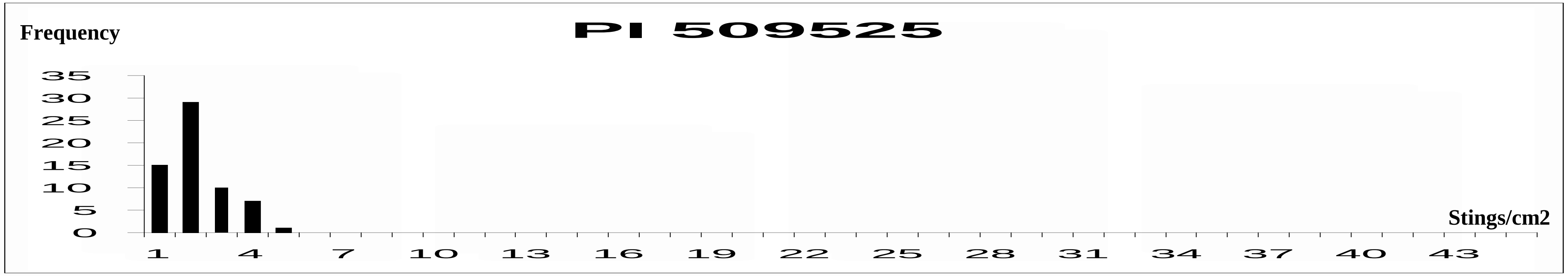
Are leafminer resistance traits heritable ?

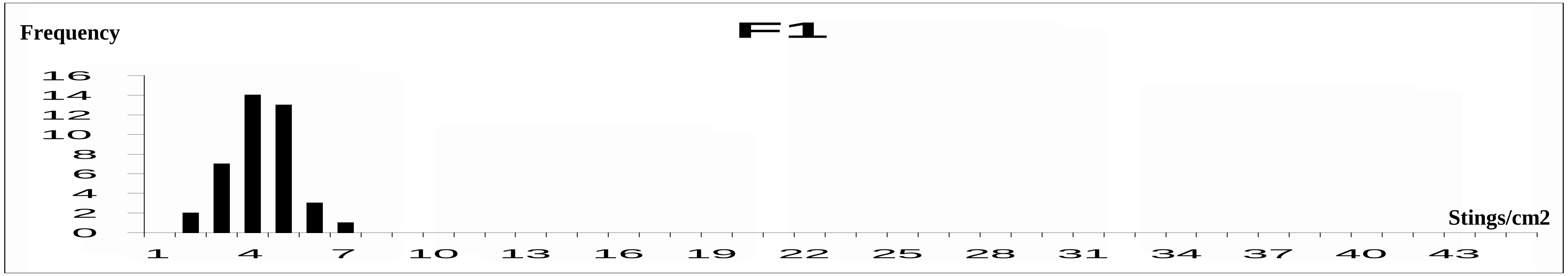
Study of the Inheritance of Leafminer Sting Density

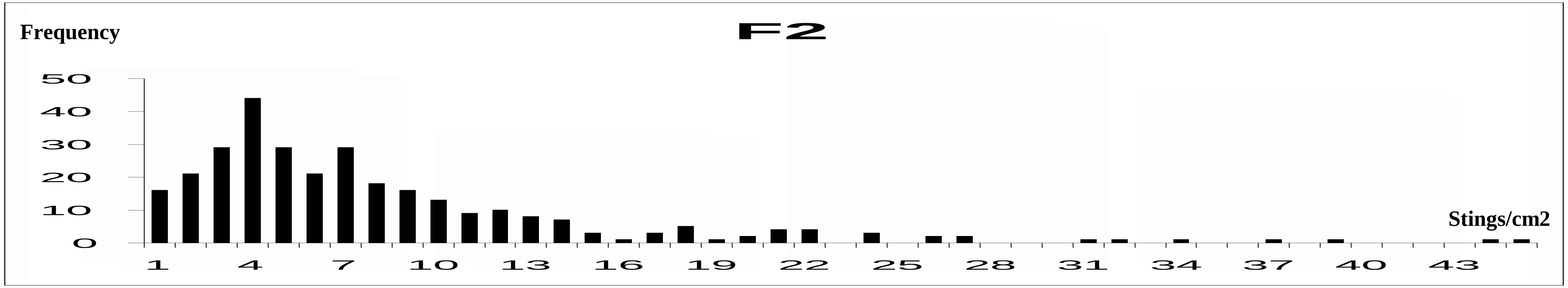
- Crosses were made between a *L. saligna* line PI 509525 (low sting density), and a stem lettuce cultivar ‘Da Ye Wo Sun’ and a butterhead cultivar ‘Margarita’(high sting density).

- Parents, F_1, and F_2 plants were transplanted in the field and number of stings per unit leaf area was recorded.


Da Ye Wo Sun


PI 509525


F1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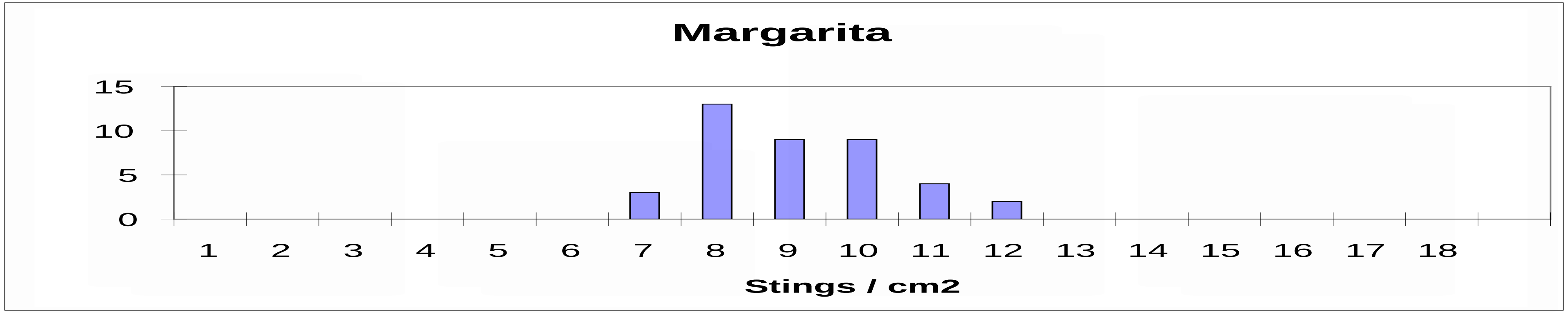
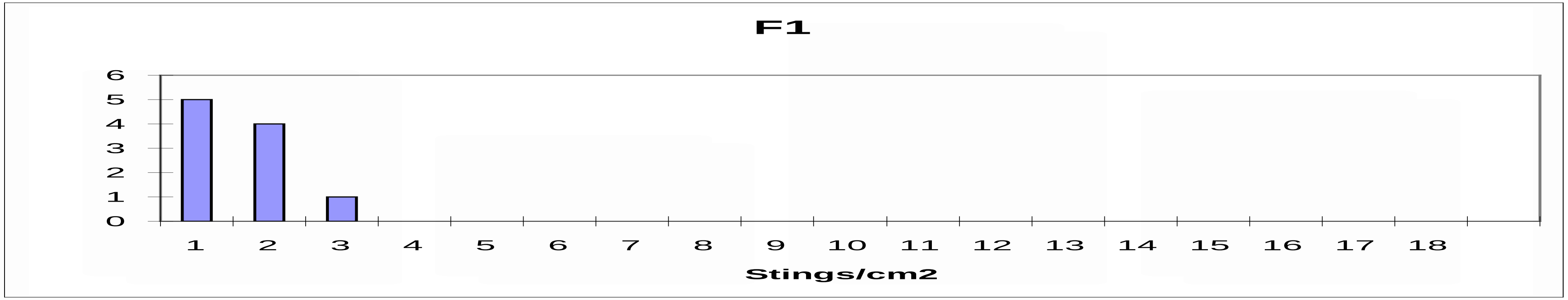

Margarita


PI 509525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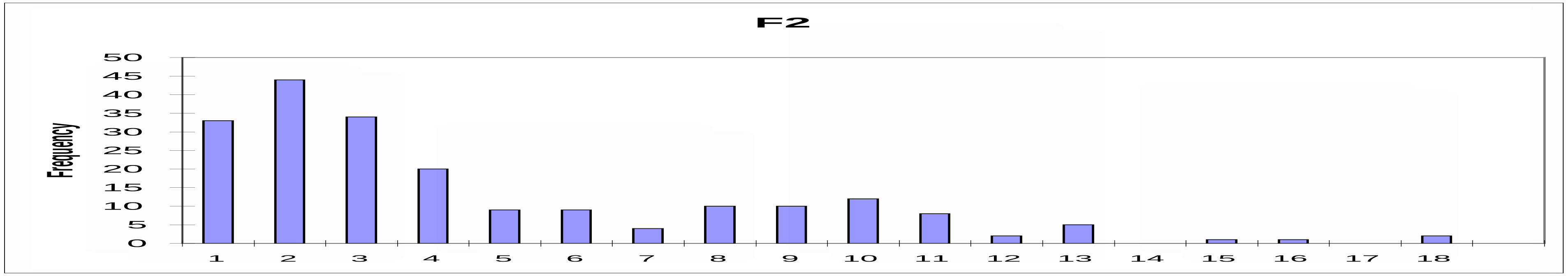

F2

Question #4

Can leafminer resistance be transferred into elite cultivars?

Leafminer/Downy Mildew Resistance Trial

Green Leaf Lettuce

		Plant	Core	Downy
Genotype	Stings/cm^2	Wt. (g)	length (cm)	mildew
Waldmann’s Green	3.5 A	380.3 AB	7.7 A	3.3 B
Iceberg	3.1 A	367.5 AB	5.3 B	1.8 C
Grand Rapids	2.3 B	273.2 B	5.4 B	2.0 C
Two Star	2.3 B	306.4 AB	4.9 B	4.0 A
Shining Star	2.3 B	288.2 B	5.6 B	4.0 A
MU18-283 (*cor*)	0.4 C	436.0 A	4.7 B	1.5 C
MU18-247 (*cor*)	0.3 C	287.0 B	4.5 B	2.0 C

Leafminer/Downy Mildew Resistance Trial

Red Leaf Lettuce

Genotype	Stings/cm^2.	Plant Wt. (g)	Core length (cm)	Downy mildew
Big Red	4.4 A	374.3 B	7.0 A	3.0 B
Prizehead	3.8 A	414.5 B	5.9 A	4.0 A
Red Fox	3.8 A	591.3 A	7.8 A	3.5 AB
Lolla Rossa	2.0 B	180.8 C	7.3 A	2.0 C
Merlot	1.8 B	205.1 C	7.9 A	1.3 D
MU19-281	0.5 C	235.8 C	6.0 A	1.5 CD

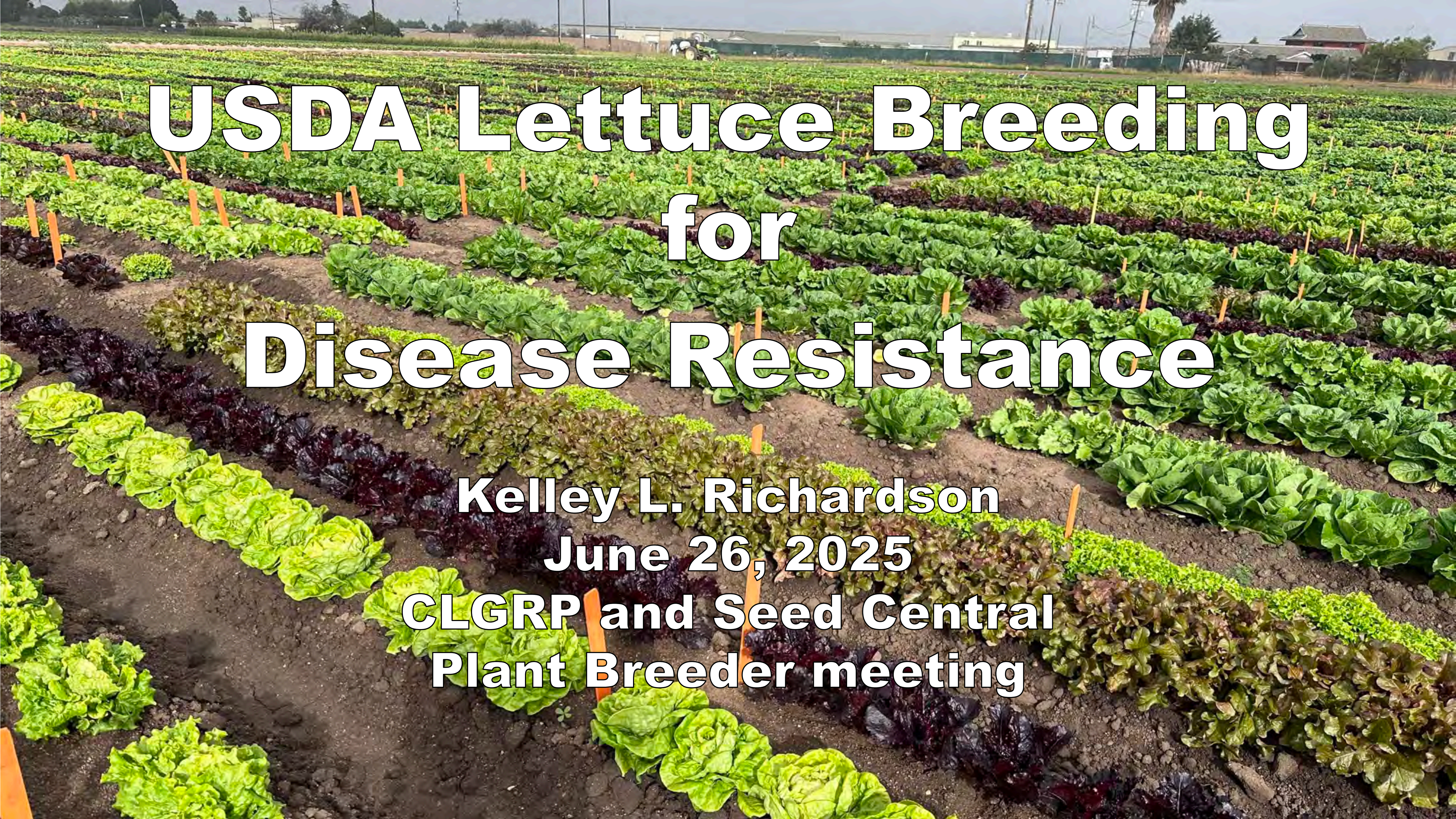
Leafminer Resistance Trial

Romaine Lettuce

Genotype	Stings/cm^2	Plant Wt. (g)	Core length (cm)	Tipburn
Red Hot	5.2 A	600.2 ABC	6.5 B.	0.3 BC
Green Forest	3.6 B	756.9 A	7.8 A	0.0 C
Parris Island Cos	3.6 B	295.6 D	5.3 C	0.0 C
Heart's Delight	3.5 B	441.7 CD	6.0 BC	1.6 AB
Valmaine	3.3 B	483.4 BCD	6.1 BC	2.4 A
Green Towers	2.7 C	453.0 CD	5.6 BC	0.4 BC
MU19-371 (*cor*)	0.3 D	699.7 AB	6.6 AB	1.3 ABC
MU19-290 (*cor*)	0.3 D	746.0 A	6.7 AB	0.0 C

Summary

1. There are significant differences in leafminer stings and mines among lettuce genotypes.
2. Multiple mechanisms of leafminer resistance exist in lettuce varieties. Leafminer sting density is positively correlated to sucrose concentration, but negatively associated with concentrations of phenolic compounds.
3. The low leafminer sting density in PI 509525 is controlled by a single dominant gene.
4. Different types of lettuce with leafminer and multiple resistances have been developed and released by the USDA-ARS.

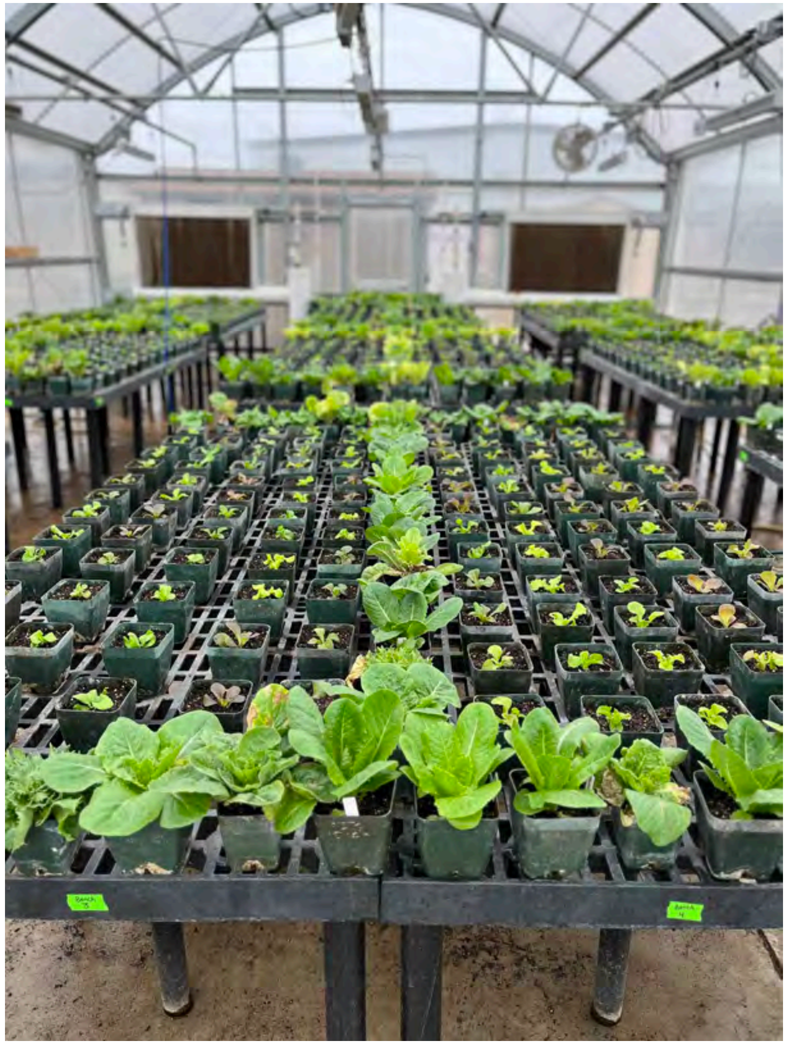


USDA Lettuce Breeding for Disease Resistance

Kelley L. Richardson
June 26, 2025
CLGRP and Seed Central
Plant Breeder meeting

Today’s Presentation

- **USDA-ARS mission and our role in breeding**
- **2 Case studies of ongoing industry collaboration**
    - **INSV**
    - **Fusarium**
- **Opportunities for future collaboration**



USDA Agricultural Research Service

- USDA-ARS Mission:
  - Deliver cutting-edge, scientific tools and innovative solutions for US growers, industry, and communities
- Industry has historically asked us to serve as pre-breeders
- Develop strategic plans to meet stakeholders' needs and support USDA's mission
- Scientists frequently collaborate with universities, companies, other organizations, and other countries
- We share research results at conferences, field days, grower meetings, publications

Review of INSV breeding efforts

- **Minor INSV in Monterey county prior to 2015**
- **In 2018, saw a significant increase in INSV in commercial and research fields**
- **Implemented a field evaluation protocol in 2020**

INSV severity rating

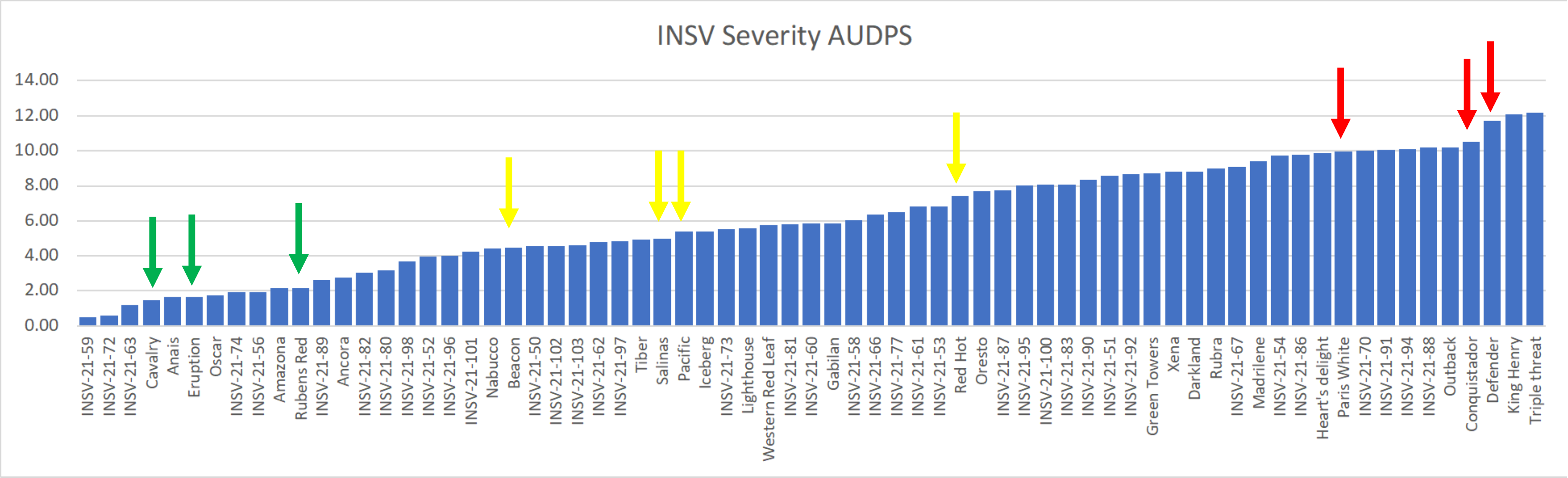
- 10 plants per seedline flagged for weekly evaluation
- Rated each plant for INSV severity (0-5) at 6, 7, 8, and 9 weeks after planting

	Plant 1	Plant 2	Plant 3	Plant 4	Plant 5	Plant 6	Plant 7	Plant 8	Plant 9	Plant 10	Date	MEAN
Week 1	0	1	0	0	0	0	0	0	0	1	9/28	.2
Week 2	1	2	1	1	1	0	0	1	0	1	10/5	.8
Week 3	2	2	2	2	2	1	1	2	1	1	10/1	1.6
Week 4	2	2	2	2	2	2	2	2	2	2	10/1	2
Week 5	2	2	2	3	2	3	2	2	2	4	10/2	2.4

Hasegawa and Del Pozo-Valdivia. 2023. Plant Disease 107

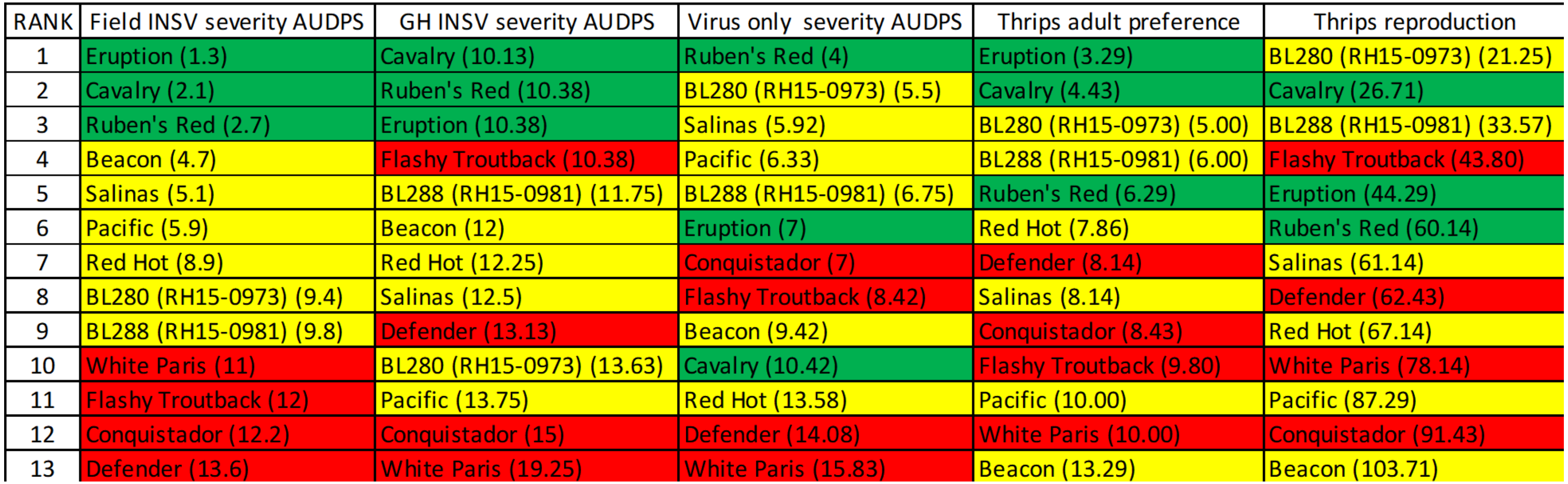
Germplasm evaluation

- 2021, 2022, (and 2023) June and August plantings at Spence Farm
- Tested breeding lines, commercial varieties, and wild material of any color and head type
- From BASF, Enza Zaden, GreenGo, Nipomo Native Seed, Sakata, Seminis, Vilmorin-Mikado
- Selected material consistently resistant, intermediate, or susceptible

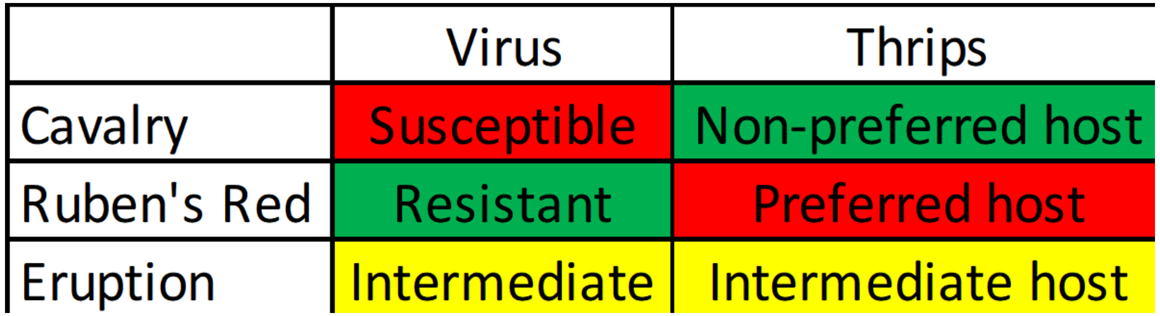


Dissecting mechanisms of resistance

- **Selected material tested in the greenhouse and growth room**
- **INSV severity AUDPS in the field, greenhouse, and virus only**
- **Number of adult (preference) and immature (reproduction) thrips**

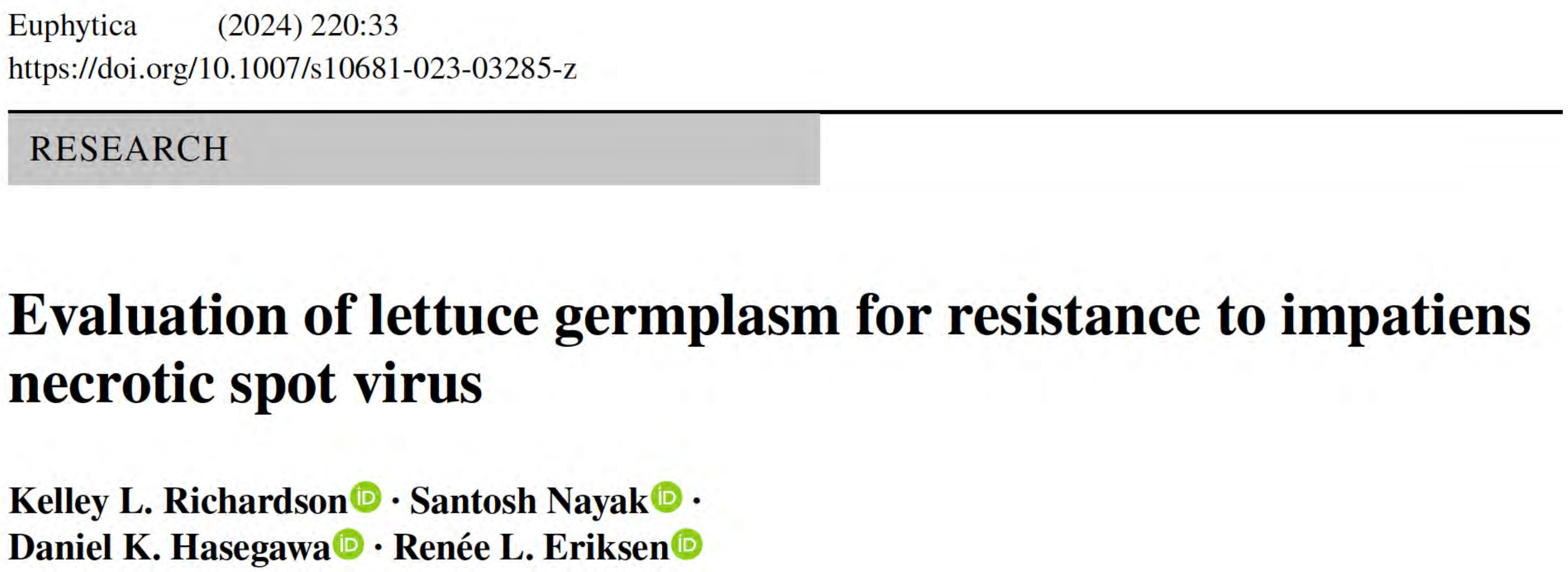


RANK	Field INSV severity AUDPS	GH INSV severity AUDPS	Virus only severity AUDPS	Thrips adult preference	Thrips reproduction
1	Eruption (1.3)	Cavalry (10.13)	Ruben's Red (4)	Eruption (3.29)	BL280 (RH15-0973) (21.25)
2	Cavalry (2.1)	Ruben's Red (10.38)	BL280 (RH15-0973) (5.5)	Cavalry (4.43)	Cavalry (26.71)
3	Ruben's Red (2.7)	Eruption (10.38)	Salinas (5.92)	BL280 (RH15-0973) (5.00)	BL288 (RH15-0981) (33.57)
4	Beacon (4.7)	Flashy Troutback (10.38)	Pacific (6.33)	BL288 (RH15-0981) (6.00)	Flashy Troutback (43.80)
5	Salinas (5.1)	BL288 (RH15-0981) (11.75)	BL288 (RH15-0981) (6.75)	Ruben's Red (6.29)	Eruption (44.29)
6	Pacific (5.9)	Beacon (12)	Eruption (7)	Red Hot (7.86)	Ruben's Red (60.14)
7	Red Hot (8.9)	Red Hot (12.25)	Conquistador (7)	Defender (8.14)	Salinas (61.14)
8	BL280 (RH15-0973) (9.4)	Salinas (12.5)	Flashy Troutback (8.42)	Salinas (8.14)	Defender (62.43)
9	BL288 (RH15-0981) (9.8)	Defender (13.13)	Beacon (9.42)	Conquistador (8.43)	Red Hot (67.14)
10	White Paris (11)	BL280 (RH15-0973) (13.63)	Cavalry (10.42)	Flashy Troutback (9.80)	White Paris (78.14)
11	Flashy Troutback (12)	Pacific (13.75)	Red Hot (13.58)	Pacific (10.00)	Pacific (87.29)
12	Conquistador (12.2)	Conquistador (15)	Defender (14.08)	White Paris (10.00)	Conquistador (91.43)
13	Defender (13.6)	White Paris (19.25)	White Paris (15.83)	Beacon (13.29)	Beacon (103.71)



	Virus	Thrips
Cavalry	Susceptible	Non-preferred host
Ruben's Red	Resistant	Preferred host
Eruption	Intermediate	Intermediate host

INSV publication



Euphytica (2024) 220:33
https://doi.org/10.1007/s10681-023-03285-z

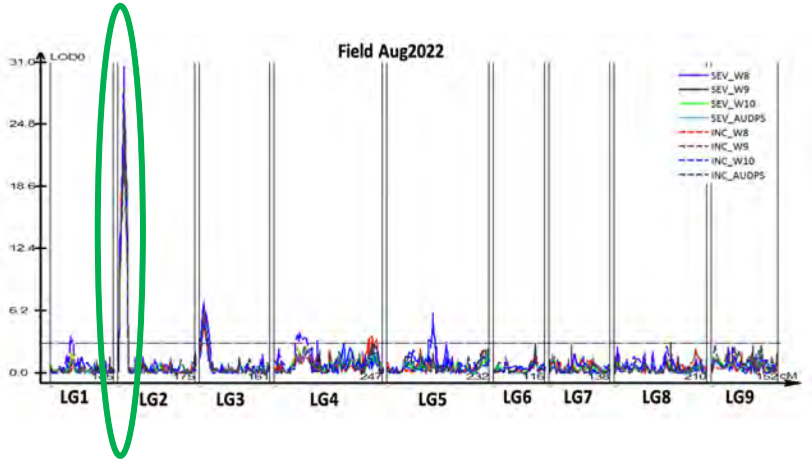
RESEARCH

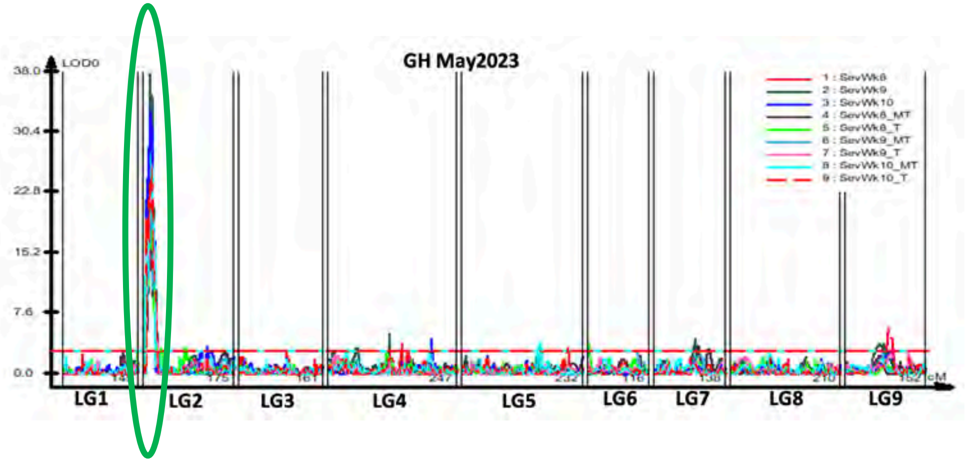
Evaluation of lettuce germplasm for resistance to impatiens necrotic spot virus

**Kelley L. Richardson · Santosh Nayak ·
Daniel K. Hasegawa · Renée L. Eriksen**

Genetic location for marker-assisted selection

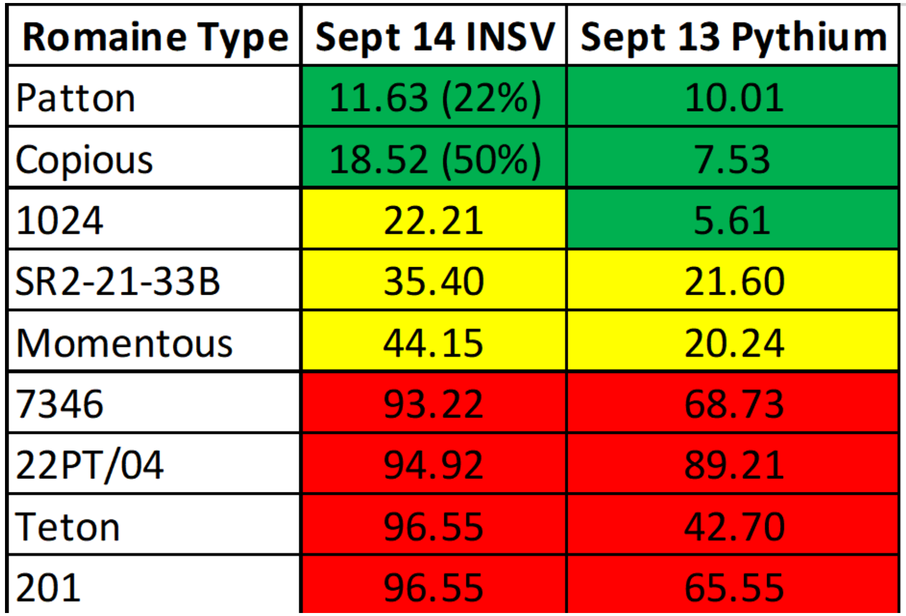
- Learn where the genes are and find linked markers
- MAS allows rapid introgression of resistance
- Pair field and greenhouse data with genetic linkage map
- Highly significant QTL on linkage group 2
- Fine mapping collaboration with Enza Zaden
- Developing MAS assay



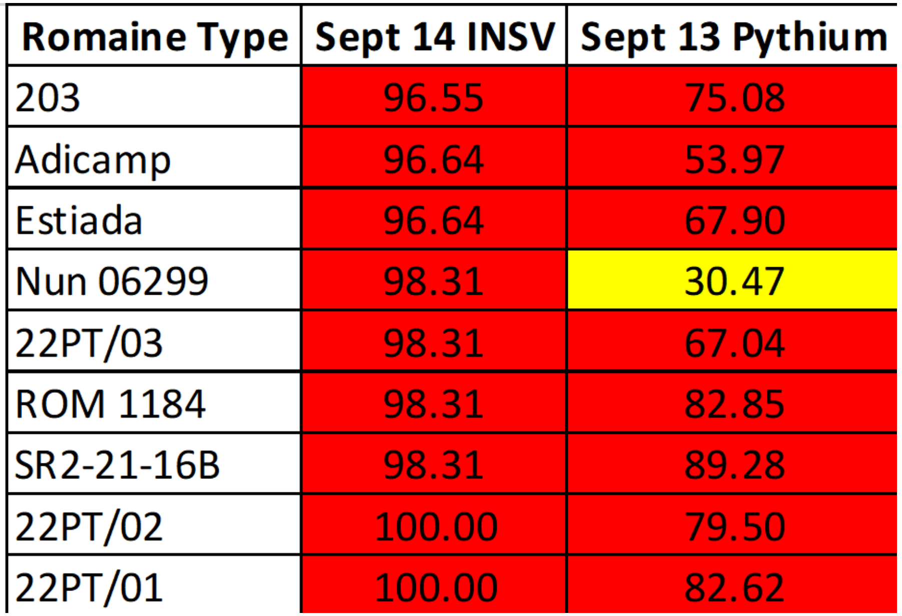


What about solutions today?

- Breeding takes time!
- Evaluate popular commercial varieties available NOW with Yu-Chen Wang and J.P. Dundore-Arias
- 2022 and 2023 Pythium/INSV variety trials



Romaine Type	Sept 14 INSV	Sept 13 Pythium
Patton	11.63 (22%)	10.01
Copious	18.52 (50%)	7.53
1024	22.21	5.61
SR2-21-33B	35.40	21.60
Momentous	44.15	20.24
7346	93.22	68.73
22PT/04	94.92	89.21
Teton	96.55	42.70
201	96.55	65.55



Romaine Type	Sept 14 INSV	Sept 13 Pythium
203	96.55	75.08
Adicamp	96.64	53.97
Estiada	96.64	67.90
Nun 06299	98.31	30.47
22PT/03	98.31	67.04
ROM 1184	98.31	82.85
SR2-21-16B	98.31	89.28
22PT/02	100.00	79.50
22PT/01	100.00	82.62

2024 and 2025 INSV Variety Trials

- **Due to low field incidence, added greenhouse test under high pressure**
- **Some did well in both field and greenhouse (green)**
- **Some have pressure-dependent resistance (yellow)**
- **Some with potential sensitivity to Pythium (pink)**
- **Lines as susceptible as the check in both field and greenhouse (no color)**

History of Fusarium in California

- Original isolate from Huron in 1990
- Imperial Valley, CA and Yuma, AZ in 2001
- Reported in all California growing areas by 2012
- Was rarely seen in cooler temperatures of Salinas until 2019

Changing pathogen population?

- **August 2021 received infected plants from Salinas Valley Seeds.**
- **Increase in *Fol* incidence and severity in past 4 years**
- **Previously resistant varieties were showing disease and susceptible varieties were showing resistance**

Root-dip inoculation protocol

Grow seedlings in pasteurized sand

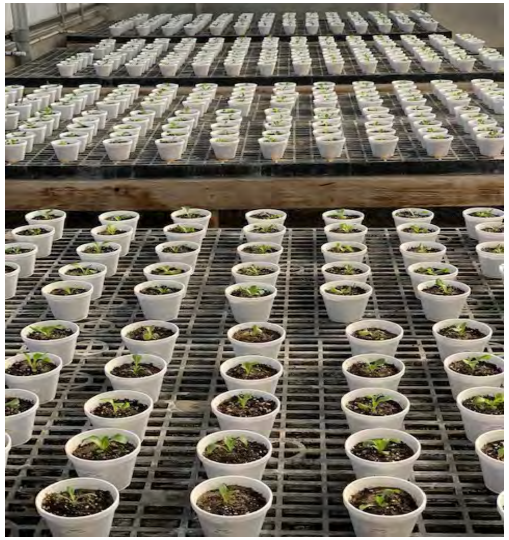
14 to 18 days old seedlings

Trim root ~ 5 mm

Dip root for 20 min (Treatments: FOL isolates and Mock)

Transplant in cups filled with pasteurized potting soil/sand mix

Greenhouse / growth room (25 ℃ / 16 h photoperiod)



Disease rating scale

Nayak et al., 2024

1= No symptom

2 = Mild stunting

3 = Stunting and some leaf yellowing and/or necrosis

4 = Dead plant

Characterization of Fusarium isolates

- **Fol321**
- **Fol621**

Recovered from infected lettuce of two different fields in Salinas

- **Fol621s**

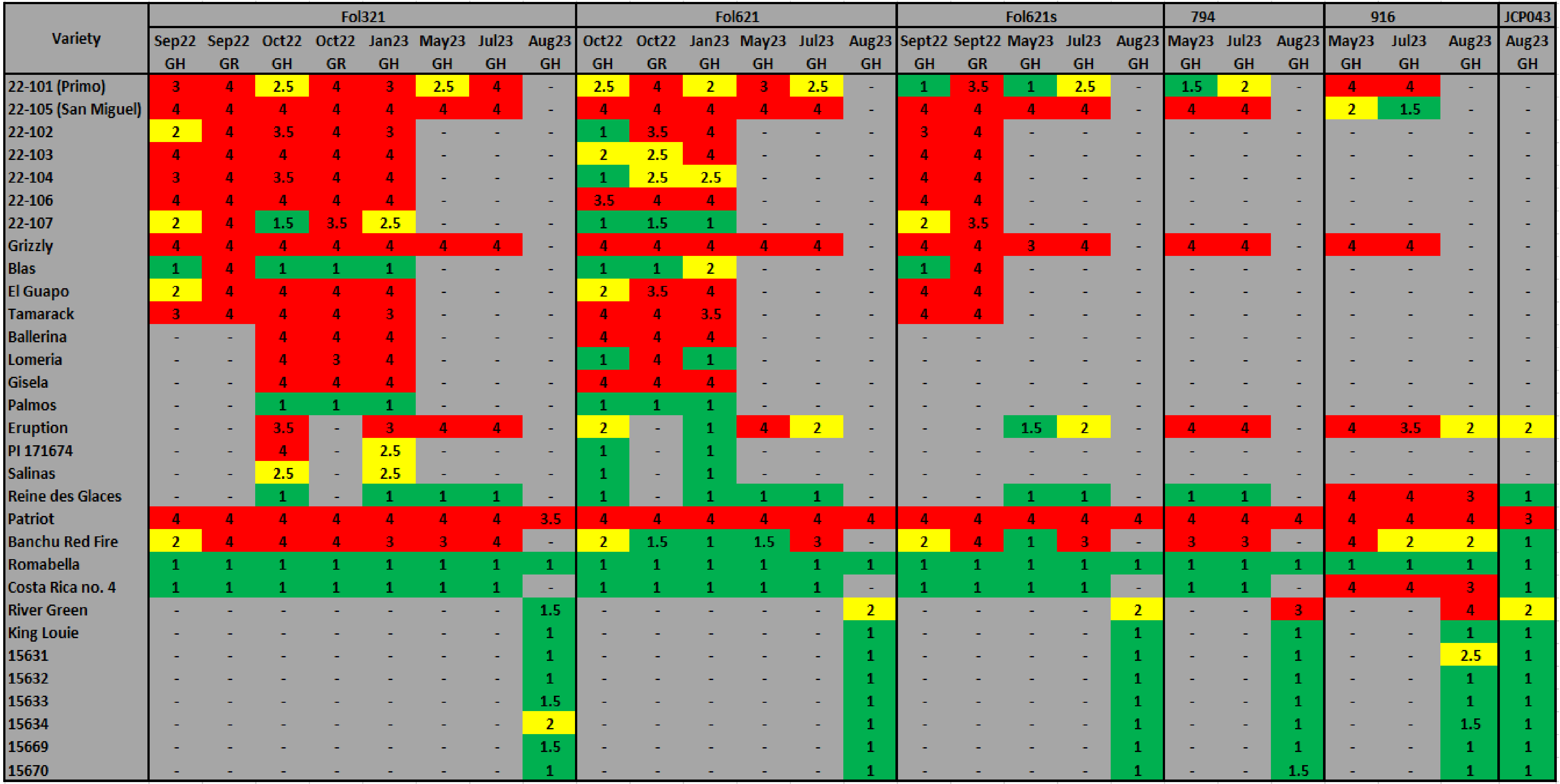
Single spore culture of Fol621

- **VSP-0916**
- **VSP-0794**

Received from Alex Putman, UC Riverside

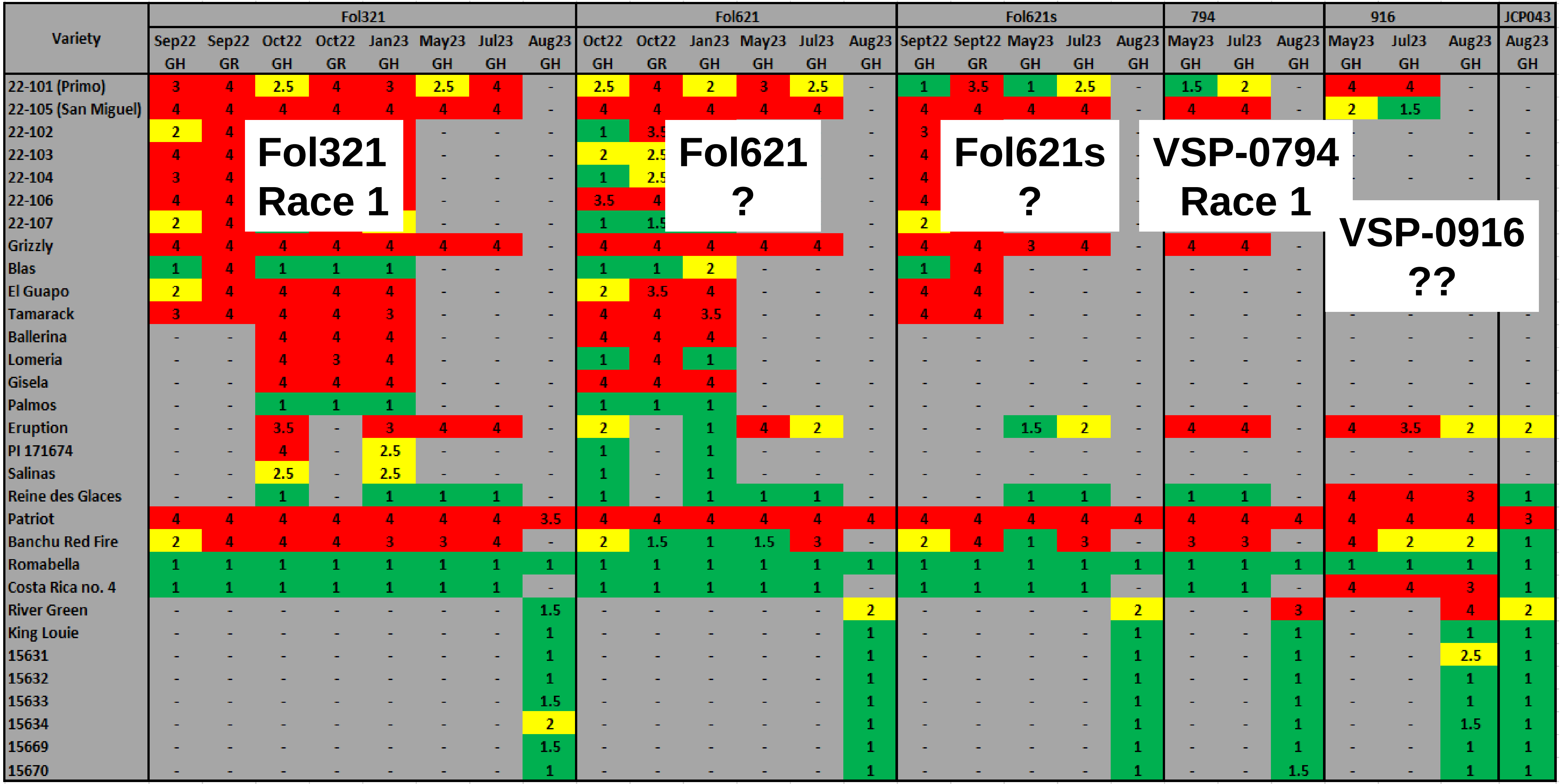
Costa Rica No. 4

Germplasm evaluation



Variety	Fol321								Fol621						Fol621s					794			916			JCP043
	Sep22	Sep22	Oct22	Oct22	Jan23	May23	Jul23	Aug23	Oct22	Oct22	Jan23	May23	Jul23	Aug23	Sept22	Sept22	May23	Jul23	Aug23	May23	Jul23	Aug23	May23	Jul23	Aug23	Aug23
	GH	GR	GH	GR	GH	GH	GH	GH	GH	GR	GH	GH	GH	GH	GH	GR	GH	GH	GH	GH	GH	GH	GH	GH	GH	GH
22-101 (Primo)	3	4	2.5	4	3	2.5	4	-	2.5	4	2	3	2.5	-	1	3.5	1	2.5	-	1.5	2	-	4	4	-	-
22-105 (San Miguel)	4	4	4	4	4	4	4	-	4	4	4	4	4	-	4	4	4	4	-	4	4	-	2	1.5	-	-
22-102	2	4	3.5	4	3	-	-	-	1	3.5	4	-	-	-	3	4	-	-	-	-	-	-	-	-	-	-
22-103	4	4	4	4	4	-	-	-	2	2.5	4	-	-	-	4	4	-	-	-	-	-	-	-	-	-	-
22-104	3	4	3.5	4	4	-	-	-	1	2.5	2.5	-	-	-	4	4	-	-	-	-	-	-	-	-	-	-
22-106	4	4	4	4	4	-	-	-	3.5	4	4	-	-	-	4	4	-	-	-	-	-	-	-	-	-	-
22-107	2	4	1.5	3.5	2.5	-	-	-	1	1.5	1	-	-	-	2	3.5	-	-	-	-	-	-	-	-	-	-
Grizzly	4	4	4	4	4	4	4	-	4	4	4	4	4	-	4	4	3	4	-	4	4	-	4	4	-	-
Blas	1	4	1	1	1	-	-	-	1	1	2	-	-	-	1	4	-	-	-	-	-	-	-	-	-	-
El Guapo	2	4	4	4	4	-	-	-	2	3.5	4	-	-	-	4	4	-	-	-	-	-	-	-	-	-	-
Tamarack	3	4	4	4	3	-	-	-	4	4	3.5	-	-	-	4	4	-	-	-	-	-	-	-	-	-	-
Ballerina	-	-	4	4	4	-	-	-	4	4	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lomeria	-	-	4	3	4	-	-	-	1	4	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Gisela	-	-	4	4	4	-	-	-	4	4	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Palmos	-	-	1	1	1	-	-	-	1	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Eruption	-	-	3.5	-	3	4	4	-	2	-	1	4	2	-	-	-	1.5	2	-	4	4	-	4	3.5	2	2
PI 171674	-	-	4	-	2.5	-	-	-	1	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Salinas	-	-	2.5	-	2.5	-	-	-	1	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Reine des Glaces	-	-	1	-	1	1	1	-	1	-	1	1	1	-	-	-	1	1	-	1	1	-	4	4	3	1
Patriot	4	4	4	4	4	4	4	3.5	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	3
Banchu Red Fire	2	4	4	4	3	3	4	-	2	1.5	1	1.5	3	-	2	4	1	3	-	3	3	-	4	2	2	1
Romabella	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Costa Rica no. 4	1	1	1	1	1	1	1	-	1	1	1	1	1	-	1	1	1	1	-	1	1	-	4	4	3	1
River Green	-	-	-	-	-	-	-	1.5	-	-	-	-	-	2	-	-	-	-	2	-	-	3	-	-	4	2
King Louie	-	-	-	-	-	-	-	1	-	-	-	-	-	1	-	-	-	-	1	-	-	1	-	-	1	1
15631	-	-	-	-	-	-	-	1	-	-	-	-	-	1	-	-	-	-	1	-	-	1	-	-	2.5	1
15632	-	-	-	-	-	-	-	1	-	-	-	-	-	1	-	-	-	-	1	-	-	1	-	-	1	1
15633	-	-	-	-	-	-	-	1.5	-	-	-	-	-	1	-	-	-	-	1	-	-	1	-	-	1	1
15634	-	-	-	-	-	-	-	2	-	-	-	-	-	1	-	-	-	-	1	-	-	1	-	-	1.5	1
15669	-	-	-	-	-	-	-	1.5	-	-	-	-	-	1	-	-	-	-	1	-	-	1	-	-	1	1
15670	-	-	-	-	-	-	-	1	-	-	-	-	-	1	-	-	-	-	1	-	-	1.5	-	-	1	1

Germplasm evaluation



Variety	Fol321								Fol621						Fol621s					794			916			JCP043
	Sep22	Sep22	Oct22	Oct22	Jan23	May23	Jul23	Aug23	Oct22	Oct22	Jan23	May23	Jul23	Aug23	Sept22	Sept22	May23	Jul23	Aug23	May23	Jul23	Aug23	May23	Jul23	Aug23	Aug23
	GH	GR	GH	GR	GH	GH	GH	GH	GH	GR	GH	GH	GH	GH	GH	GR	GH	GH	GH	GH	GH	GH	GH	GH	GH	GH
22-101 (Primo)	3	4	2.5	4	3	2.5	4	-	2.5	4	2	3	2.5	-	1	3.5	1	2.5	-	1.5	2	-	4	4	-	-
22-105 (San Miguel)	4	4	4	4	4	4	4	-	4	4	4	4	4	-	4	4	4	4	-	4	4	-	2	1.5	-	-
22-102	2	4	[illegible]	[illegible]	[illegible]	-	-	-	1	3.5	[illegible]	[illegible]	[illegible]	-	3	[illegible]	[illegible]	[illegible]	-	[illegible]	[illegible]	[illegible]	-	-	-	-
22-103	4	4	[illegible]	[illegible]	[illegible]	-	-	-	2	2.5	[illegible]	[illegible]	[illegible]	-	4	[illegible]	[illegible]	[illegible]	-	[illegible]	[illegible]	[illegible]	-	-	-	-
22-104	3	4	[illegible]	[illegible]	[illegible]	-	-	-	1	2.5	[illegible]	[illegible]	[illegible]	-	4	[illegible]	[illegible]	[illegible]	-	[illegible]	[illegible]	[illegible]	-	-	-	-
22-106	4	4	[illegible]	[illegible]	[illegible]	-	-	-	3.5	4	[illegible]	[illegible]	[illegible]	-	4	[illegible]	[illegible]	[illegible]	-	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
22-107	2	4	[illegible]	[illegible]	[illegible]	-	-	-	1	1.5	[illegible]	[illegible]	[illegible]	-	2	[illegible]	[illegible]	[illegible]	-	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Grizzly	4	4	4	4	4	4	4	-	4	4	4	4	4	-	4	4	3	4	-	4	4	-	[illegible]	[illegible]	[illegible]	[illegible]
Blas	1	4	1	1	1	-	-	-	1	1	2	-	-	-	1	4	-	-	-	-	-	-	[illegible]	[illegible]	[illegible]	[illegible]
El Guapo	2	4	4	4	4	-	-	-	2	3.5	4	-	-	-	4	4	-	-	-	-	-	-	[illegible]	[illegible]	[illegible]	[illegible]
Tamarack	3	4	4	4	3	-	-	-	4	4	3.5	-	-	-	4	4	-	-	-	-	-	-	-	-	-	-
Ballerina	-	-	4	4	4	-	-	-	4	4	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lomeria	-	-	4	3	4	-	-	-	1	4	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Gisela	-	-	4	4	4	-	-	-	4	4	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Palmos	-	-	1	1	1	-	-	-	1	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Eruption	-	-	3.5	-	3	4	4	-	2	-	1	4	2	-	-	-	1.5	2	-	4	4	-	4	3.5	2	2
PI 171674	-	-	4	-	2.5	-	-	-	1	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Salinas	-	-	2.5	-	2.5	-	-	-	1	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Reine des Glaces	-	-	1	-	1	1	1	-	1	-	1	1	1	-	-	-	1	1	-	1	1	-	4	4	3	1
Patriot	4	4	4	4	4	4	4	3.5	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	3
Banchu Red Fire	2	4	4	4	3	3	4	-	2	1.5	1	1.5	3	-	2	4	1	3	-	3	3	-	4	2	2	1
Romabella	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Costa Rica no. 4	1	1	1	1	1	1	1	-	1	1	1	1	1	-	1	1	1	1	-	1	1	-	4	4	3	1
River Green	-	-	-	-	-	-	-	1.5	-	-	-	-	-	2	-	-	-	-	2	-	-	3	-	-	4	2
King Louie	-	-	-	-	-	-	-	1	-	-	-	-	-	1	-	-	-	-	1	-	-	1	-	-	1	1
15631	-	-	-	-	-	-	-	1	-	-	-	-	-	1	-	-	-	-	1	-	-	1	-	-	2.5	1
15632	-	-	-	-	-	-	-	1	-	-	-	-	-	1	-	-	-	-	1	-	-	1	-	-	1	1
15633	-	-	-	-	-	-	-	1.5	-	-	-	-	-	1	-	-	-	-	1	-	-	1	-	-	1	1
15634	-	-	-	-	-	-	-	2	-	-	-	-	-	1	-	-	-	-	1	-	-	1	-	-	1.5	1
15669	-	-	-	-	-	-	-	1.5	-	-	-	-	-	1	-	-	-	-	1	-	-	1	-	-	1	1
15670	-	-	-	-	-	-	-	1	-	-	-	-	-	1	-	-	-	-	1	-	-	1.5	-	-	1	1

Fol321
Race 1

Fol621
?

Fol621s
?

VSP-0794
Race 1

VSP-0916
??

Fusarium publication

ORIGINAL ARTICLE

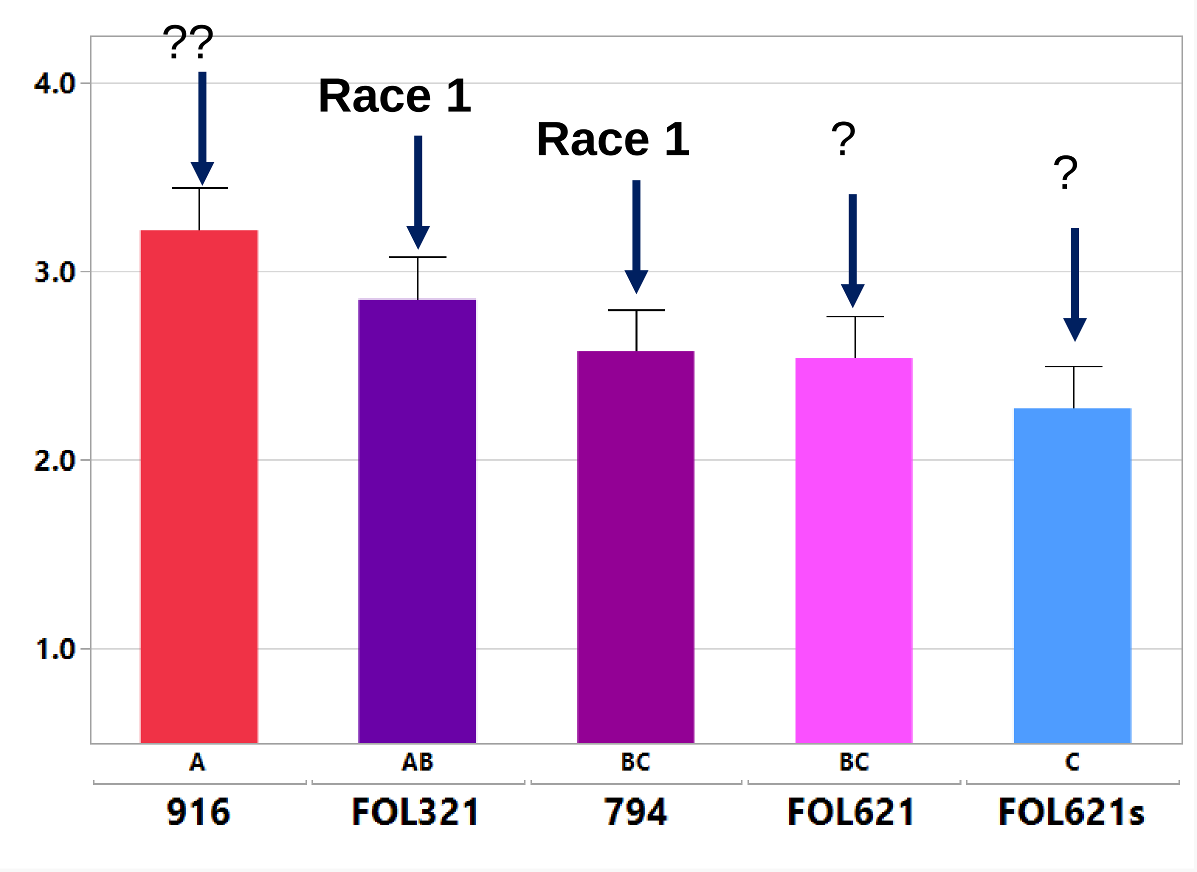
Plant Pathology WILEY

Detection of novel pathogenic variants of *Fusarium oxysporum* f. sp. *lactucae* in California

Santosh Nayak[1] | Kelley L. Richardson[1] | Alexander I. Putman[2] | Nicholas R. LeBlanc[1] | Frank N. Martin[1] | Ningxiao Li[1] | James D. McCreight[1]

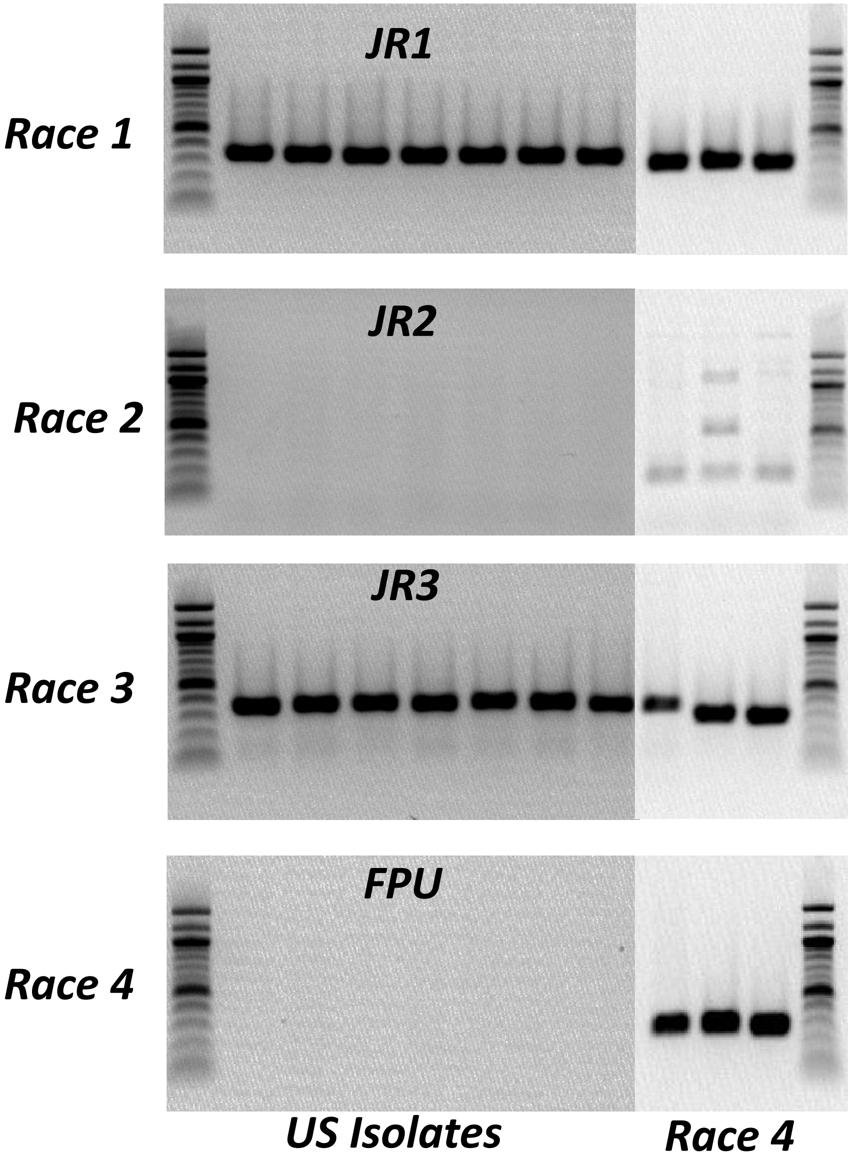
Aggressiveness of isolates

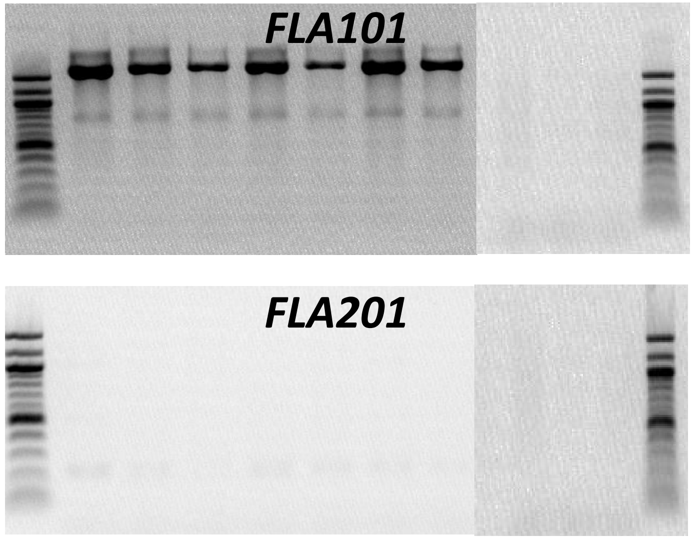
- **Ranked isolates based on disease severity of 9 cultivars across two experiments**
- **<u>VSP-0916</u> is highly aggressive**

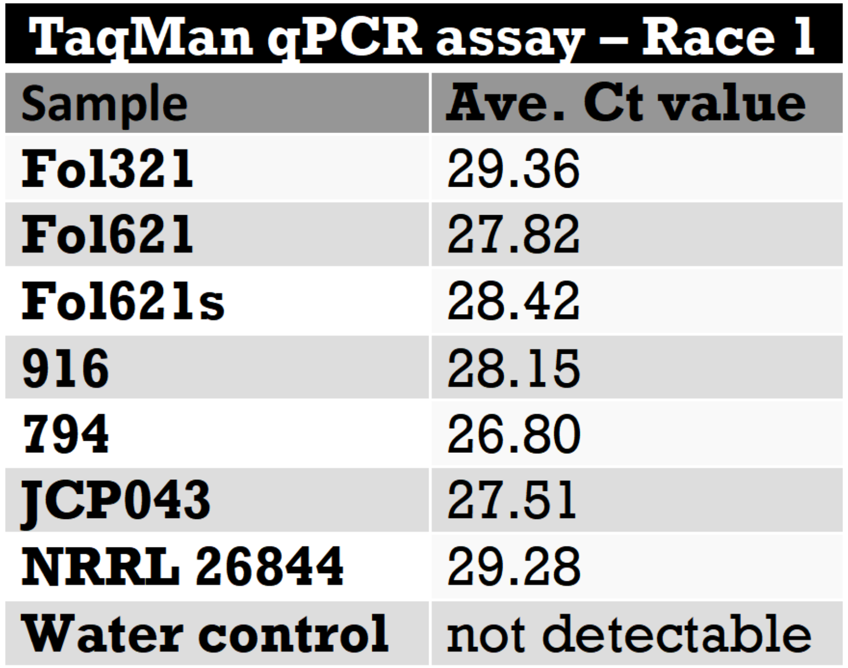


Molecular Analysis of isolates

- **Currently available tools can not differentiate our variant isolates from Race 1.**





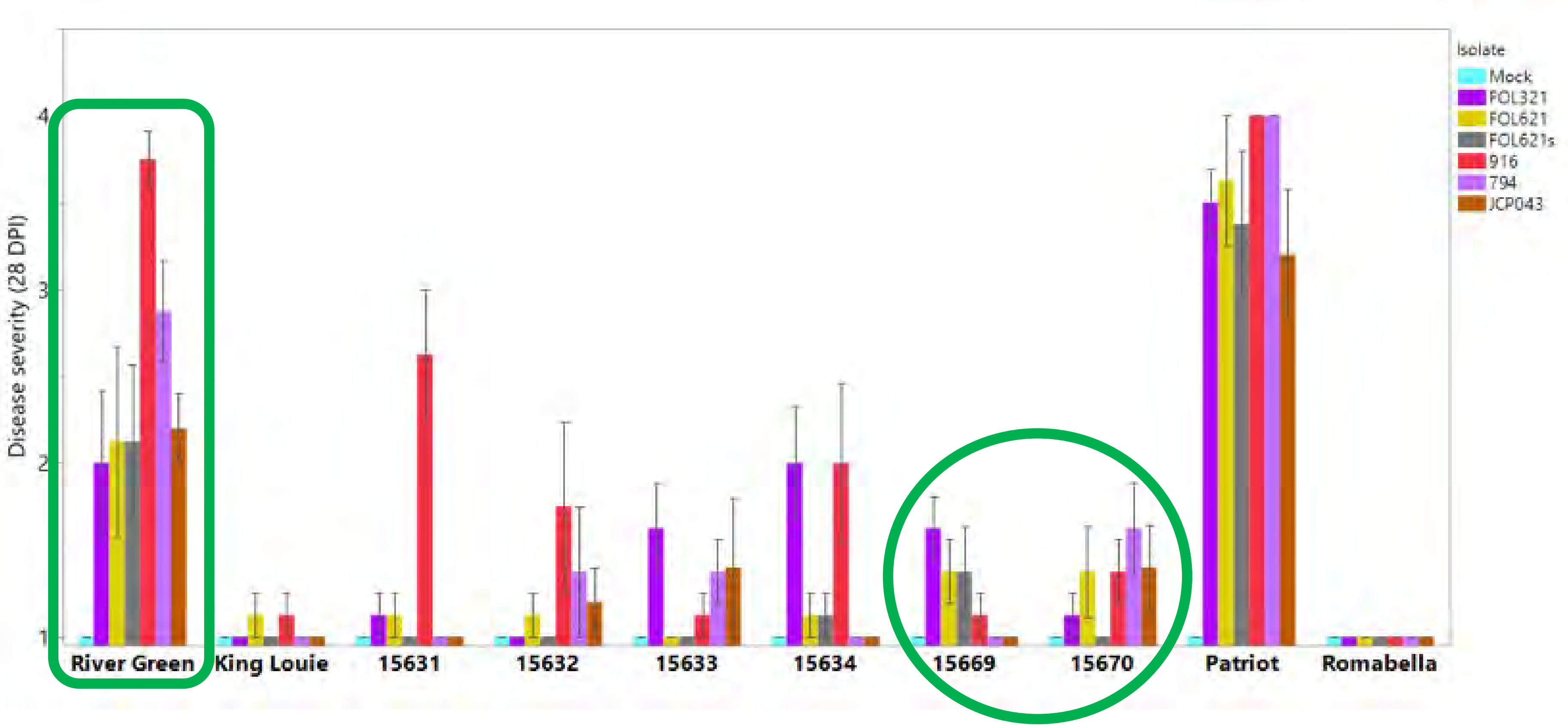


TaqMan qPCR assay – Race 1

Sample	Ave. Ct value
Fol321	29.36
Fol621	27.82
Fol621s	28.42
916	28.15
794	26.80
JCP043	27.51
NRRL 26844	29.28
Water control	not detectable

Broad-spectrum resistance

- **Evaluating race 1-resistant material under race 1 and variants.**
- **916 is race 1-breaking.**
- **Jim McCreight's released lines holding up.**



Opportunities for collaboration

- **With the uncertainty of 2025 INSV pressure, not accepting field trial entries**
- **Included a greenhouse INSV evaluation to the 2025 Pythium/INSV variety trial**
- **Looking for commercial Fusarium race 1-resistant varieties to test under the variant isolates**
- **What do you need? We're here to provide innovative solutions!**

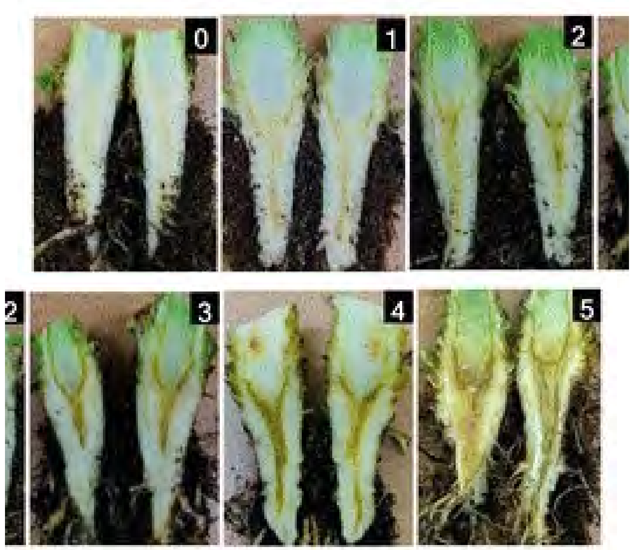
Verticillium publication

Plant Disease • 2023 • 107:3868-3876 • https://doi.org/10.1094/PDIS-09-22-2194-RE

Inheritance of Partial Resistance to Isolate VdLs17 of *Verticillium dahliae* Within *Lactuca* spp.

Santosh Nayak[†] **and Kelley Richardson**

Crop Improvement and Protection Research Unit, Agricultural Research Service, U.S. Department of Agriculture, Salinas, CA 93905



Acknowledgements

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831-512-7556

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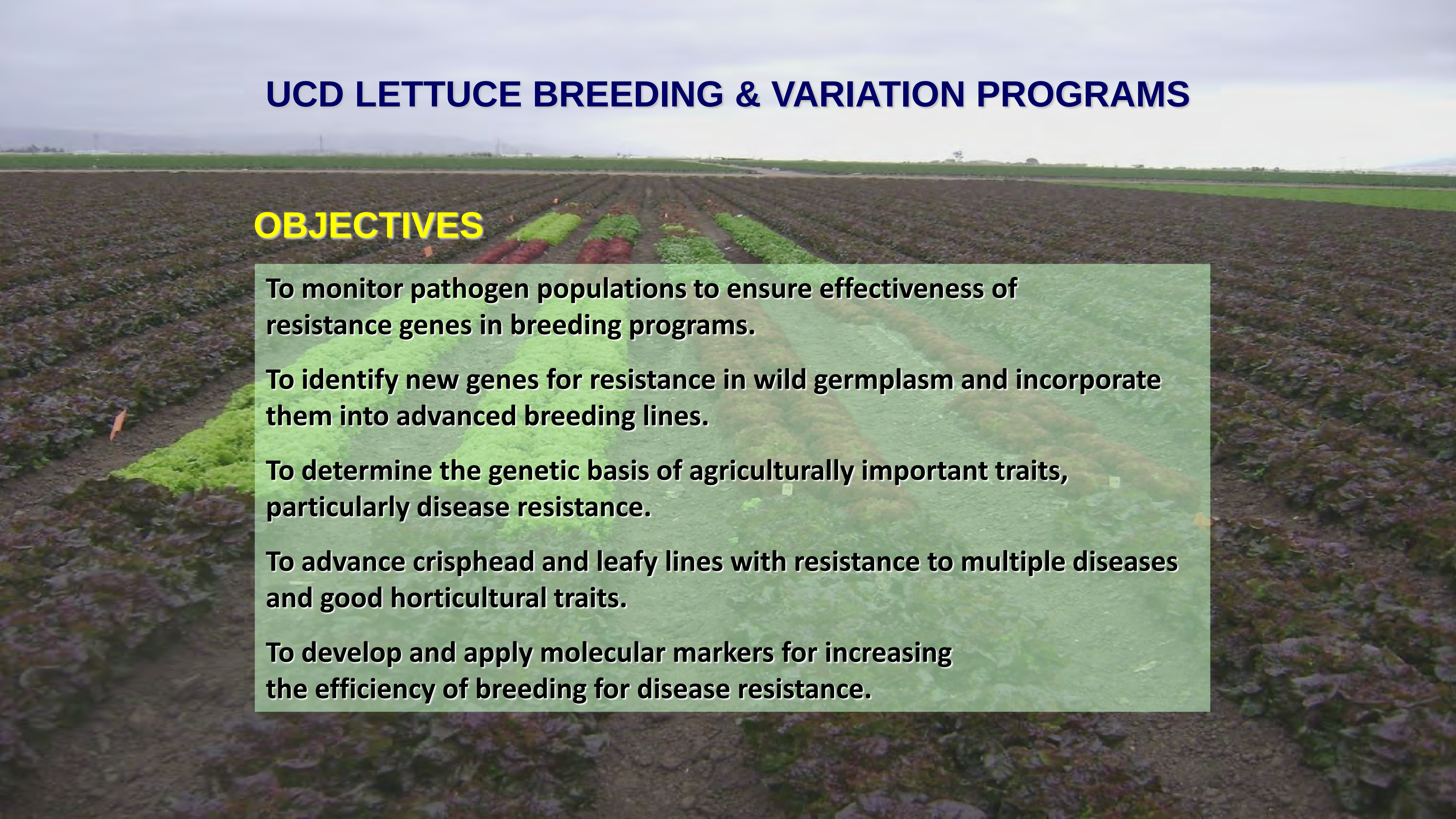
- JP Dundore-Arias
- Renee Eriksen
- Isidora Garcia Hernandez
- Nick LeBlanc
- Ningxiao Li
- Frank Martin
- Jim McCreight
- Lorraine Meza
- Richard Michelmore
- Jose Orozco
- Alex Putman
- David Saavedra
- Ivan Simko
- Stephanie Slinski
- Richard Smith
- Yu-Chen Wang

Thank you!

UCD LETTUCE BREEDING & VARIATION PROGRAMS



OBJECTIVES

To monitor pathogen populations to ensure effectiveness of resistance genes in breeding programs.

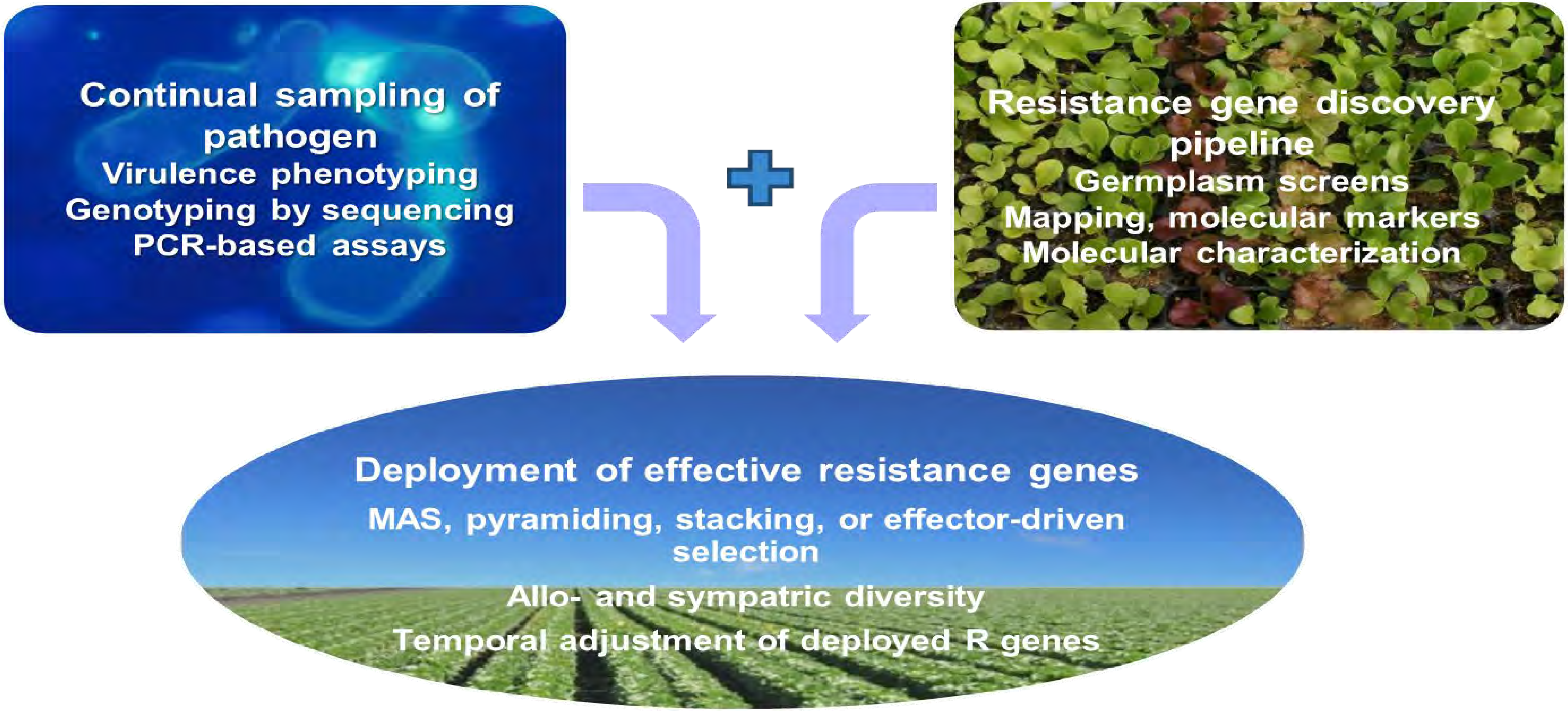
To identify new genes for resistance in wild germplasm and incorporate them into advanced breeding lines.

To determine the genetic basis of agriculturally important traits, particularly disease resistance.

To advance crisphead and leafy lines with resistance to multiple diseases and good horticultural traits.

To develop and apply molecular markers for increasing the efficiency of breeding for disease resistance.

The influenza paradigm: deployment of resistance genes driven by knowledge of pathogen population genetics



Continual sampling of pathogen

Virulence phenotyping

Genotyping by sequencing

PCR-based assays

+

Resistance gene discovery pipeline

Germplasm screens

Mapping, molecular markers

Molecular characterization

Deployment of effective resistance genes

MAS, pyramiding, stacking, or effector-driven selection

Allo- and sympatric diversity

Temporal adjustment of deployed R genes

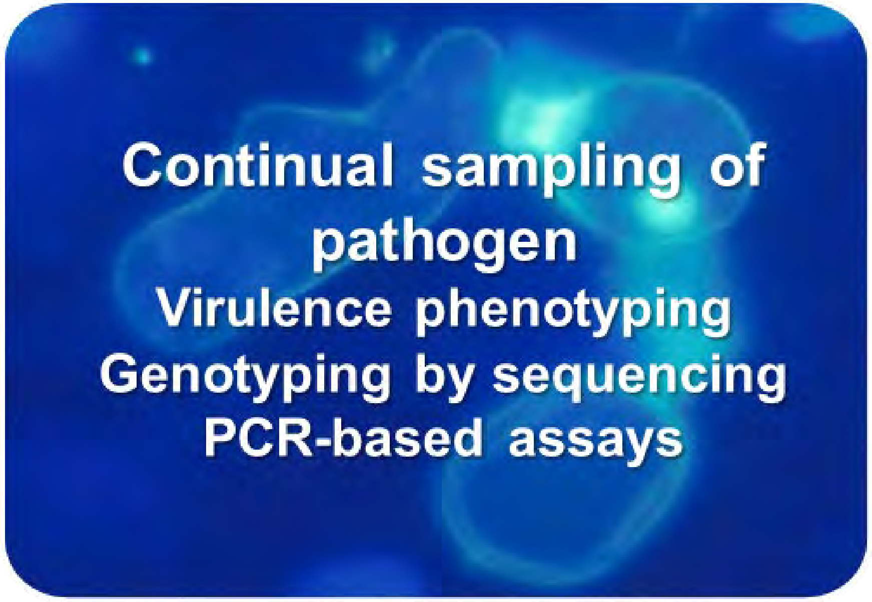
Johnson (1984). A critical analysis of durable resistance. *Ann. Rev. Phytopathol*. **22**:309-30.

Michelmore, Christopoulou, & Caldwell. (2013). Impacts of resistance gene genetics, function, and evolution on a durable future. *Ann. Rev. Phytopathol*. **51**: 291–319.

Target Diseases

Working on resistance to ***Bremia lactucae*** **(downy mildew),** ***Fusarium oxysporum*** **(wilts),** ***Pythium uncinulatum,*** **Impatiens necrotic spot virus (INSV),** ***Rhizorhapis suberifaciens*** **(corky root),** *Verticillium dahliae, Alternaria solani*, (*Phytophthora cryptogea*).

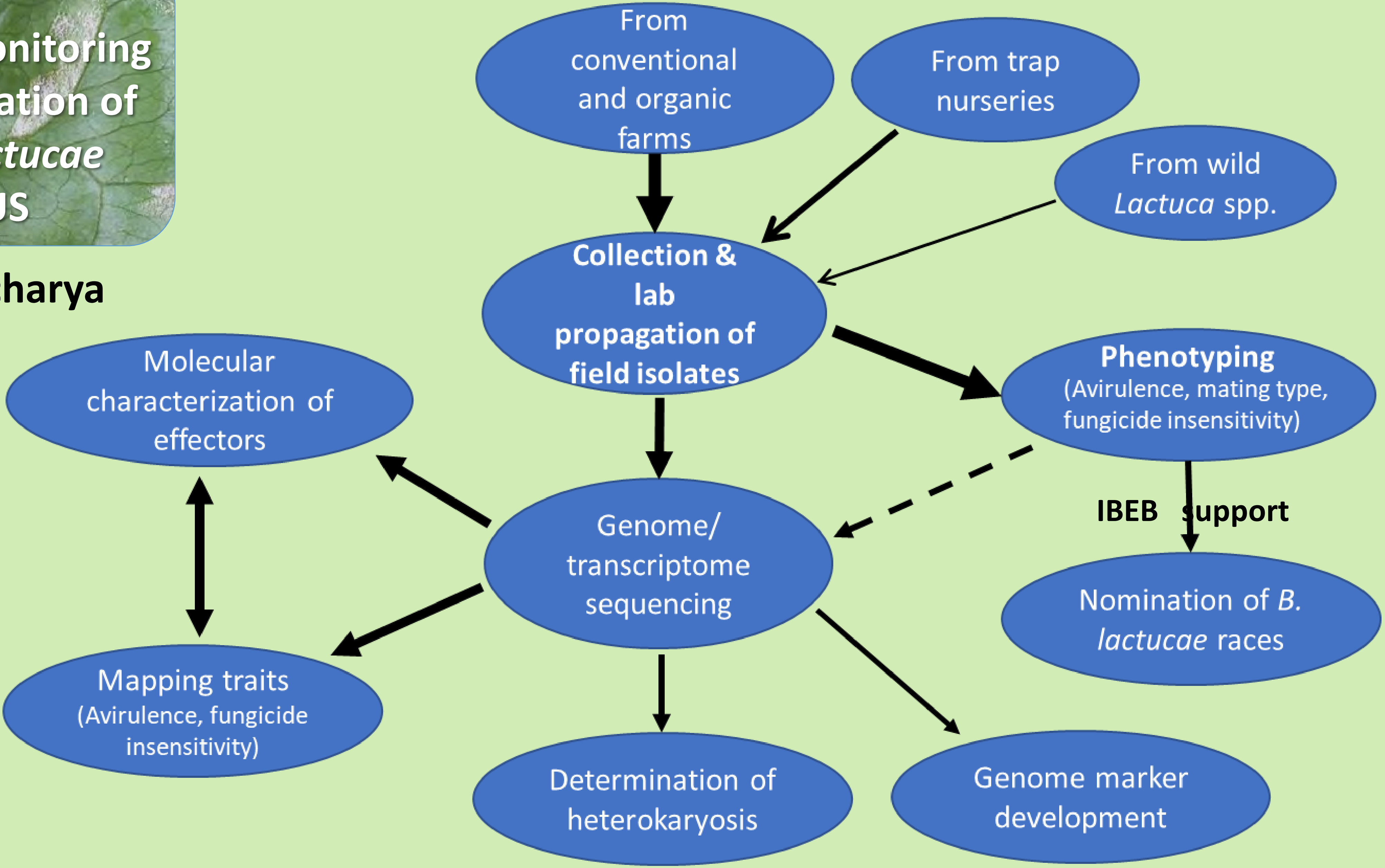
Key component, understanding pathogen variability requires:



Continual sampling of pathogen
Virulence phenotyping
Genotyping by sequencing
PCR-based assays

Continual monitoring and investigation of *Bremia lactucae* IBEB-US

Charlotte Acharya



From conventional and organic farms

From trap nurseries

From wild *Lactuca* spp.

Collection & lab propagation of field isolates

Phenotyping
(Avirulence, mating type, fungicide insensitivity)

IBEB support

Nomination of *B. lactucae* races

Genome/ transcriptome sequencing

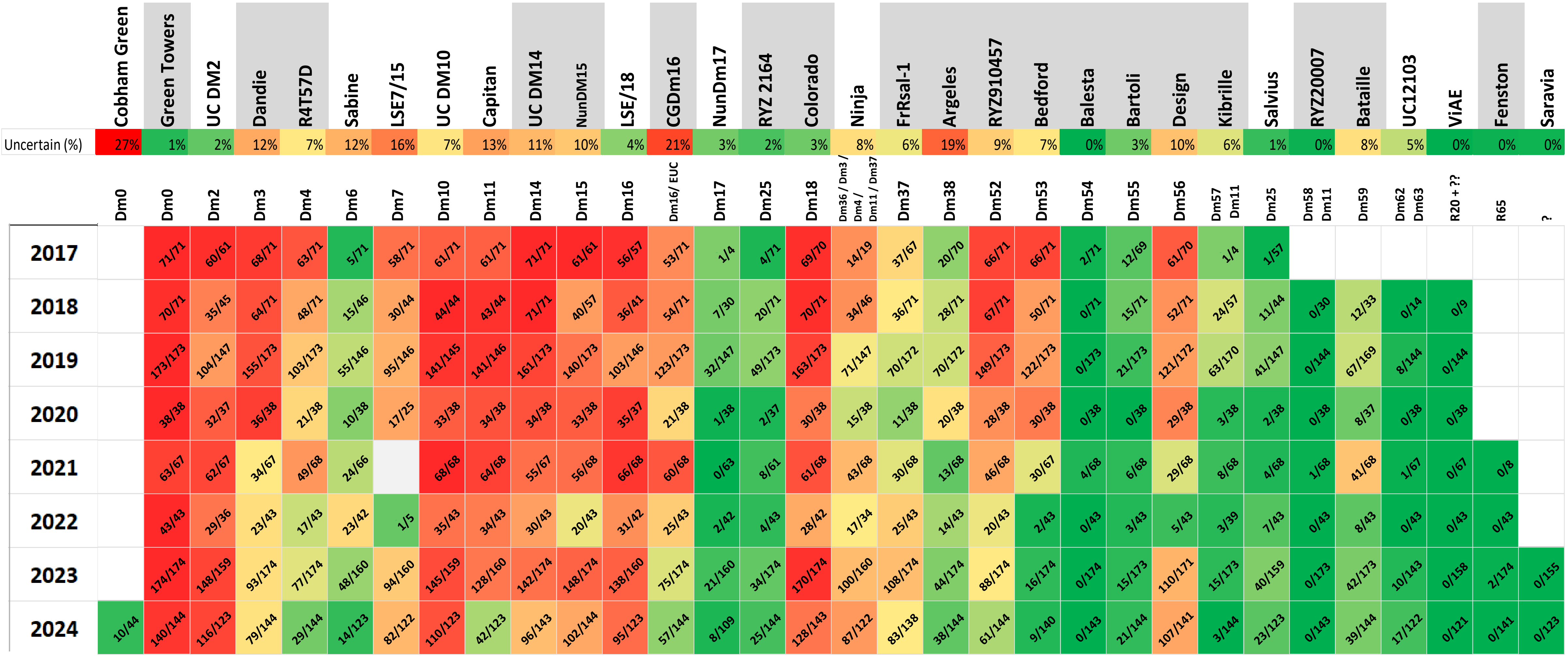
Molecular characterization of effectors

Mapping traits
(Avirulence, fungicide insensitivity)

Determination of heterokaryosis

Genome marker development

Annual frequency of (a)virulence reactions



	Cobham Green	Green Towers	UC DM2	Dandie	R4T57D	Sabine	LSE7/15	UC DM10	Capitan	UC DM14	NunDM15	LSE/18	CGDm16	NunDm17	RYZ 2164	Colorado
Uncertain (%)	27%	1%	2%	12%	7%	12%	16%	7%	13%	11%	10%	4%	21%	3%	2%	3%
	Dm0	Dm0	Dm2	Dm3	Dm4	Dm6	Dm7	Dm10	Dm11	Dm14	Dm15	Dm16	Dm16/ EUC	Dm17	Dm25	Dm18
2017		71/71	60/61	68/71	63/71	5/71	58/71	61/71	61/71	71/71	61/61	56/57	53/71	1/4	4/71	69/70
2018		70/71	35/45	64/71	48/71	15/46	30/44	44/44	43/44	71/71	40/57	36/41	54/71	7/30	20/71	70/71
2019		173/173	104/147	155/173	103/173	55/146	95/146	141/145	141/146	161/173	140/173	103/146	123/173	32/147	49/173	163/173
2020		38/38	32/37	36/38	21/38	10/38	17/25	33/38	34/38	34/38	33/38	35/37	21/38	1/38	2/37	30/38
2021		63/67	62/67	34/67	49/68	24/66		68/68	64/68	55/67	56/68	66/68	60/68	0/63	8/61	61/68
2022		43/43	29/36	23/43	17/43	23/42	1/5	35/43	34/43	30/43	20/43	31/42	25/43	2/42	4/43	28/42
2023		174/174	148/159	93/174	77/174	48/160	94/160	145/159	128/160	142/174	148/174	138/160	75/174	21/160	34/174	170/174
2024	10/44	140/144	116/123	79/144	29/144	14/123	82/122	110/123	42/123	96/143	102/144	95/123	57/144	8/109	25/144	128/143

	Ninja	FrRsal-1	Argeles	RYZ910457	Bedford	Balesta	Bartoli	Design	Kibrille	Salvius	RYZ20007	Bataille	UC12103	ViAE	Fenston	Saravia
Uncertain (%)	8%	6%	19%	9%	7%	0%	3%	10%	6%	1%	0%	8%	5%	0%	0%	0%
	Dm36 / Dm3 / Dm4 / Dm11 / Dm37	Dm37	Dm38	Dm52	Dm53	Dm54	Dm55	Dm56	Dm57 Dm11	Dm25	Dm58 Dm11	Dm59	Dm62 Dm63	R20 + ??	R65	?
2017	14/19	37/67	20/70	66/71	66/71	2/71	12/69	61/70	1/4	1/57						
2018	34/46	36/71	28/71	67/71	50/71	0/71	15/71	52/71	24/57	11/44	0/30	12/33	0/14	0/9		
2019	71/147	70/172	70/172	149/173	122/173	0/173	21/173	121/172	63/170	41/147	0/144	67/169	8/144	0/144		
2020	15/38	11/38	20/38	28/38	30/38	0/38	0/38	29/38	3/38	2/38	0/38	8/37	0/38	0/38		
2021	43/68	30/68	13/68	46/68	30/67	4/68	6/68	29/68	8/68	4/68	1/68	41/68	1/67	0/67	0/8	
2022	17/34	25/43	14/43	20/43	2/43	0/43	3/43	5/43	3/39	7/43	0/43	8/43	0/43	0/43	0/43	
2023	100/160	108/174	44/174	88/174	16/174	0/174	15/173	110/171	15/173	40/159	0/173	42/173	10/143	0/158	2/174	0/155
2024	87/122	83/138	38/144	61/144	9/140	0/143	21/144	107/141	3/144	23/123	0/143	39/144	17/122	0/121	0/141	0/123

Cultivar susceptible Cultivar resistant

More isolates sporulate <----------> Fewer isolates sporulate

*EU-D set highlighted in grey

Number of isolates sporulating/total number of isolates tested.

Charlotte Acharya

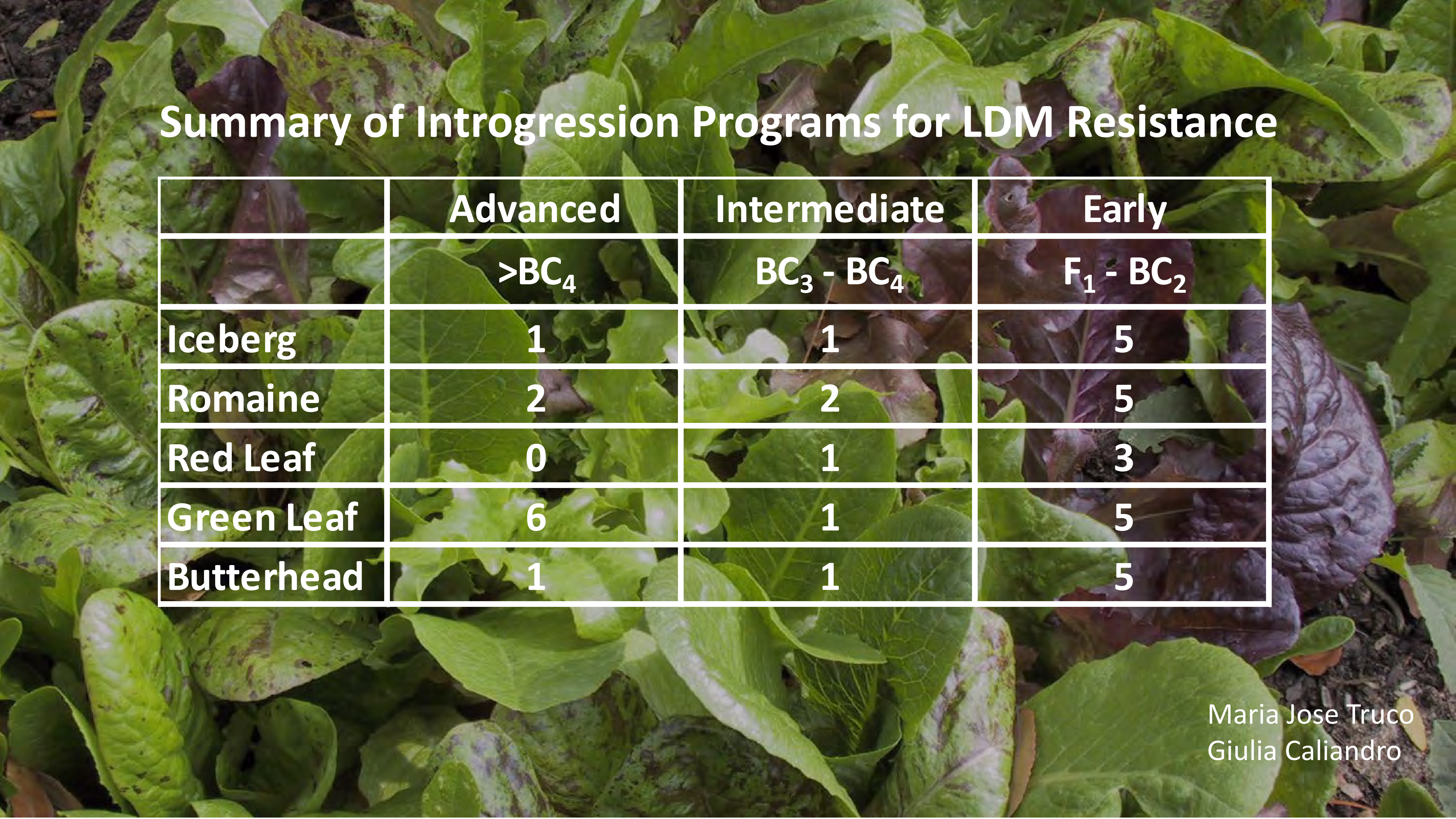
Trap Nurseries 2024/2025

- **Summer 2024, Salinas, CA, Miguel Macias, Sakata Seed**
  - **3 isolates collected and tested: all B2 mating types, with 2 novels and 1 VP-300**
- **Summer/Fall 2024, Watsonville, CA, Ryan McMillen, BASF**
  - **28 isolates collected: 7 novel and 1 VP-300**
- **Fall 2024, Holtville, CA, Ana Pastrana, UC Cooperative Extension**
  - **Planted in late October – no Bremia**
- **Winter 2024, Yuma, AZ, Stephanie Slinski, YCEDA**
  - **Planted mid-November – no Bremia**
- **Winter 2024, Yuma, AZ, Jonny McIntier, Sakata Seed**
  - **Planted mid-November – no Bremia**
- **Winter 2025, Salinas, CA, Mel Mekonnen, Syngenta**
  - **Planted late January**
- **Spring 2025, Nipomo, CA, Bill Waycott, Nipomo Native Seeds**
  - **Planted mid-February**

Thanks to all these cooperators

Emma Deuitch

Summary of Introgression Programs for LDM Resistance

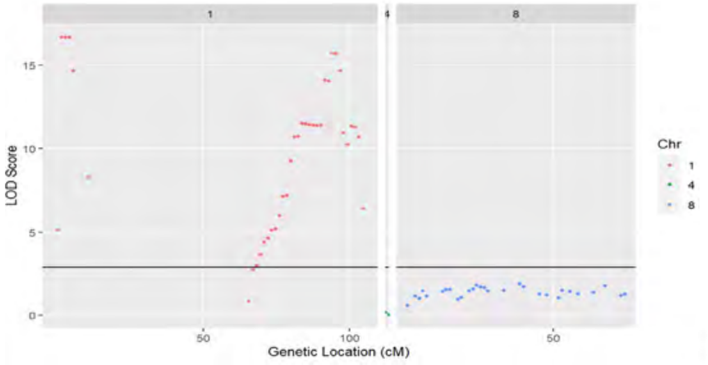


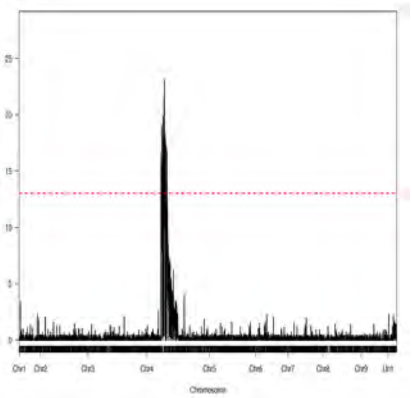
	Advanced	Intermediate	Early
	$>BC_4$	BC_3 - BC_4	F_1 - BC_2
Iceberg	1	1	5
Romaine	2	2	5
Red Leaf	0	1	3
Green Leaf	6	1	5
Butterhead	1	1	5

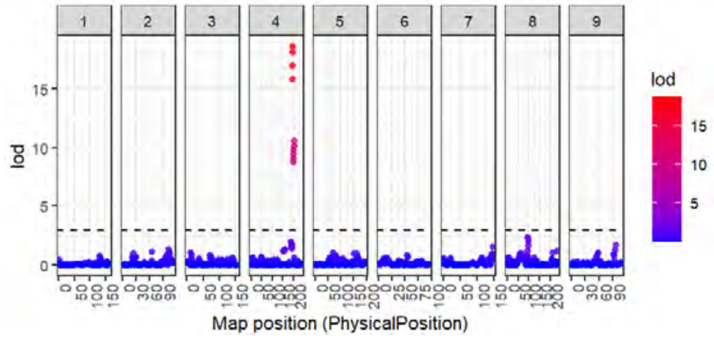
Maria Jose Truco
Giulia Caliandro

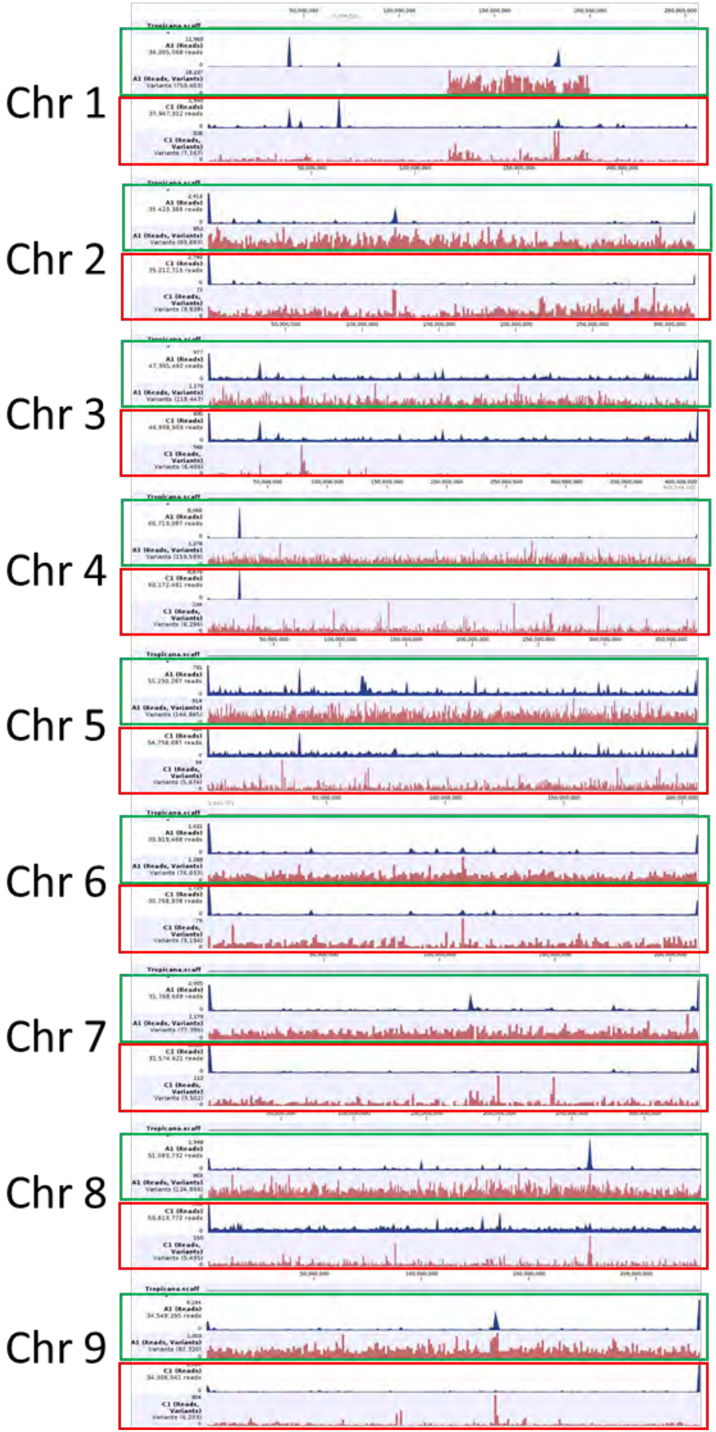
Advanced resistant breeding lines for Lettuce Downy Mildew

- Advanced Backcross lines from *L. saligna* & *L. serriola* into *L. sativ*a background
- GBS genotyping of segregating BCxSx progenies
- Bulked Segregant Analysis of R & S bulks









Work in progress:

- 2 lines resistant QTL MRC Chr 4 & 2 lines resistant QTL MRC Chr 1
- Fine mapping of the region associated with resistance
- Development of markers for MAS

Maria Jose Truco
Yuxin Bai
Pelin Yuksel
Samjhana Khanal

Fusarium oxysporum (Fusarium wilt)

Maria José Truco

Collaboration with Stephanie Slinski (Yuma Center of Excellence for Desert Agriculture)

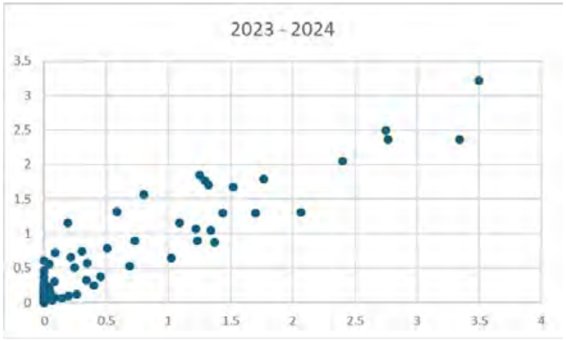
Screening segregating populations for resistance

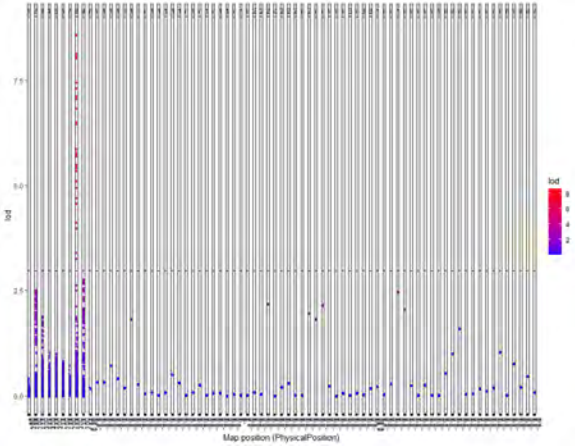
Release of Fusarium resistance lines from single plant selections

Screening wild germplasm for resistance (*L. serriola* & *L. saligna*)

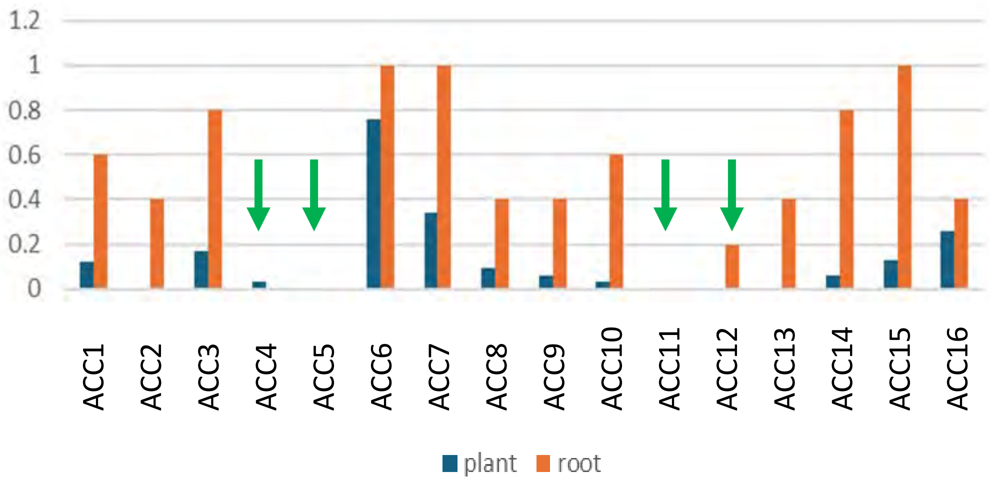
Genetic characterization of Fusarium resistance in segregating populations

- Five segregating populations
- Screening in multiple years & environments
- Multiple QTL in most of the populations
- 1 population with a single QTL in chr 8





L. saligna accessions



Globisporangium (*Pythium*) *uncinulatum* wilt

Yuxin Bai

Collaboration with JP Dundore-Arias, CSUMB

Sequencing genomes – assessing variation
– diagnostic markers
- isolates for screening for resistance

Screening germplasm for resistance

Steve Koike

Lettuce lines screened so far for resistance to *G. uncinulatum* SKP43

As susceptible as Green Towers:

- 19 *L. sativa* cultivars
- 3 *L. serriola* accessions

Slightly less susceptible than Green Towers

- 6 *L. sativa* cultivars
- 1 *L. aculeata* accession

As resistant as Coolguard (subject to confirmation)

- 1 *L. sativa* cultivar
- 2 *L. serriola* accessions
- 9 *L. saligna* accessions

Will test lines identified by JP Dundore-Arias as resistant in the field.
Will test for resistance to other isolates of *G. uncinulatum.*
Will provide resistant lines to JP and Kelly Richardson for testing in field.

Yuxin Bai

Identification of Lettuce Germplasm Resistant to Impatiens Necrotic Spot Virus and Sequencing INSV Isolates in CA and AZ Lettuce Production Areas

Ali Ahmed

Collaborators
Steve Koike (TriCal Diagnostics), Daniel Hasegawa (USDA-Salinas, Kelly Richardson (USDA-Salinas), Jose Pablo Dundore-Arias (CSU, Monterey), Bill Waycott (Nipomo Native seeds), Oscar Crispin (Enza Zaden), Yu-Chen Wang (USDA-Salinas), Juan Marroquin (Univ. of Arizona), Hanu Pappu (Washington State University).

Call for germplasm and samples:

Let me know if you have germplasm that you would like screened.

Interested in receiving samples of infected plants from throughout CA & AZ.

Identification of Lettuce Germplasm Resistant to Impatiens Necrotic Spot Virus and Sequencing INSV Isolates in CA and AZ Lettuce Production Areas

Goals:

- Assess variation of INSV isolates in lettuce production areas
- Identify multiple sources of resistance to INSV
- Introgression of resistance into cultivated lettuce
- Determine genetic basis of resistance
- Monitor for resistance-breaking strains

Approach:

- Build on what known for TSWV
- Sequence 100s of isolates of INSV from CA and AZ
- Clone the five INSV genes
- *Agrobacterium*-mediated transient expression of the five INSV proteins to lettuce germplasm looking for HR.
- Identification of sequence variants of recognized proteins.
- Testing recognition of variant proteins to assess the possibility of resistance-breaking strains

Received/collected 256 INSV symptomatic samples

Sample locations	Submitted by	Number of samples
USDA, Salinas, CA	Daniel Hasegawa	22
USDA Salinas green house experiment	Kelly Richardson	12
USDA Salinas (Spence Field, Salinas, Gonzales, Soledad, Chualan, Pajaro, Castroville)	Yu-Chen Wang	18
Downy Mildew Trap crops, Salinas, CA and Yuma, AZ	Emma Deuitch	14
UC Davis greenhouses (4 different greenhouses)	Maria Truco/Giulia Caliandro	11
UC Davis field trial	Luis Salazar	8
San Juan Bautista, CA	Bill Waycott	1
San Juan Bautista, CA	Oscar Crispin	1
University of Arizona Agricultural Center, Yuma, AZ	Juan Marroquin	4
Monterey, CA	Jose Pablo Dundore-Arias	6
West Santa Maria, Salinas, Spence area Monterey County, Gonzalez, Castroville, San Benito County, Ventura County, Santa Maria, Oxnard, Soledad, King City, South County Monterey, Blanco Salinas Valley, Santa Barbara, Watsonville	Steve Koike (TriCal Diagnostics)	159
Total		**256**

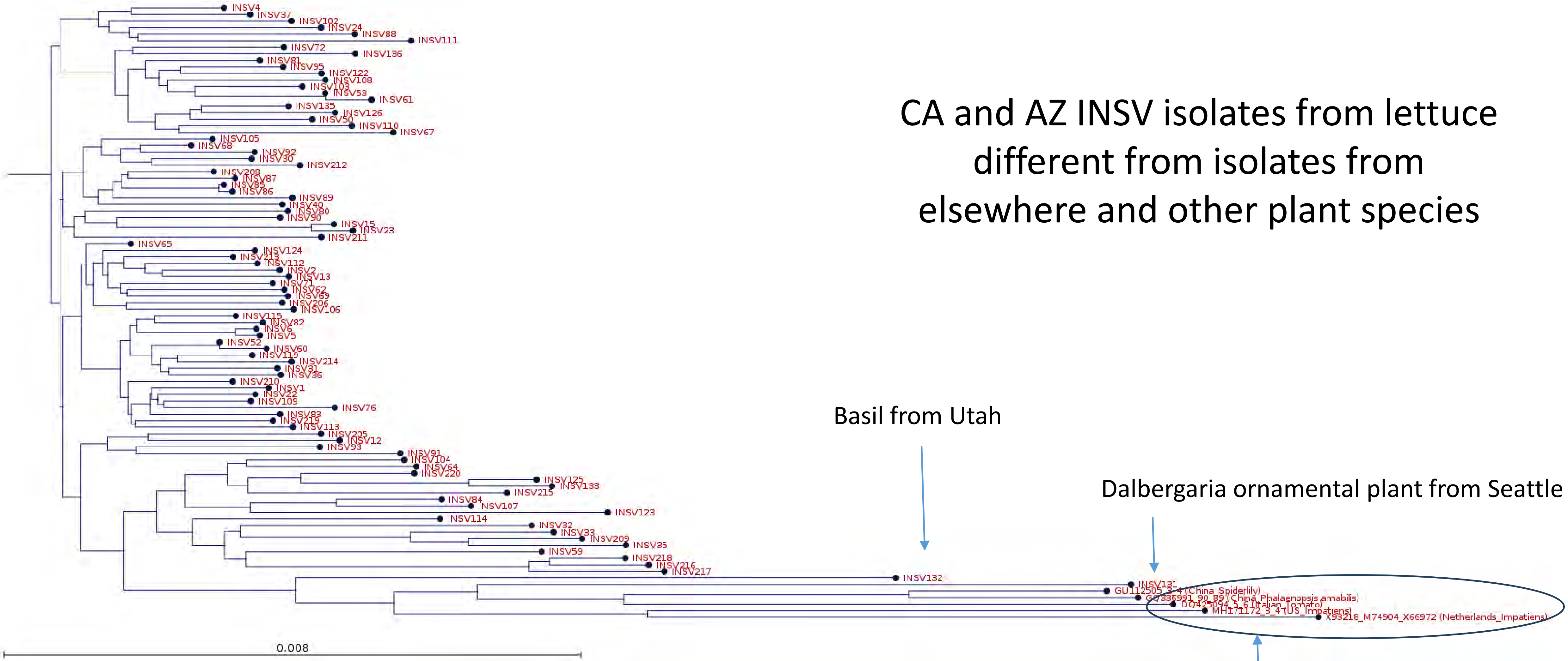
- 3 Basil samples (Utah)
- One Dalbergaria (ornamental) sample (Seattle, Wa)
- One Celery sample (Salinas, CA)
- Two Spinach sample (Monterey county & Salinas, CA)
- One Quinoa (Salinas, CA)
- One Spurry (Yuma, AZ)

- 2008_1 sample
- 2009_1 sample
- 2019_1 sample
- 2020_2 samples
- 2021_2 samples
- 2022_14 samples
- 2023_129 samples
- 2024_106 samples

- Mexico_3 samples
- Utah_ 3 samples
- WA_1 sample
- AZ_13 samples
- CA_240 samples

Whole genome neighbor joining phylogenic tree between 89 recent INSV isolates, and 5 published isolates

CA and AZ INSV isolates from lettuce different from isolates from elsewhere and other plant species



Basil from Utah

Dalbergaria ornamental plant from Seattle

Published isolates from other hosts and different regions

Kraken analysis identified other viruses

		# samples	
Varicosavirus lactucae	Lettuce big-vein associated virus (LBVaV)	40	Most samples have significant INSV reads
Ophiovirus mirafioriense	Mirafiori lettuce big-vein virus (MLBVV)	32	Most samples have significant INSV reads
Cytorhabdovirus lactucamaculante	Lettuce Yellow Mottle Virus	2	Low INSV reads
Lettuce Mosaic Virus		1	No INSV reads
Celery mosaic virus		1	No INSV reads
Ocimum basilicum RNA virus_1 Ocimum basilicum RNA virus_2	Basil RNA virus	2	Contains significant INSV reads

- 25 Samples co-infection by LBVaV and MLBVV inaddition to INSV
- 3 samples with very low INSV reads
- Will do assembly and phylogenetic analysis

Kuntal Dey

Summary and future studies for sequencing component

- Assembled 89 complete single isolate INSV genomes (87_lettuce, one_basil, one_Dalbergaria)
- INSV from lettuce very different from TSWV
- CA & AZ INSV isolates from lettuce different from isolates from elsewhere and other plant hosts
- Three MX isolates cluster together and distinct from CA & AZ isolates
- MX isolates more variable and distinct in their M genome compared with CA & AZ isolates

- 100 more samples submitted for sequencing
- Wil continue sequencing samples to assess variation throughout CA
- Monitor emergence of resistance breaking isolates
- Will analyze recombination and reassortment events among INSV isolates using RDP software
- Analysis of variation in predicted protein structures using AlphaFold

Identification of Lettuce Germplasm Resistant to INSV

- *Agrobacterium*-mediated transient expression of five genes in lettuce germplasm looking for HR
- Cloned all five genes into an expression vector & transformed into C58 strain of *Agrobacterium tumefaciens*
- Whole plant vacuum infiltration of 3 to 5-week-old seedlings with pool of five *Agrobacterium* strains
- Score for necrotic resistance response resulting from recognition of one or more INSV proteins

42 lettuce accessions among 189 tested showed necrosis in response to the pool of five INSV proteins

Reacting line 1

Reacting line 2

Non- reacting line

~150 accessions
show no HR reaction

Four plants infiltrated per accession

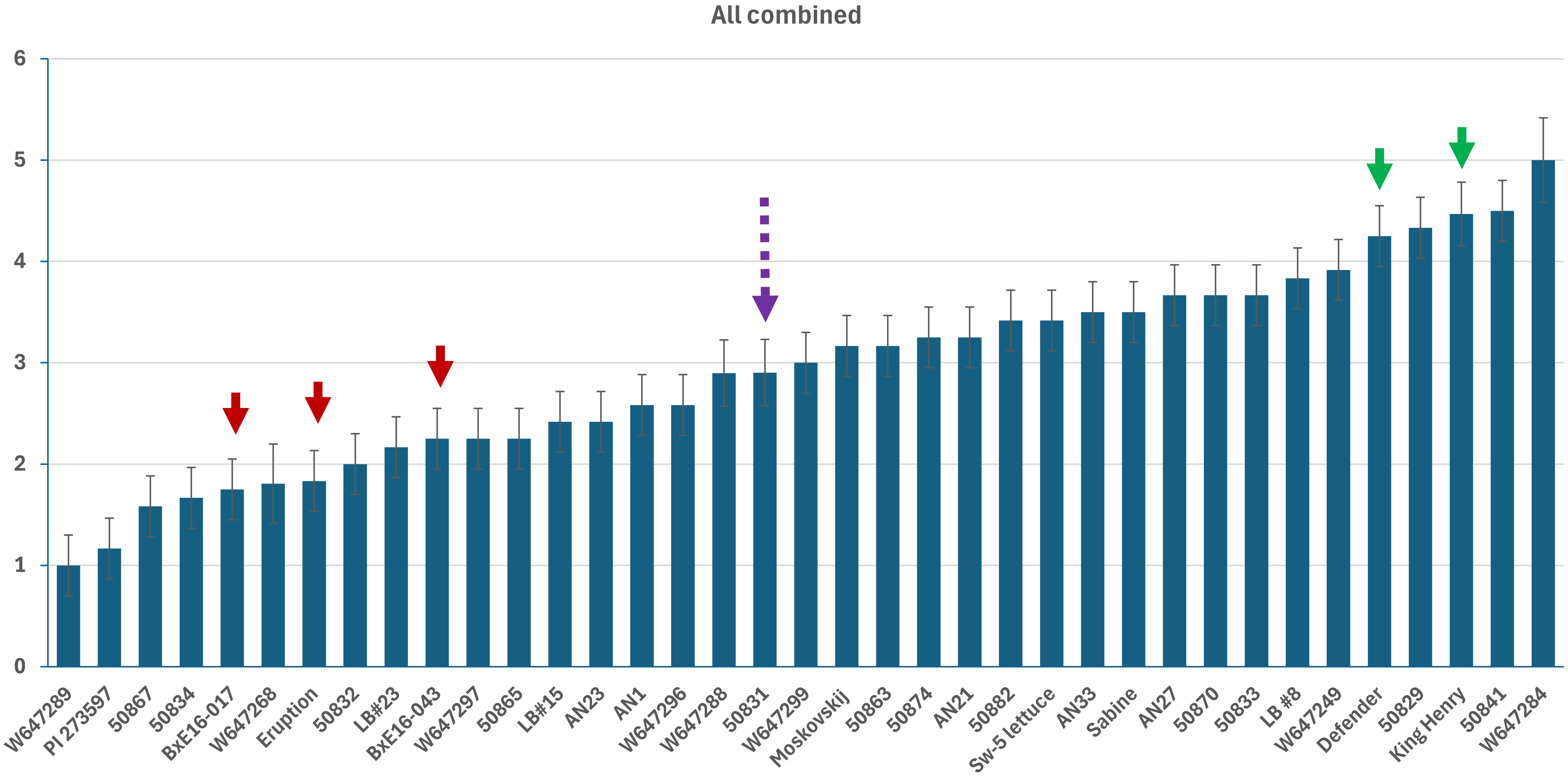
14 Accessions among 31 Tested Showed Resistance to live INSV virus via Thrips and Mechanical Inoculation

- 31 promising lines tested at USDA/Salinas green house (Kelley Richardson group)
- Four plant per line per replicate tested. 3 replicates
- Four-week old seedlings challenged with INSV
- First scored 2 weeks after infection. A total of five scoring taken
- 0 -5 scoring: 0 = no symptoms, 5 = completely necrotic or dead plant

- 14 accessions scored < 3 score
- 14 accessions between 3 – 4
- 3 accessions between 4 -5
- Positive control Eruption scored 1.83

D. Hasegawa

Overall mean of each accession



- Eruption under field condition is close to score 0
- Eruption : 1.83
- QTL line1: 1.75
- QTL line2: 2.25
- Too much disease pressure. They put every week new batches of viruliferous Thrips

Kelly Richardson

Summary and future studies for identification of resistance to INSV

- Identified 14 accessions resistant to INSV
- Germplasm shared with Kelly Richardson
- Currently being crossed to cultivated lettuce
- Will investigate genetic basis of resistance

- Continuing germplasm screening against pool of five viral proteins
- Testing resistant lines for detection of individual viral proteins
- Will test resistant lines with variants of detected proteins

Ali Ahmed

Bacterial corky root caused by *Rhizorhapis suberifaciens*

Maria Ferrer

Collaboration with Isolde Francis (CSUB), Steve Koike (TriCal Diagnostics), Stephanie Slinski (U Arizona) & Yu Chen Wang (UCANR)

- Assembled the complete genomes of 16 strains of *R. suberifaciens* and related species.
  - ~3-4 Mb genomes with 0 to 5 plasmids
- Validated CA1 strain for screening for resistance.
  - screening for knockouts in candidate susceptibility genes
  - PCR based assays for chromosome and plasmids
- Reports of corky root in Arizona and Salinas valley.
  - Received ~50 samples of roots with symptoms of corky root

Symptomatic lettuce roots collected by Stephanie Slinski

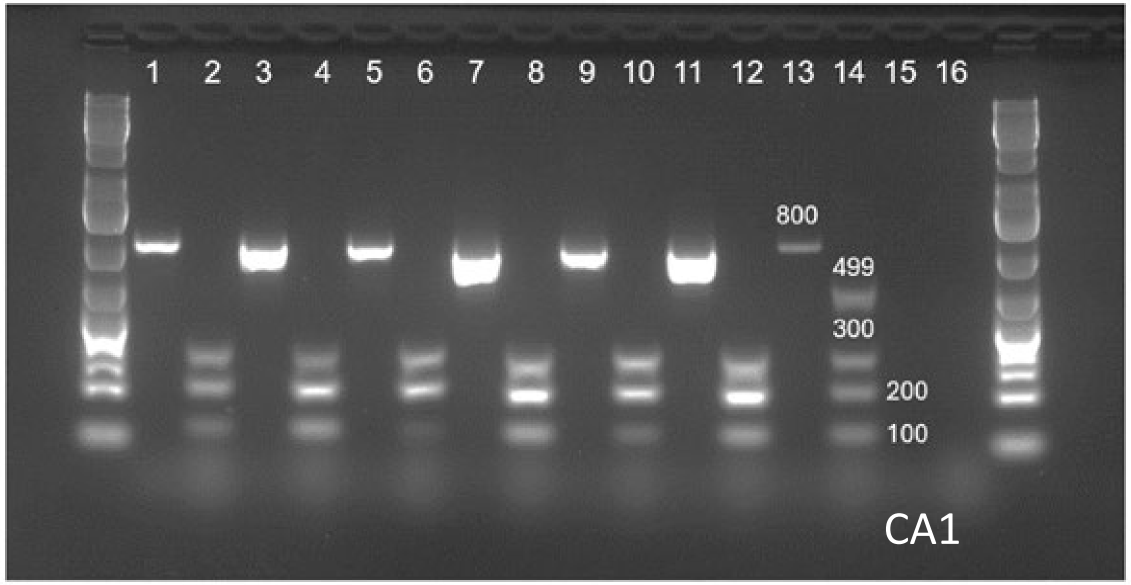
Roots collected in South Gila Valley of Yuma

Roots collected in Wellton

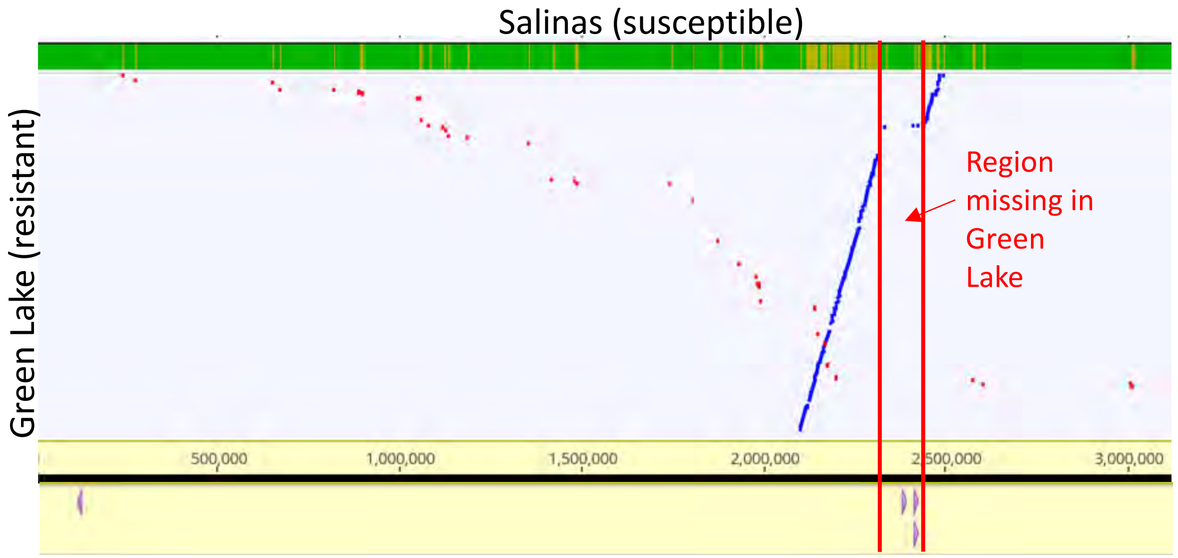
DNA extracted from 36 symptomatic roots coming from Yuma, Wellton and Roll (AZ)

Maria Ferrer

PCR using chromosomal and plasmid primers on DNA extracted from 6 & 4 symptomatic roots provided by Steve Koike & Yu-Chen Wang



gDNA of CA1 = positive control.
The PCRs for the genomic fragment and the plasmids conducted separately.
All primer pairs successfully amplified their respective target fragments, except for the 499 bp amplicon of the largest plasmid.



Identified deletion on resistant cvs vs Salinas reference
Design primers to amplify 2 genes on Salinas corresponding to deleted region in Green Lake

Maria Ferrer

Corky root summary

		Lettuce markers			*Rhizorhapis suberifaciens* markers			Root WGS	Corky root	
		PDS & actin	Gene 1	Gene 2	Chr 800 bp	16SrDNA	Plasmids (100, 200, 300bp)	"CA1" sequences	symptoms	
Susceptible	Salinas	+ +	+	+					+	
cultivars	Iceberg	+ +	+	+					+	
	La Brillante	+ +	+	+					+	
Resistant	Green Lake	+ +	-	-					-	
genotypes	Tropicana	+ +	-	-					-	
	US96UC23	+ +	-	-					-	
Symtomatic	24082303-2	+ +	+	+	+	+	+ + +	-	+	
root	UCCE-120	+ +	+	+	+	+	+ + +	-	+	
samples	24080701	+ +	+	+	+	+	+ + +	+ +	Weak	
	24082804	+ +	+	+	+	+	+ + +	+ + +	Weak	
	No name	+ +	+	+	+	+	+ + +	+ +	Weak	
	24072907	+ +	+	+	+	+	+ + +	+ + +	+	
	24082104	+ +	+	+	+	+	+ + +	+ + +	+	
	24080903	+ +	-	-	+	+	+ + +	-	+	
	UCCE-121&110	+ +	-	-	+	+	+ + +	+ +	+	
	SS Yuma (2-8)	NA +	+	NA	+	+	+ + +	NA	+	
	SS Yuma (1)	NA +	+	NA	-	+	- - -	NA	+	Fusarium?

Sample data

Maria Ferrer

Transcripts from six washed, CR symptomatic, *R. suberifaciens* positive roots.

	Sample name	2411910_3	SGV Yuma 1	INSV_infected_ root	9-ROLL A2	24091708	24100701
	Origin	**AZ**	**Yuma**	**Salinas**	**Roll, AZ**	**San Benito**	**Monterey**
	Total reads	**24,310,352**	**17,222,594**	**100,533,314**	**61,711,918**	**13,250,929**	**15,101,852**
Soilborne pathogens	*Verticillium dahliae*	138	36	338	424	214	490
	Fusarium oxysporum spp.	14,449	246	11,185	38,877	2,789	4,034
	Globisporangium uncinulatum	0	0	0	2	0	0
	Sclerotinia sclerotiorum	129	136	815	482	77	399
	Thielaviopsis basicola	0	0	2	0	0	0
Viruses	*Rhizoctonia solani*	196	136	917	613	112	439
	INSV	8	2	30,453	27	9	69,317
	LBVaV	1	6	0	1	703	17,252
	MLBVV	0	0	0	0	0	0

Most reads from lettuce

No reads from *R. suberifaciens* because library of poly A mRNA

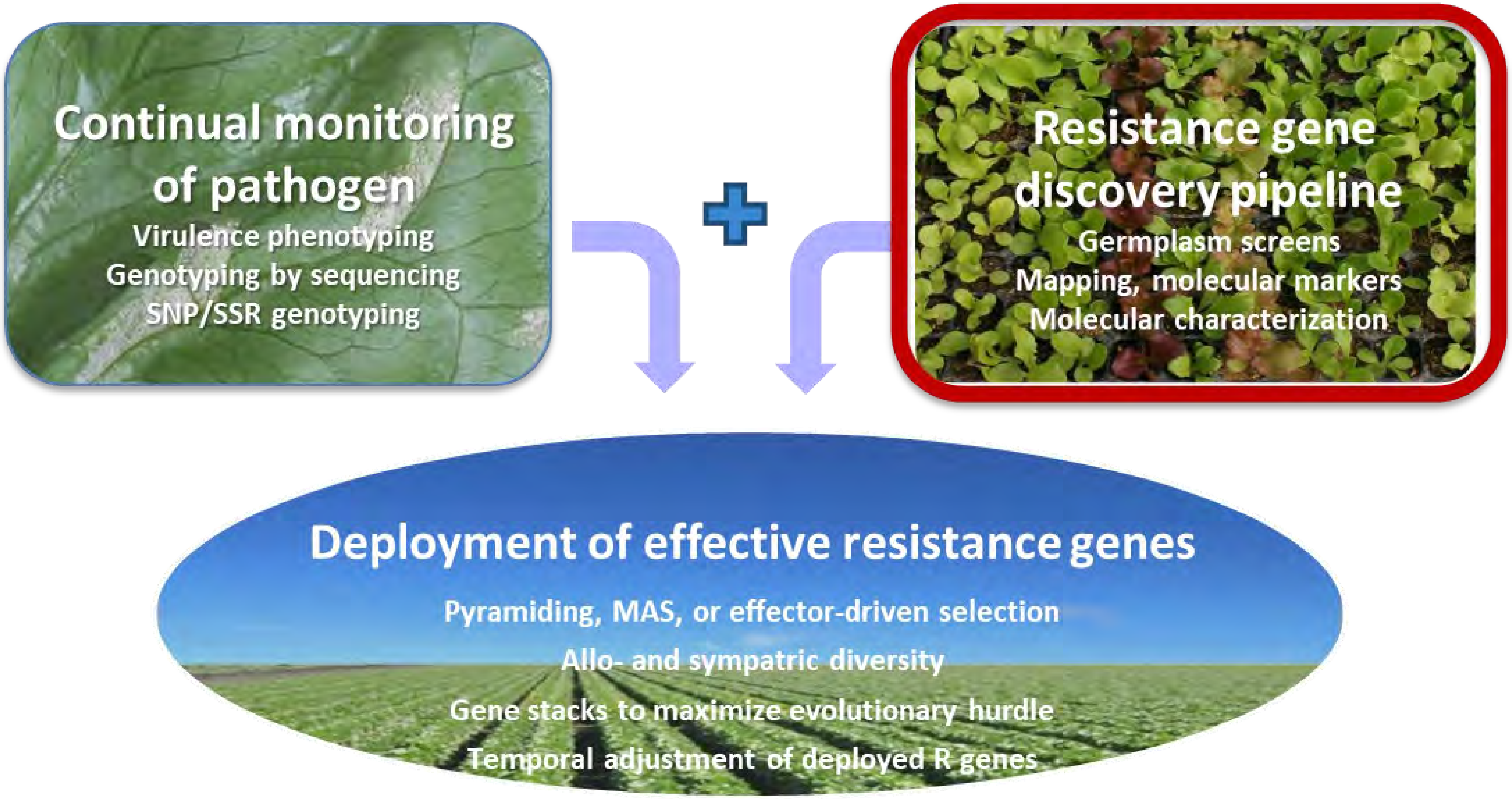
Several other pathogens are associated with symptomatic roots.

Maria Ferrer

Future directions for corky root resistance project.

- Determination of sensitivity and specificity of PCR assay for *R. suberfaciens*.
- Reference-guided assembly of contemporary isolates using sequences of infected roots.
- Sequence comparisons of contemporary assemblies with CA1.
- Attempt to isolate causal bacterium (difficult).
- Further PCR analysis with primers for the *cor* resistance region.
- Sequencing of additional symptomatic samples.
- Analysis of asymptomatic roots from multiple locations.
- Interested in receiving symptomatic and asymptomatic root samples.

The influenza paradigm: deployment of resistance genes driven by knowledge of pathogen population genetics



Continual monitoring of pathogen

Virulence phenotyping
Genotyping by sequencing
SNP/SSR genotyping

Resistance gene discovery pipeline

Germplasm screens
Mapping, molecular markers
Molecular characterization

Deployment of effective resistance genes

Pyramiding, MAS, or effector-driven selection
Allo- and sympatric diversity
Gene stacks to maximize evolutionary hurdle
Temporal adjustment of deployed R genes

100+ lines being sequenced on the PacBio Revio

Mapping parents

e.g. L. sativa Eruption, Ninja, PI251246, *L. serriola* US96UC23, Armenian 999, ….

Lines representing all known sources of resistance to *Bremia lactucae* and other diseases

e.g. IBEB differential series, lines with *Dm* genes designated by Parra *et al*., breeding sources, ….

Lines with interesting developmental phenotypes (LettuceKnow)

e.g. Ostinata, Olof, Tesy, Ibis, Lovina, Mystic, Cocarde, Crna, Chicon Batavia, ……

Lines suggested by SCRI participants/diversity panel

e.g. Merlot, Iceberg, Lolla Rosa, Margarita,

Lines of interest to ILGC companies

e.g. Palmos, Icebazaar, Icevic, Presidential

Others? Suggestions welcome

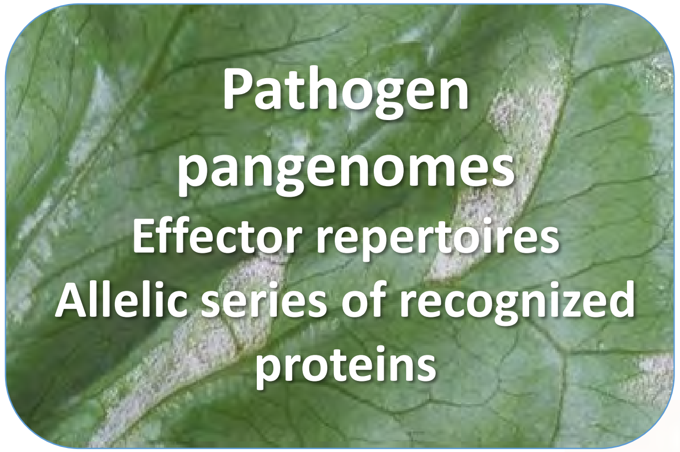
= Highly divergent set of lines of *L. sativa*, *L. serriola*, *L. aculeata*, *L. saligna*, *L. virosa*, *L. tartarica*, & *L. perennis*. Reference templates for short reads of diversity panels.

T2T Sequencing Summary

L. sativa
  Reference genome: cv. Salinas, gapless, 2.59 Gb
  20 cultivars, ~ 90-300 gaps
L. serriola
  Reference genome: US96UC23, ~gapless, 2.59 Gb
  4 accessions, ~ 60-150 gaps
L. saligna
  Reference genome: CGN05327, ~gapless, 2.3 Gb
  6 accessions, ~ 80-120 gaps
L. virosa
  Reference genome: CGN4683, ~gapless (93 gaps), 3.4 Gb
  2 accessions, ~ 600 gaps

Long-term goal: Deploying resistance genes encoding novel recognition capabilities

Disease resistance in plants is initiated by extracellular and intracellular receptors



Pathogen pangenomes

Effector repertoires

Allelic series of recognized proteins

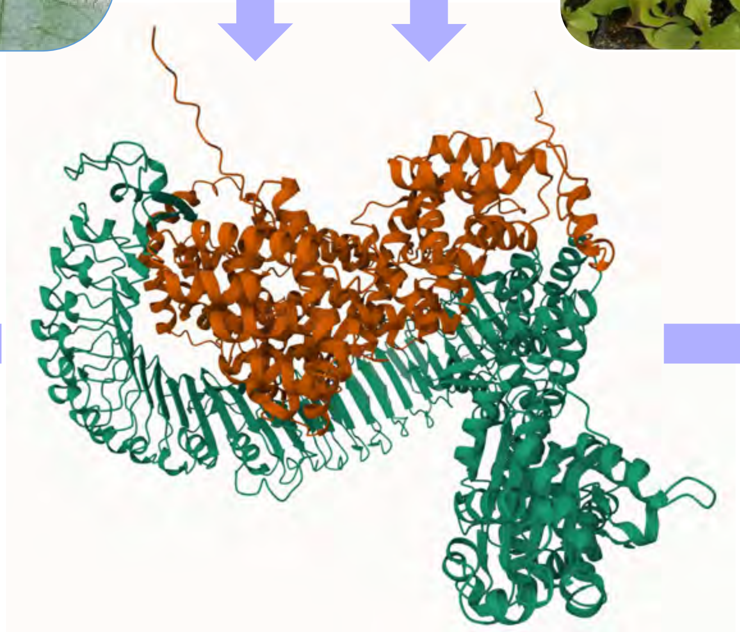
Alfafold

CryoEM

Crop pangenome

Resistance gene repertoire

Allelic series of receptor proteins



Transient assays

Modification validation

Base/prime editing

Crop deployment

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Hanhui Kuang, Brigitte Masionneuve,
& many others for specific clones & materials

Funding

California Leafy Greens Research Board
International Lettuce Genomics Consortium
USDA NRI, AFRI, SCRI
Foundation for Food and Agricultural Research

THANKS TO THE CALIFORNIA LEAFY GREENS RESEARCH BOARD FOR FOUNDATIONAL SUPPORT *

Strategies to reduce lettuce susceptibility to biotic stressors

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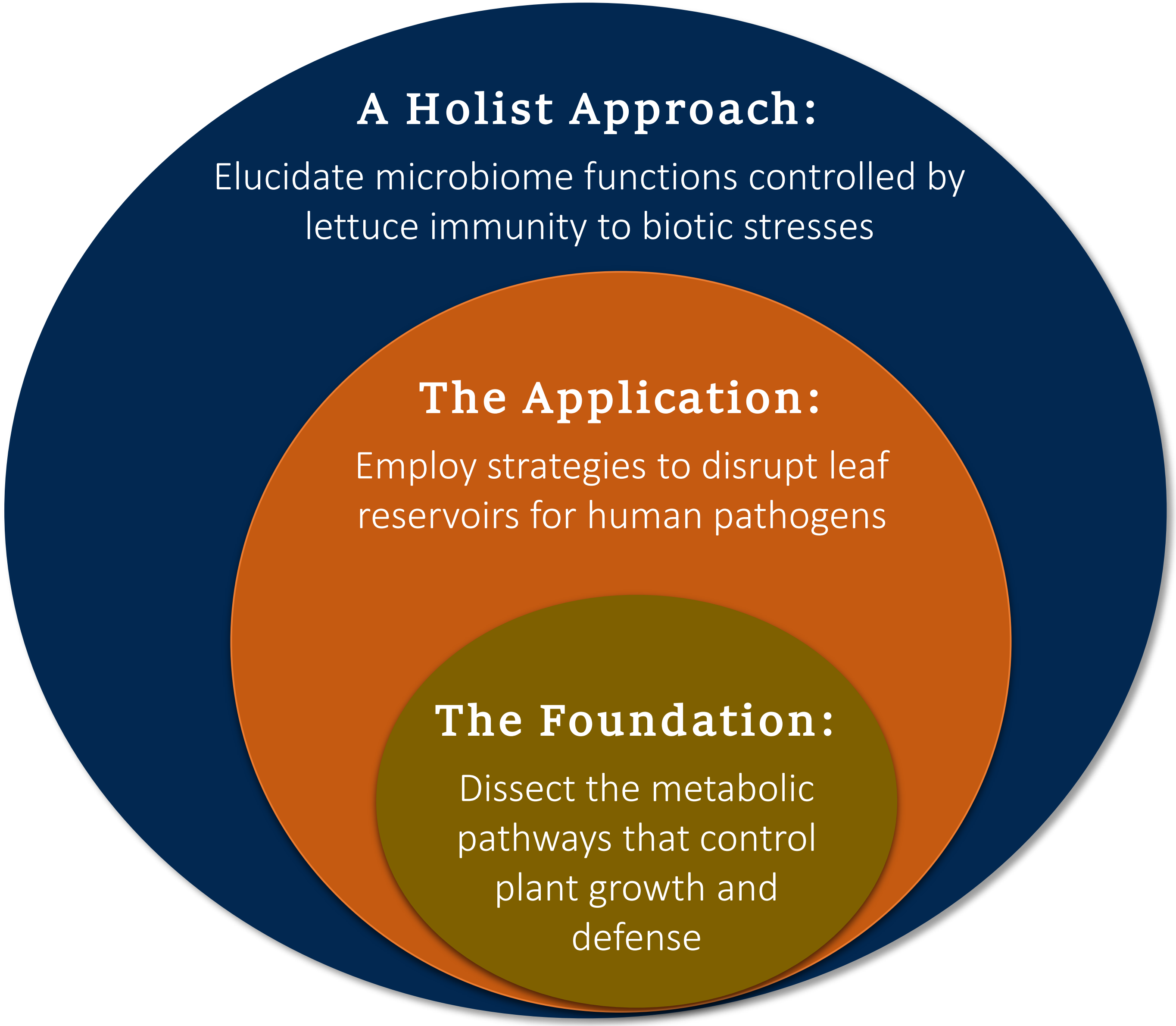
UCDAVIS
DEPARTMENT OF PLANT SCIENCES
College of Agricultural and Environmental Sciences

Research Program and Goals



A Holist Approach:

Elucidate microbiome functions controlled by lettuce immunity to biotic stresses

The Application:

Employ strategies to disrupt leaf reservoirs for human pathogens

The Foundation:

Dissect the metabolic pathways that control plant growth and defense

2020 –
Agricultural microbiome functions

2011 –
Human pathogens on plants with focus on lettuce

2003 –
Model systems to study plant-pathogen interactions

Employing strategies to disrupt leaf reservoirs for human pathogens

(funded by CA Leafy Greens Research Program, Henry A. Jastro Research Awards, BARD, USDA-NIFA-AFRI awards)

Relevant Articles:

1. Jacob C, **Melotto M**. 2025. BMC Plant Biology 25:635.
2. Student J, Weitz T, Blewett T, Yaron S, **Melotto M**. 2024. J Food Protection 87:100334.
3. Jacob C, Student J, Bridges DF, Weiping C, Porwollik S, McClelland M, **Melotto M**. 2024. Front Plant Sci. 15:1302047.
4. **Melotto M**, Zhang W, Teplitski M. 2023. eBook: Breeding crops for enhanced food safety. Lausanne: Frontiers Media SA.
5. Jacob C, Velasquez AC, Josh N, Settles M, He SY, **Melotto M**. 2021. G3: Genes|Genomes|Genetics 11(12):jkab331.
6. **Melotto M**, Brandl MT, Jacob C, Jay-Russell M, Micallef SA, Warburton M, Van Deynze A. 2020. Front Plant Sci 11:428.
7. Montano J, Rossidivito G, Torreano J, Porwollik S, Sela S (Saldinger), McClelland M, **Melotto M**. 2020. Front Microbiol 11:6.
8. Oblessuc PR, Matiolli CC, **Melotto M**. 2020. BMC Plant Biology 20:16.
9. Jacob C, **Melotto M**. 2020. Front Plant Sci 10:1769
10. Oblessuc PR, Bisneta MV, **Melotto M**. 2019. FEMS Microbiol Letters 366:fnz197.
11. Roy D, Panchal S, Rosa BA, **Melotto M**. 2013. Phytopathology 103:326-332. (*Editor's Pick*).
12. Melotto M, Underwood W, Koczan J, He SY. 2006. Cell 126:969-980.

Why do we care? Public health concern and economic burden

Contents lists available at ScienceDirect

Journal of Food Protection

journal homepage: www.elsevier.com/locate/jfp

Research Paper

Foodborne Illnesses from Leafy Greens in the United States: Attribution, Burden, and Cost

Xuerui Yang *, Robert Scharff

Department of Human Science, The Ohio State University, Columbus, OH, USA

ARTICLE INFO

Keywords:
Burden of illness
Cost of illness
Food attribution
Leafy greens
Norovirus
Shiga toxin-producing *Escherichia coli*

2024

ABSTRACT

Leafy green vegetables are a major source of foodborne illnesses. Nevertheless, few studies have attempted to estimate attribution and burden of illness estimates for leafy greens. This study combines results from three outbreak-based attribution models with illness incidence and economic cost models to develop comprehensive pathogen-specific burden estimates for leafy greens and their subcategories in the United States. We find that up to 9.18% (90% CI: 5.81%-15.18%) of foodborne illnesses linked to identified pathogens are attributed to leafy greens. Including 'Unknown' illnesses not linked to specific pathogens, leafy greens account for as many as 2,307,558 (90% CI: 1,077,815–4,075,642) illnesses annually in the United States. The economic cost of these illnesses is estimated to be up to $5.278 billion (90% CI: $3.230-$8.221 billion) annually. Excluding the pathogens with small outbreak sizes, Norovirus, Shiga toxin-producing *Escherichia coli* (both non-O157 and O157:H7), *Campylobacter* spp., and nontyphoidal *Salmonella*, are associated with the highest number of illnesses and greatest costs from leafy greens. While lettuce (romaine, iceberg, "other lettuce") takes 60.8% of leafy green outbreaks, it accounts for up to 75.7% of leafy green foodborne illnesses and 70% of costs. Finally, we highlighted that 19.8% of Shiga toxin-producing *Escherichia coli* O157:H7 illnesses are associated with romaine among all food commodities, resulting in 12,496 estimated illnesses and $324.64 million annually in the United States.

Annually, 9.2% of outbreaks in the US are attributed to leafy greens:

- > 2 million illnesses
- > $5.2 billion cost

Main etiological agents are: Norovirus and three bacteria (STEC, Campylobacter, and Salmonella)

Within leafy greens, lettuce accounts for:

- 61% of outbreaks
- 75% of the illnesses
- 70% of the cost

2019 NIFA-UC Davis Workshop
Breeding Crops for Enhanced Food Safety

Organizers:

Allen Van Deynze
Plant Breeding

Michele Jay-Russell
Food Safety

Maeli Melotto
Plant-Microbe Interactions

USDA NIFA

Western Center for Food Safety WCFS UC DAVIS

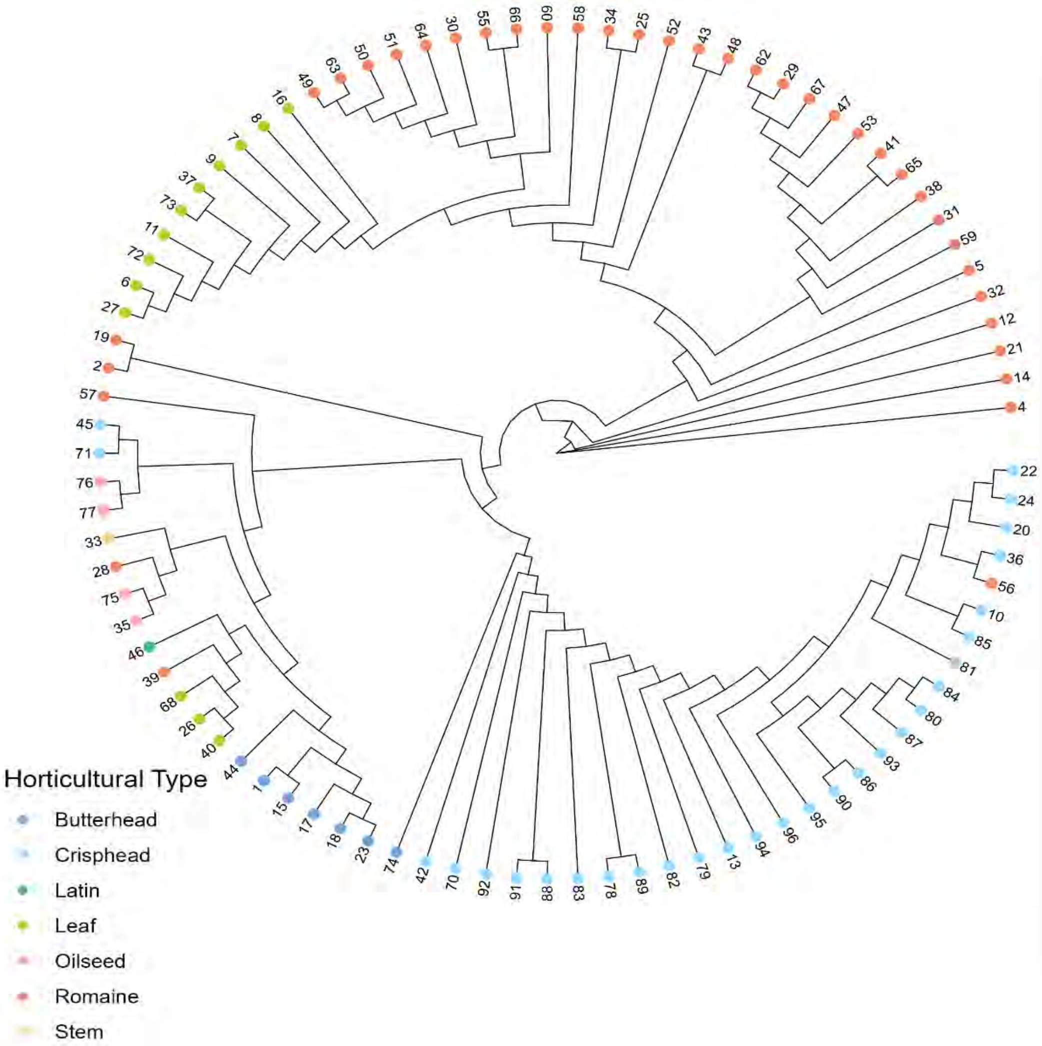
UC DAVIS Plant Breeding Center

CALIFORNIA LEAFY GREENS RESEARCH PROGRAM

UC DAVIS DEPARTMENT OF PLANT SCIENCES
College of Agricultural and Environmental Sciences

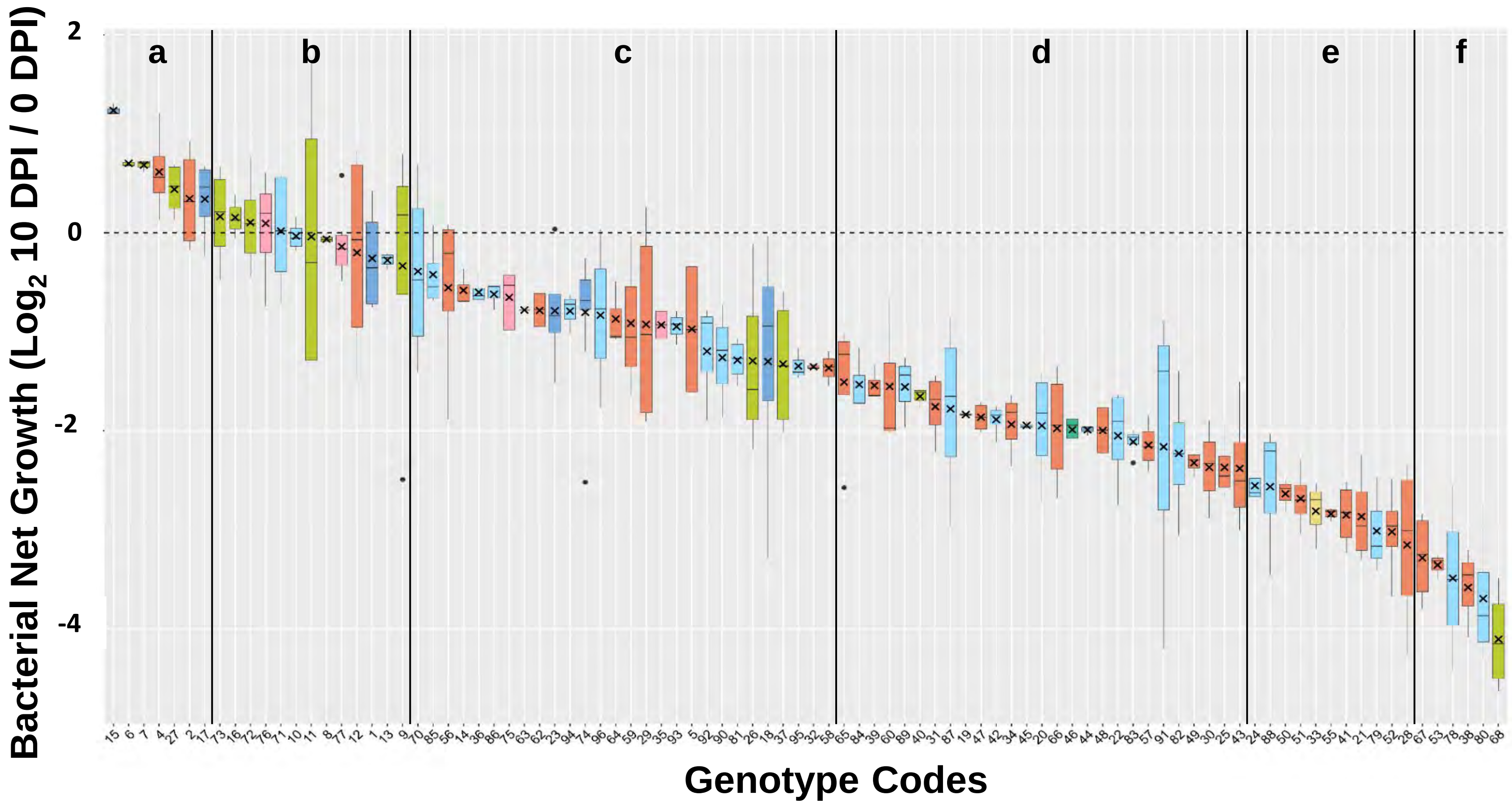
O157:H7 persistence as target for breeding

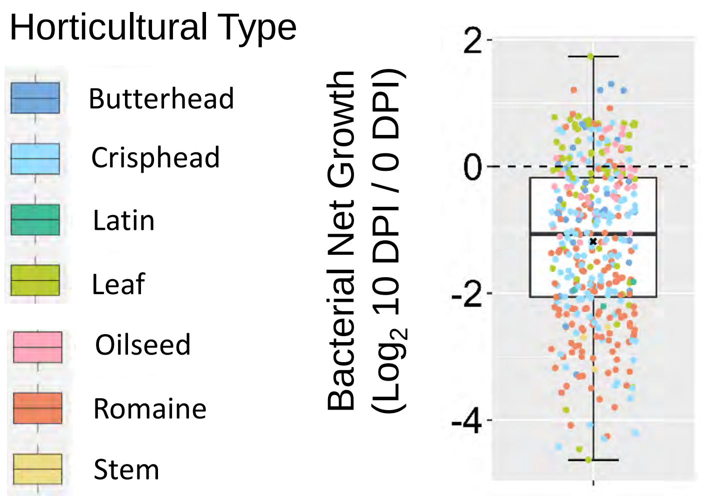
Screening of 96 lettuce genotypes of various horticultural types

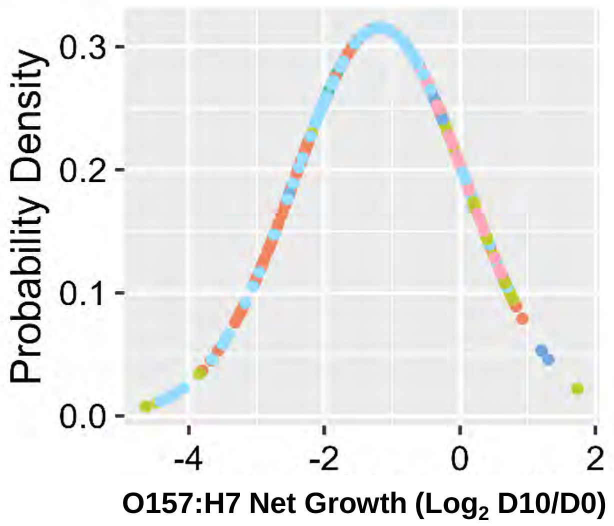


O157:H7 persistence is a quantitative trait

Screening of 96 lettuce genotypes of various horticultural types



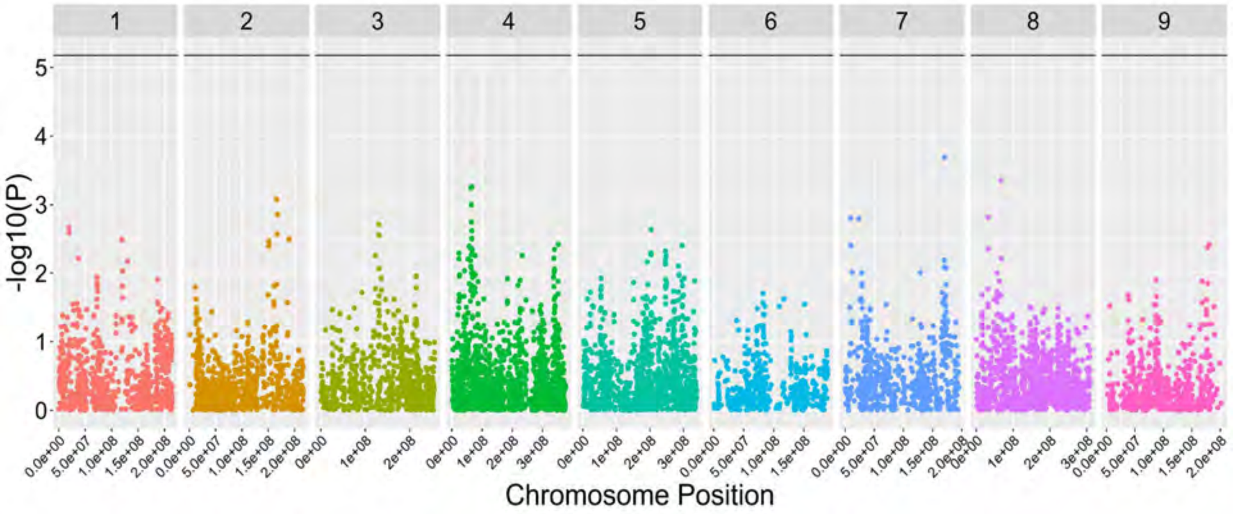




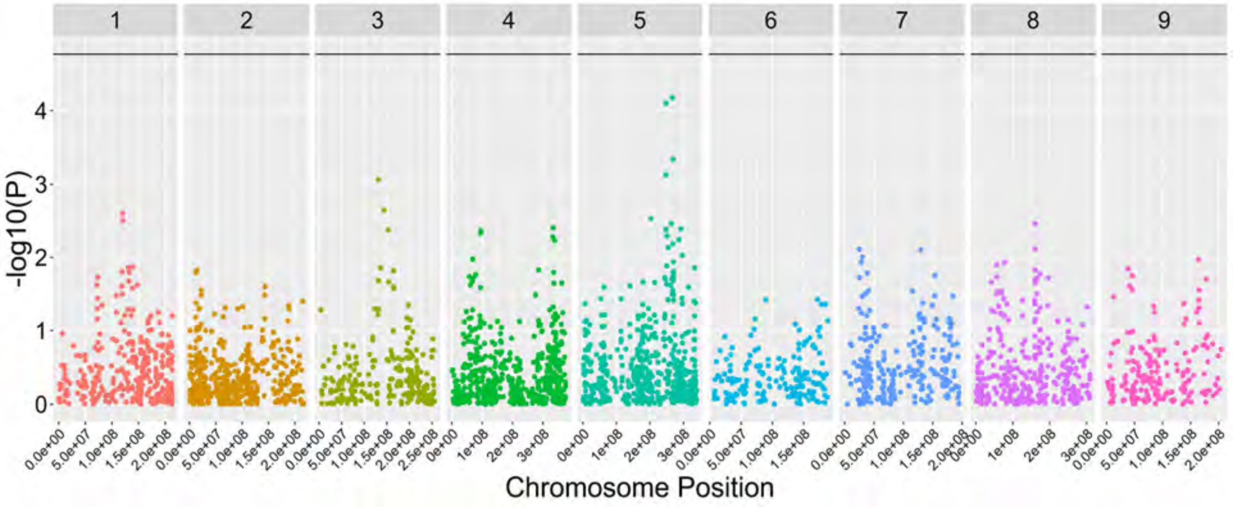
Searching for genes associated with O157:H7 persistence

Genome Wide Association Studies (GWAS) connecting phenotypic variability with genetic variability

Full Screen GWAS

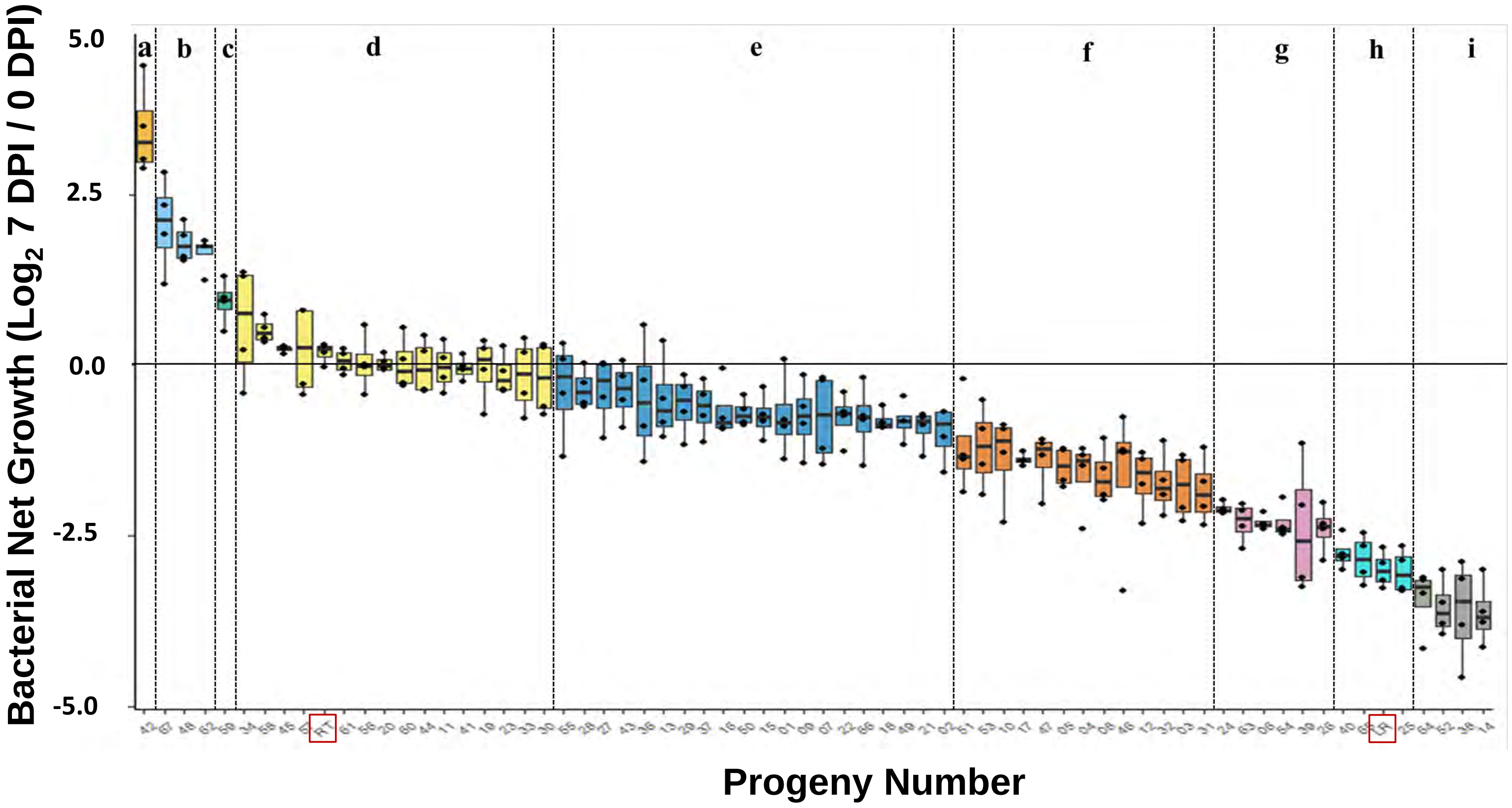


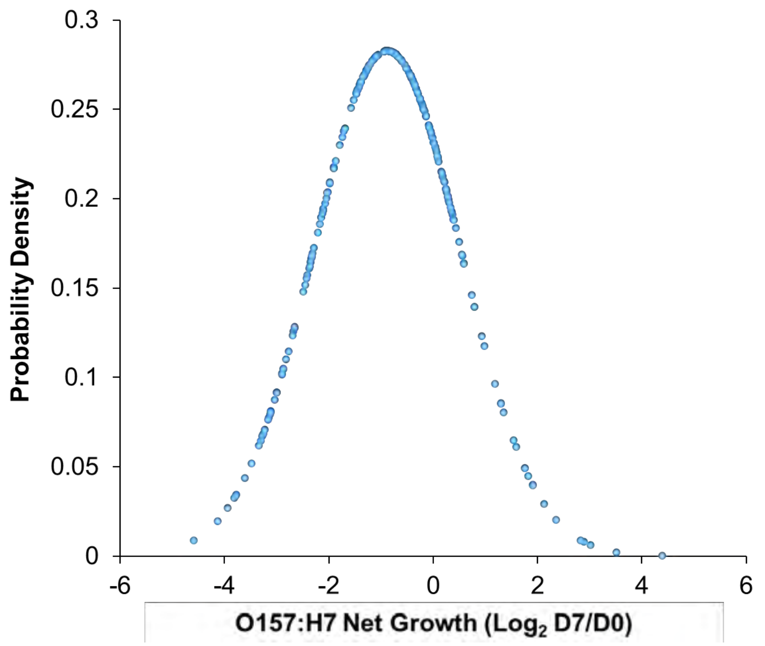
Romaine and Leaf Lettuce GWAS



O157:H7 persistence is a quantitative trait

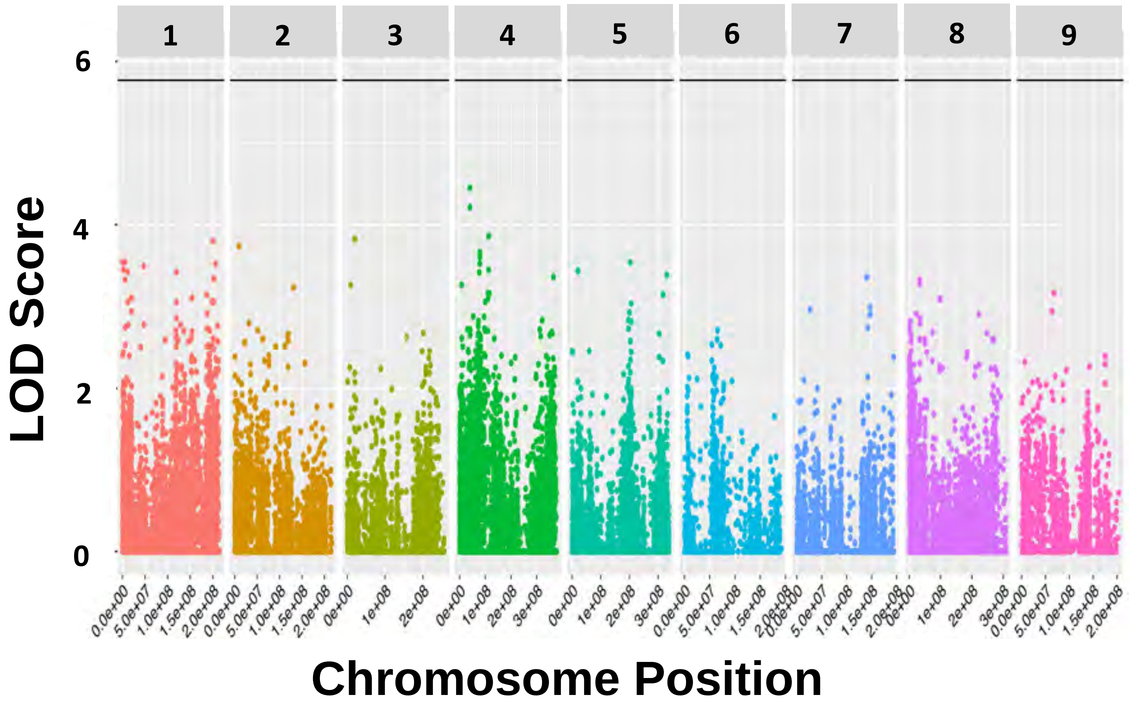
Screening of 67 $F_{2:3}$ families derived from the Lollo Rossa x Red Tide cross



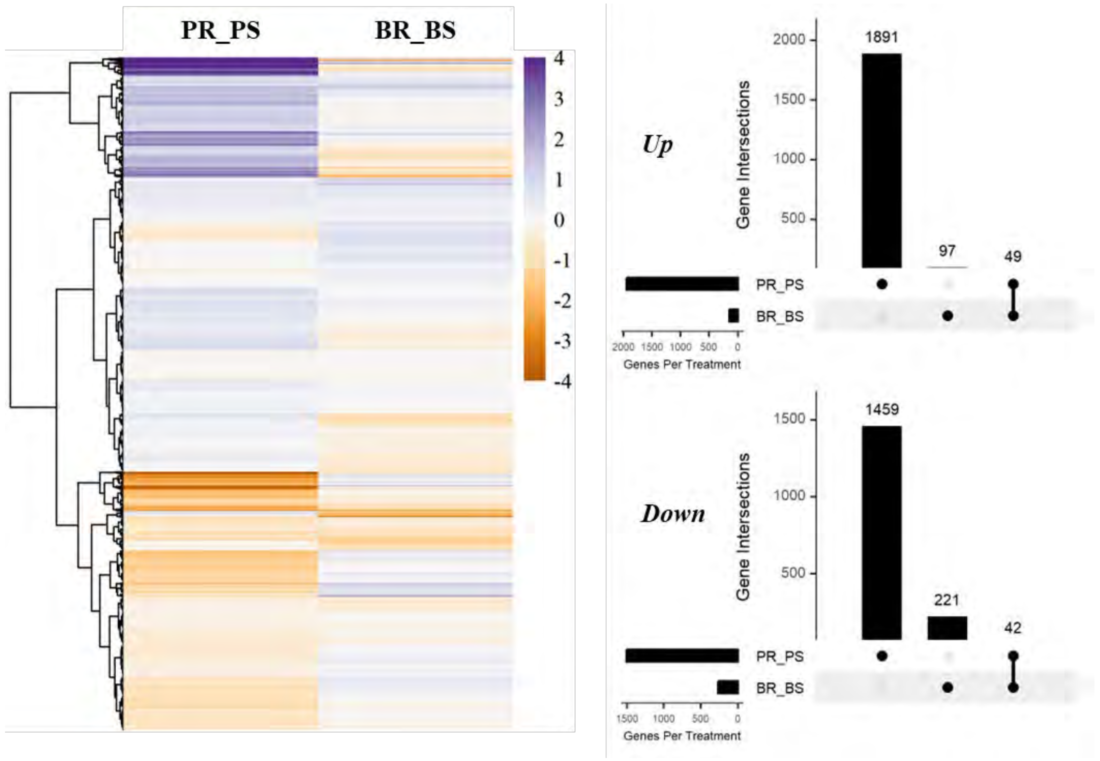


Searching for genes associated with O157:H7 persistence

Regression analysis

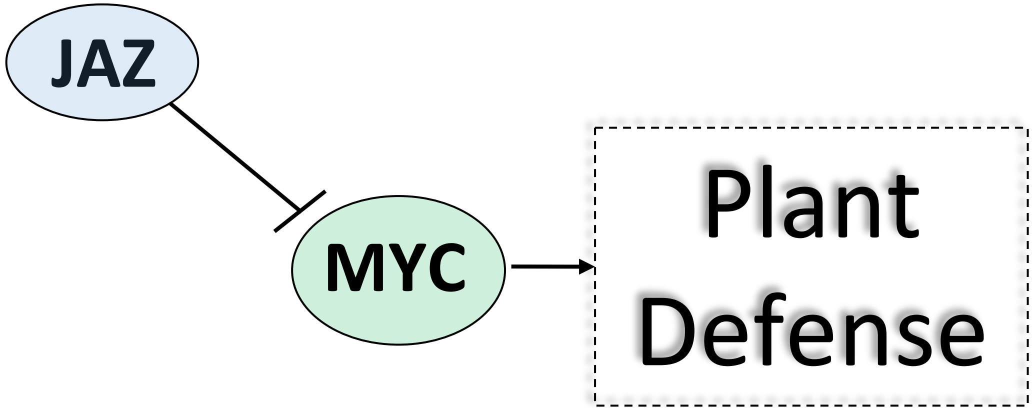


RNA-seq bulk segregant analysis



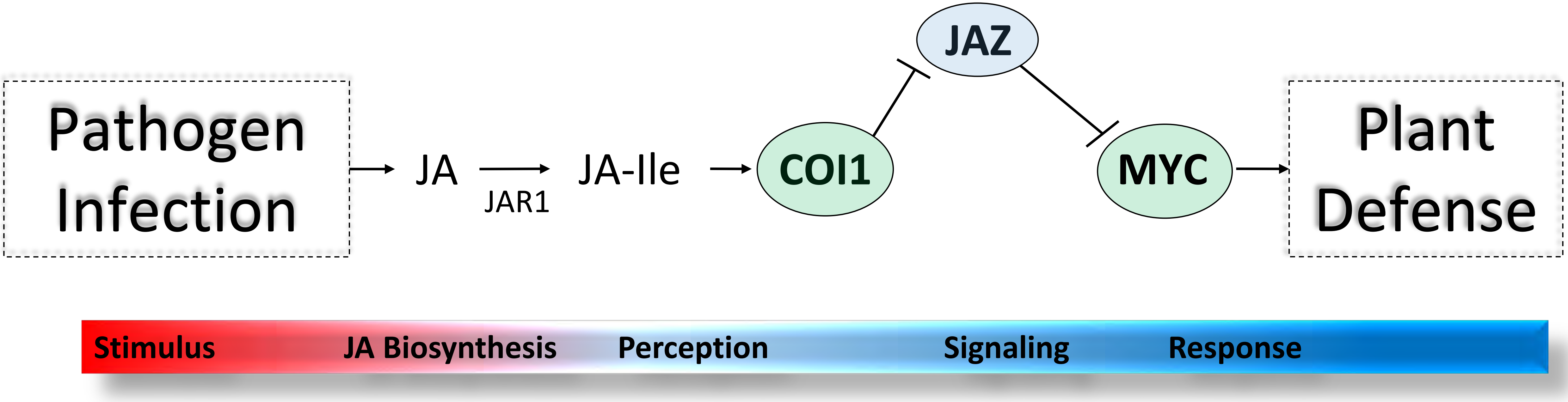
JAZ proteins put the breaks on defenses

Resting State



Jasmonate signaling potentiates defenses

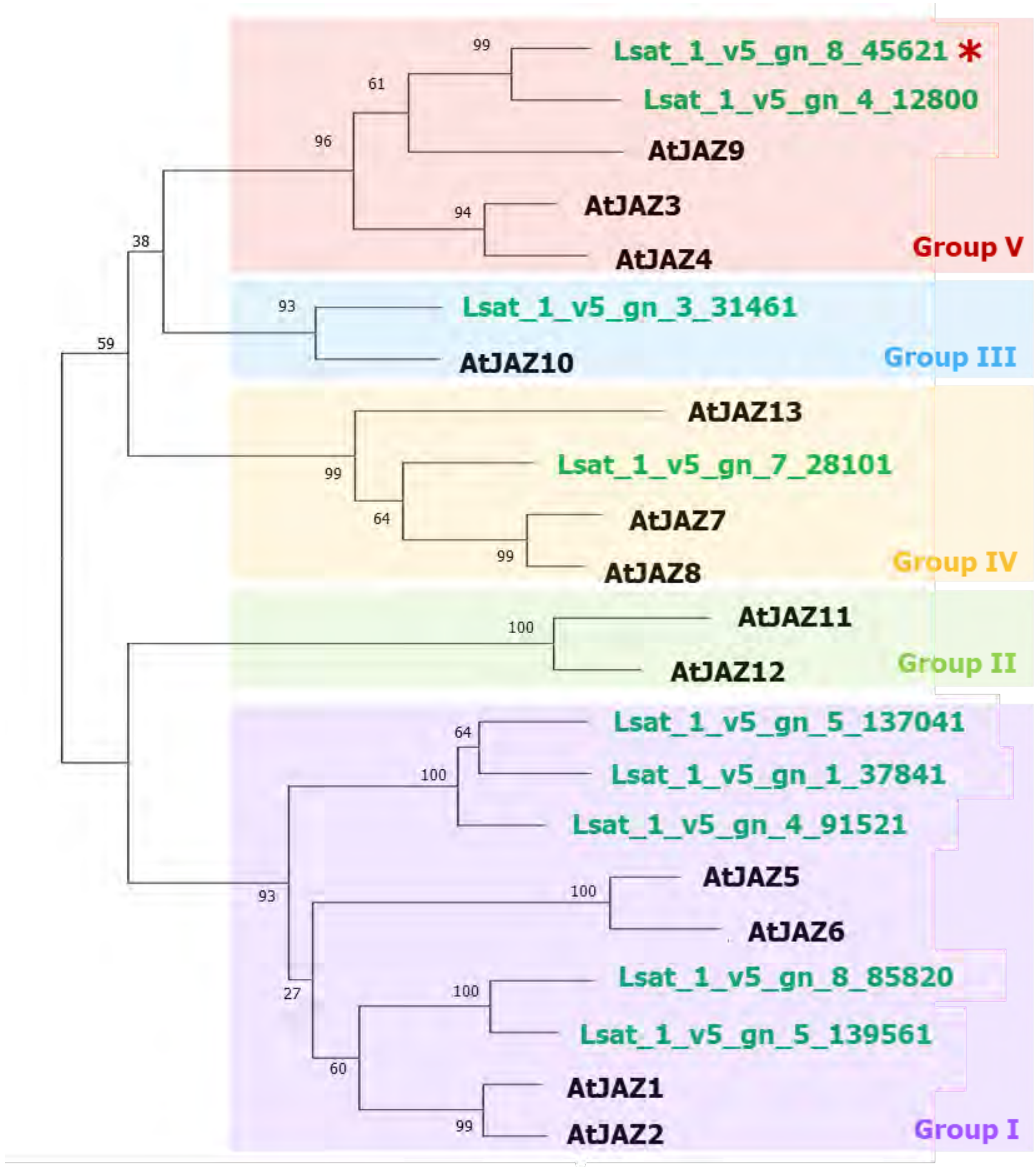
Activation State



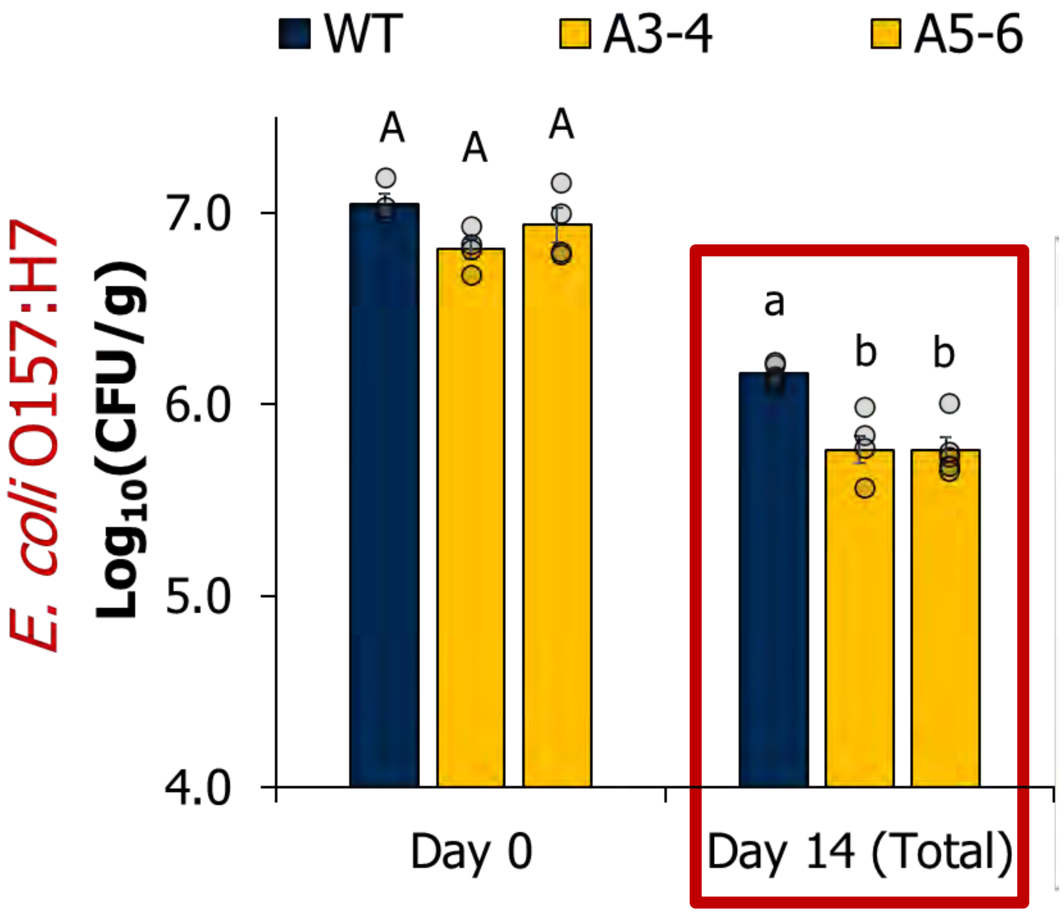
Identification of these proteins required a comprehensive approach and extensive effort by multiple research teams.

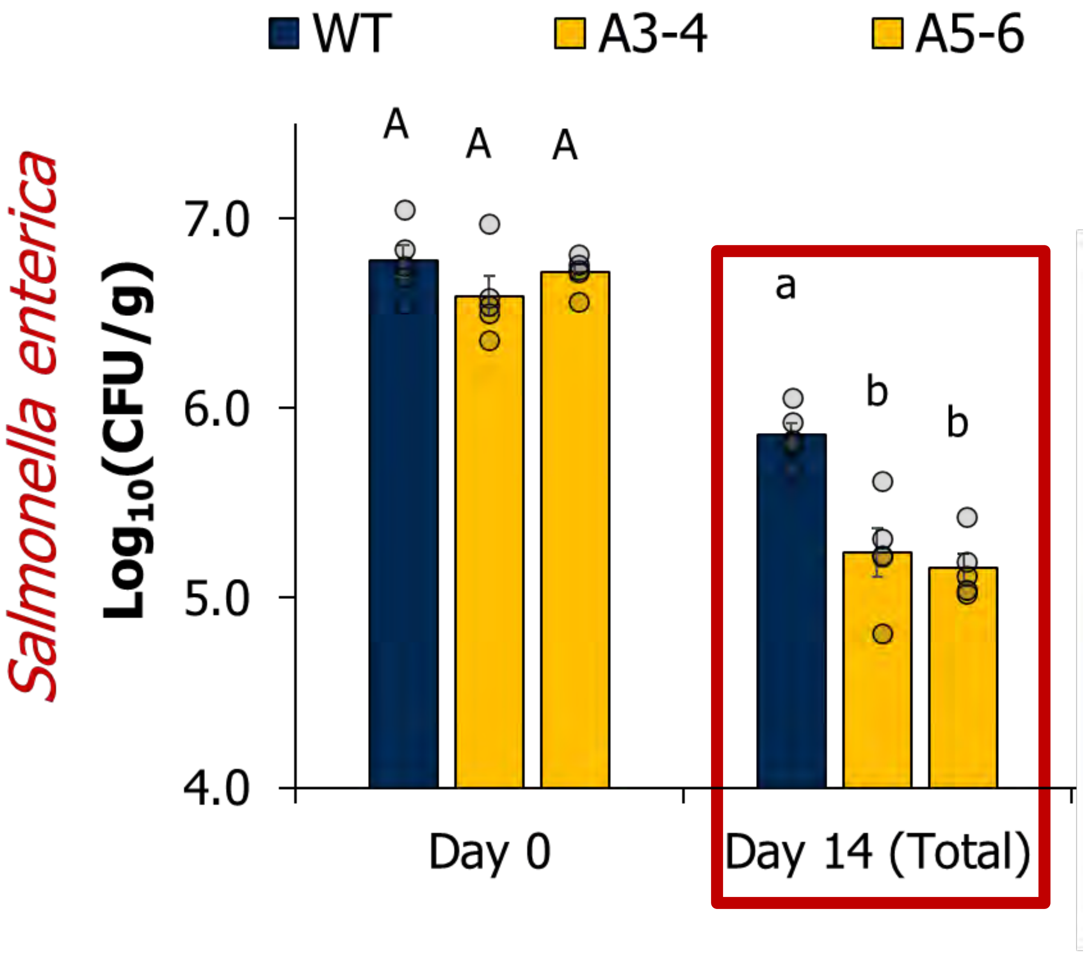
Thines, Katzir, Melotto et al. (Nature 2007); Chini et al. (Nature 2007)

Lettuce genome has nine LsJAZ encoding genes

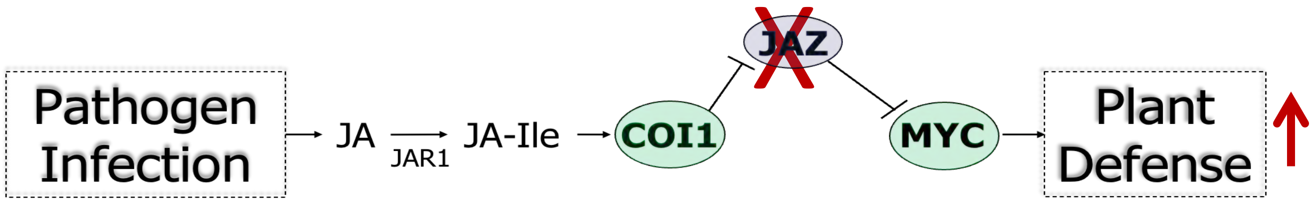


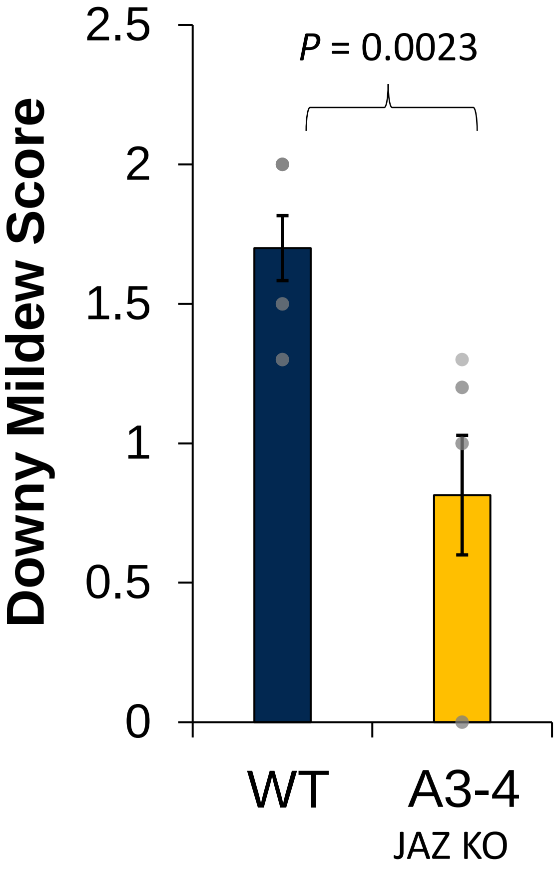
Ls45621 contributes to HP persistence





By releasing the JA signaling, lettuce becomes more resistant to multiple stressors in the field


Pathogen Infection
JA
JAR1
JA-Ile
COI1
JAZ
MYC
Plant Defense



Final thoughts…

- *Traits in development* = human pathogen persistence in leaves and overall resistance to field biotic stressors.
- *Germplasm, markers, or screening tools* = exploratory genome-wide approaches to develop markers and targeted approaches to search for causal genes.
- *Relevant breeding goals or upcoming opportunities* = safer and healthier crop through targeted genome editing.
- *Thoughts on where collaboration with industry could be valuable* = commercially relevant cultivars and development of mapping populations for screening.

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CA Leafy Greens Research Program
CA&ES Programmatic Initiatives
Henry Jastro Research Awards
Industry Gifts and Awards
NASA Space Biology Program
NIH-NIAID
USDA-NIFA-AFRI

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Yulin Du
Zach Jaramillo
Maria Miccono
Ho-Wen Yang

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Visiting Professors
Postdoctoral Fellows
Graduate Students
Junior Specialists
Undergraduates
High School Students

Improving Nitrogen Use Efficiency (NUE) in Spinach

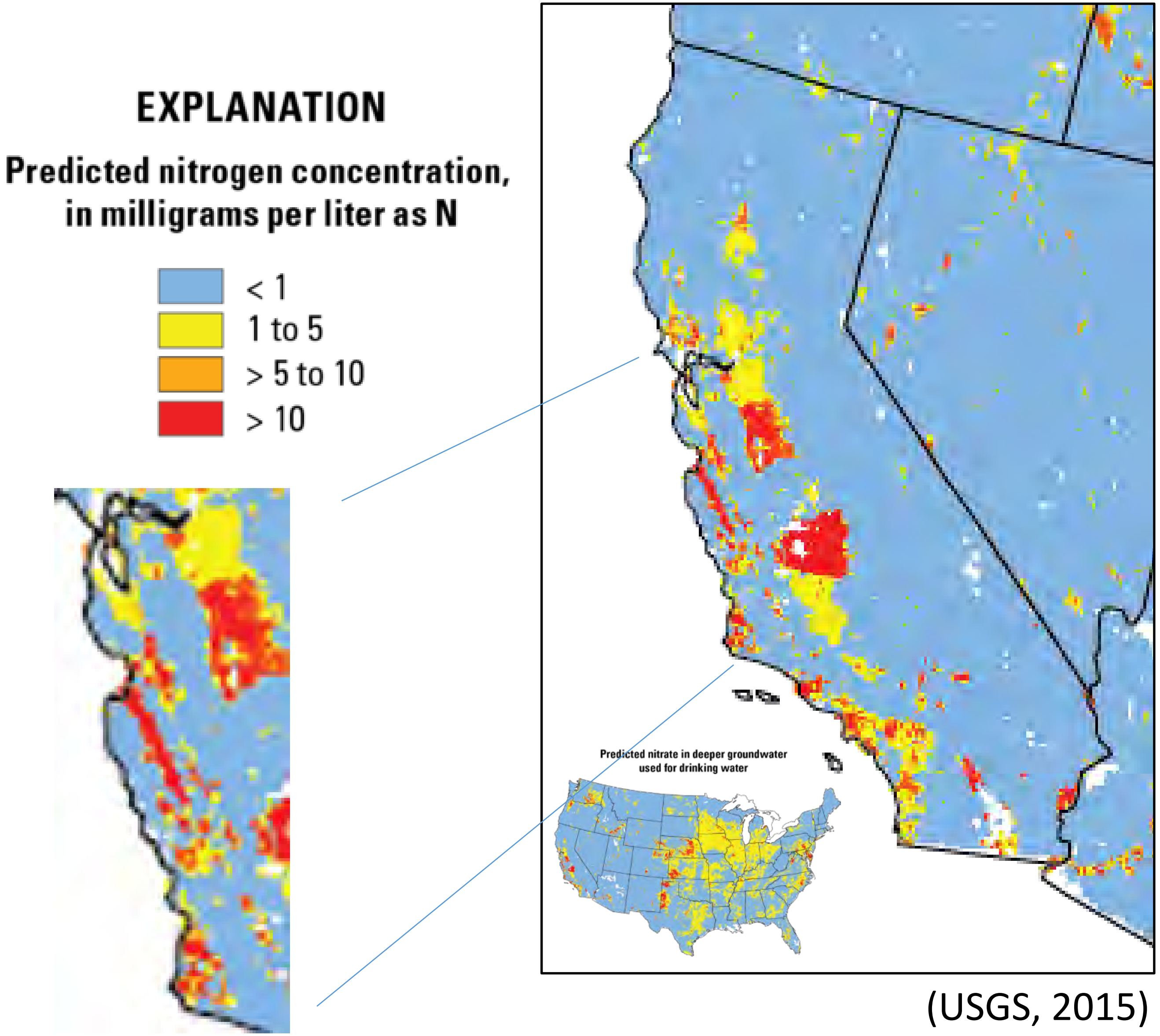

Charlie Brummer
UC Davis


Allen Van Deynze
UC Davis

Charlie Brummer, Allen Van Deynze, Oon-ha Shin,


Oon-Ha Shin
Postdoc – Breeder

Why Study Nitrogen Use Efficiency?

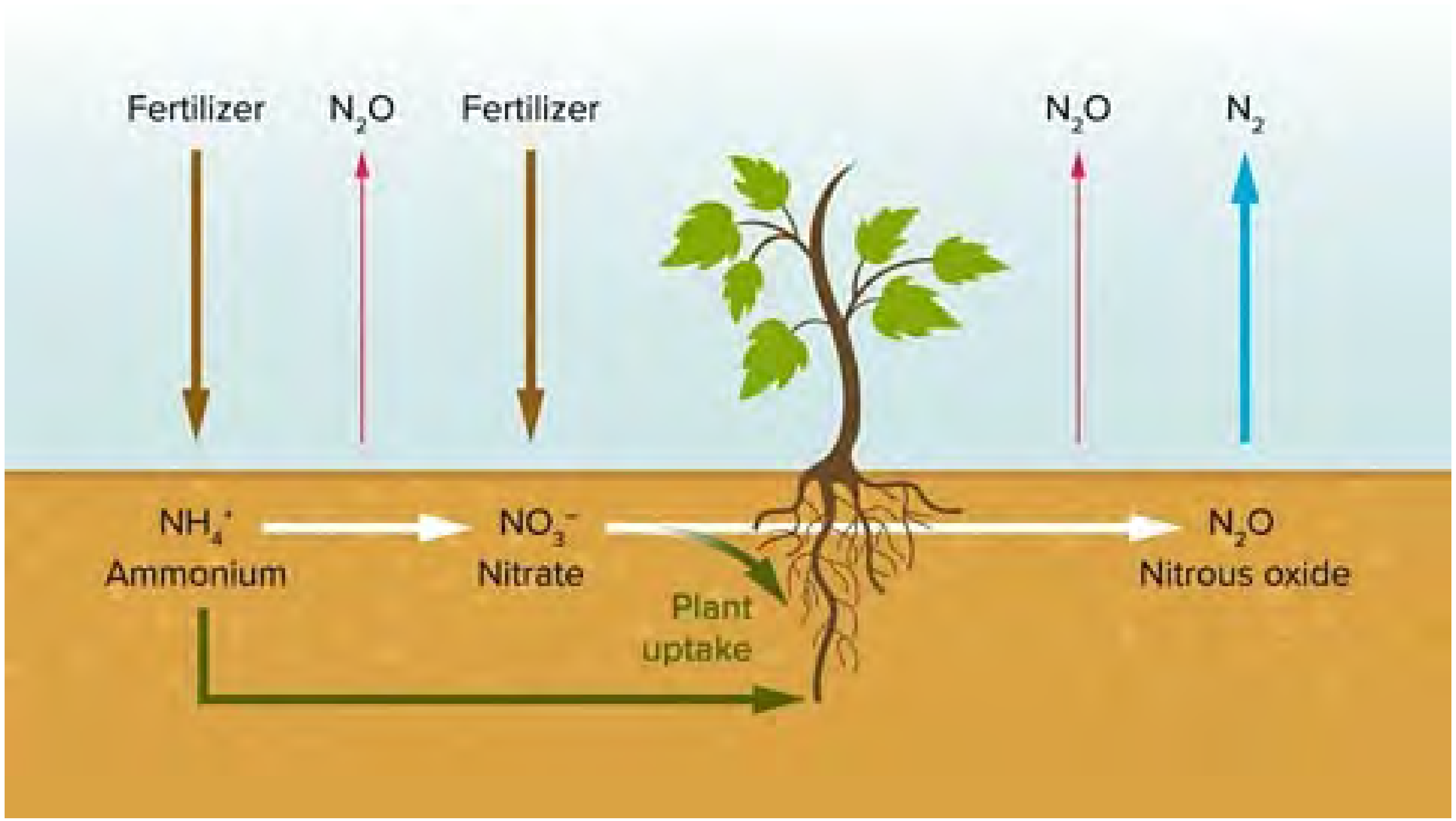


(USGS, 2015)

- CA produces 70% US spinach
- Two thirds of nitrogen pollution in the US is caused by agriculture runoff
- Nitrogen pollution in ground water is an issue for drinking water
- Fertilizer costs account for nearly 20% of the total operating cost of spinach production

Nitrous oxides (N_20) are 300 times more potent than Carbon

- ~1/2 of nitrogen fertilizer is used by plants
- N_20 accounts for 2-3% of greenhouse gases, but are 300 times more potent than carbon in refracting C02 and degrading ozone.
- N_20 emissions increases with dry soils and placement above 2 inches in soil in both conventional and low tillage



When plant roots don’t take up all the nutrients from fertiliser, the greenhouse gas N_2O is released (Credit: E. Verhoeven et al/California Agriculture 2017/Knowable Magazine)

Improving Nitrogen Use Efficiency is one of the most effective methods of counteracting effects of nitrogen on environment and to maximize yield

van Kessel, et al. 2013. Global Change Biology 19:33-44

Screening for Nitrogen Use Efficiency

2019 - 2021

Germplasm accessions from USDA, CGN, IPK

Hybrid cultivars

- 344 gene-bank accessions with high marketability under low N were selected for breeding
- Fresh/dry biomass, chlorophyll concentration, total N, leaf shape were measured under low N (~50 lbs/acre)

- Advanced breeding strategies explored using genomic and remote sensing data from the trials

Breeding for Nitrogen Use Efficiency

Evaluate single plant or families

Recombine best

Advance population

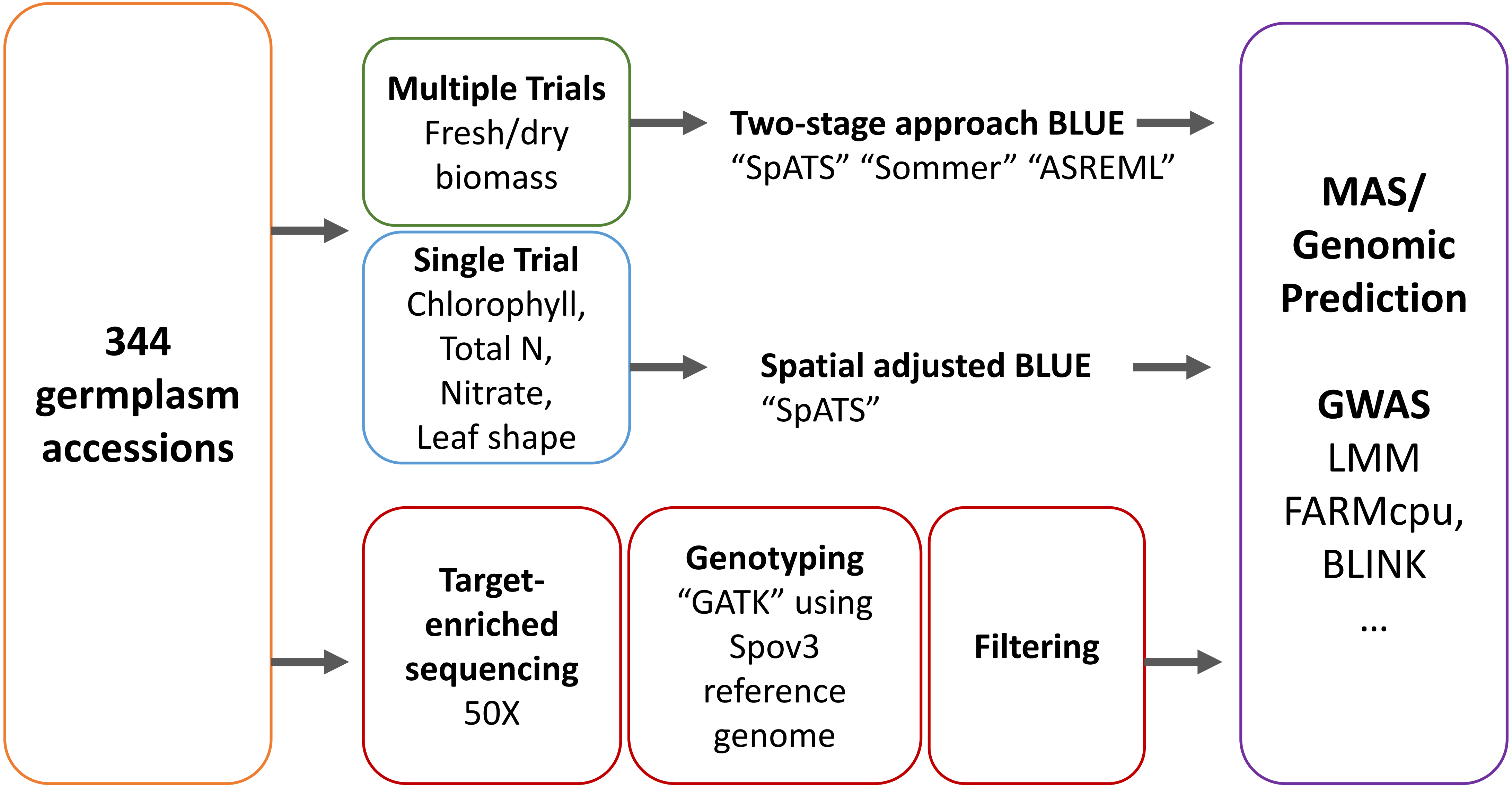
<Recurrent selection>

	NUE POPULATION				
Generation/Cycle	**2021**	**2022**	**2023**	**2024**	**2025**
New crosses	61	8	49	-	-
Syn1 – Syn4 (not evaluated)		13	16	11	11
Cycle 1			5	10	7
Cycle 2					3
Cycle 3					
Total	**61**	**21**	**70**	**21**	**21**


(Salinas, CA)

- Promising populations were developed by recombining best families or single plants

GENOMIC ANALYSIS APROACH



344 germplasm accessions

Multiple Trials
Fresh/dry
biomass

Two-stage approach BLUE
“SpATS” “Sommer” “ASREML”

Single Trial
Chlorophyll,
Total N,
Nitrate,
Leaf shape

Spatial adjusted BLUE
“SpATS”

Target-enriched sequencing
50X

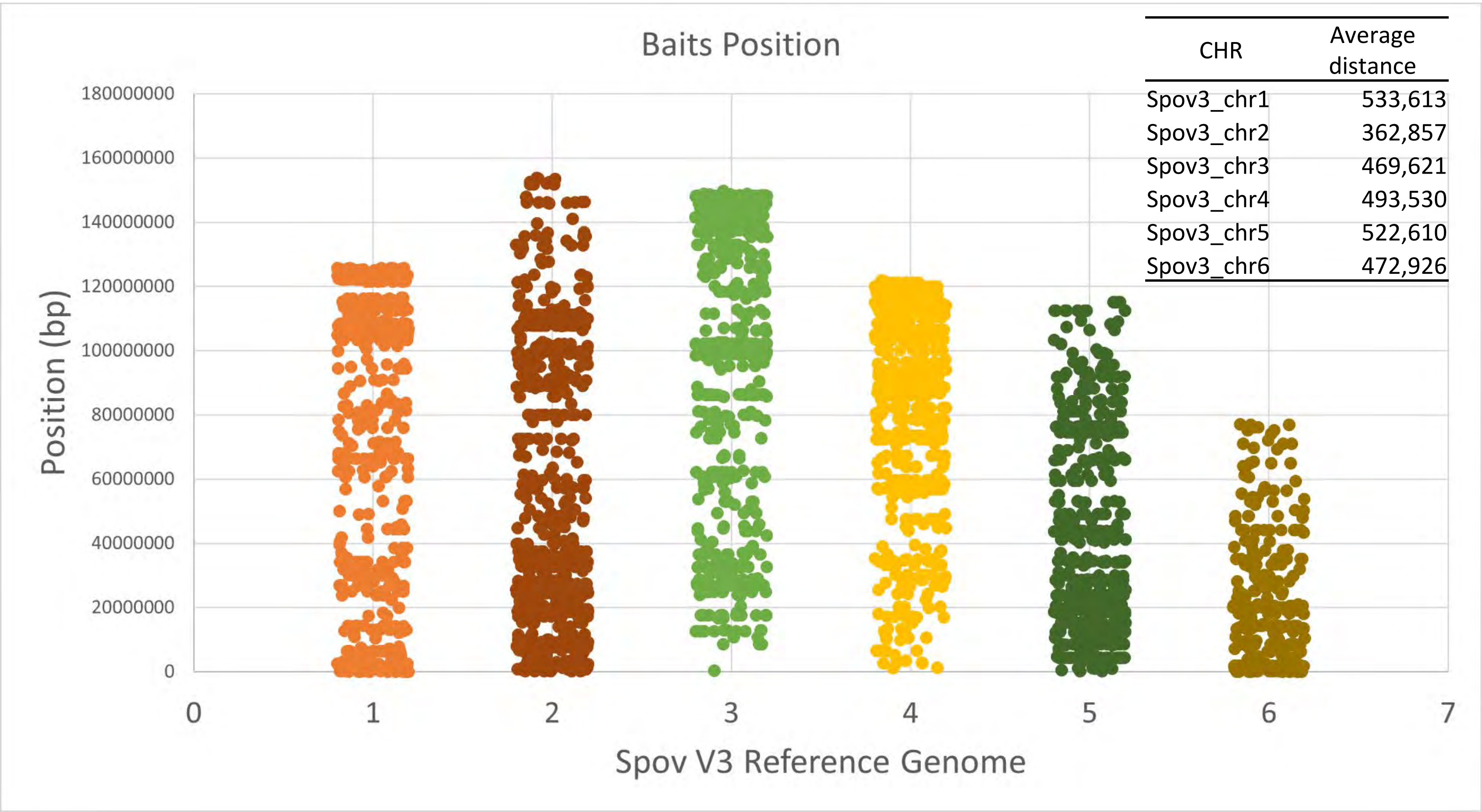
Genotyping
“GATK” using
Spov3
reference
genome

Filtering

MAS/
Genomic
Prediction

GWAS
LMM
FARMcpu,
BLINK
...

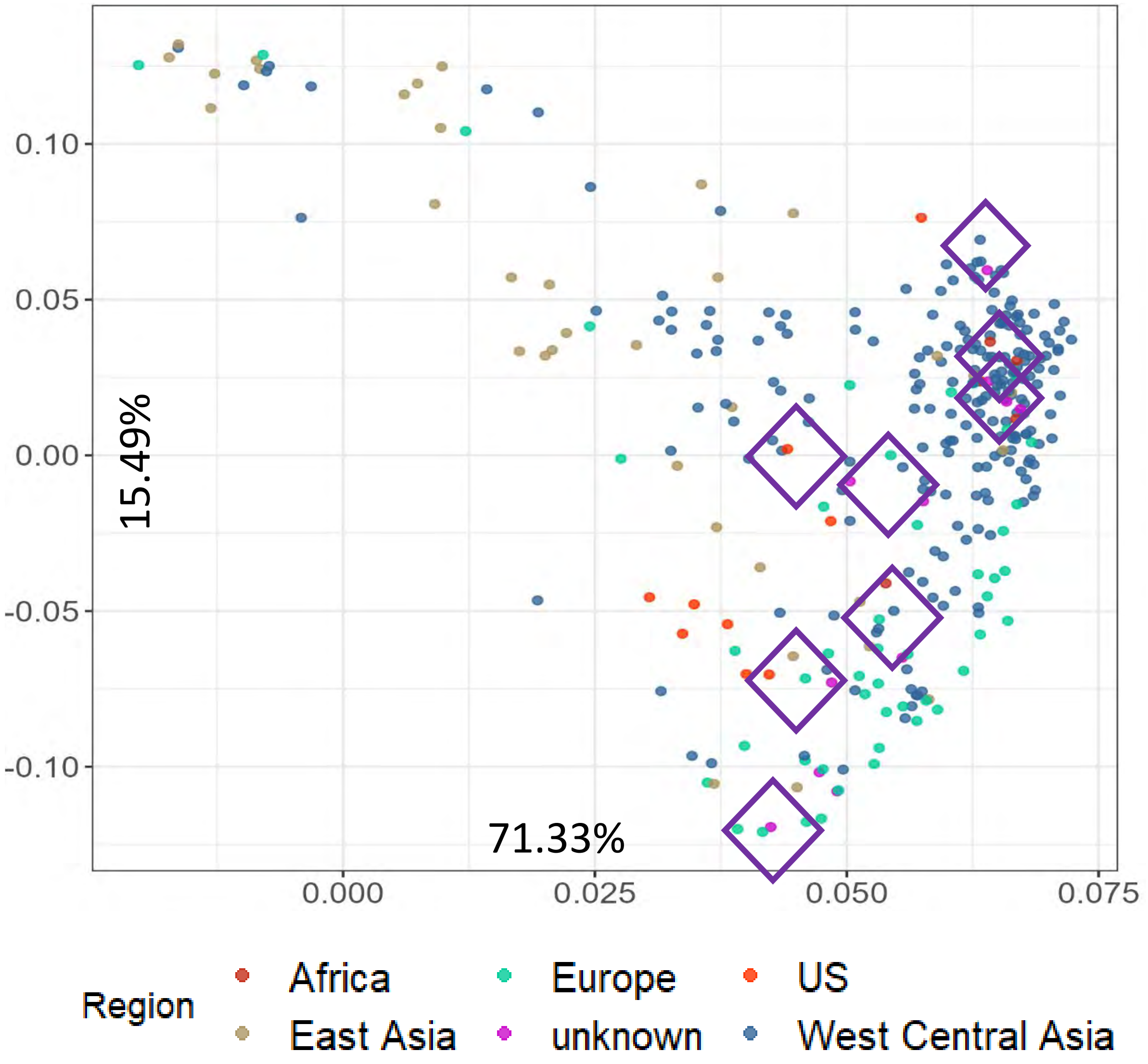
GENOMIC ANALYSIS APROACH



CHR	Average distance
Spov3_chr1	533,613
Spov3_chr2	362,857
Spov3_chr3	469,621
Spov3_chr4	493,530
Spov3_chr5	522,610
Spov3_chr6	472,926

- Target-enriched sequencing method
- 20k baits designed by Van Deyne’s lab to capture key genes that are associated with spinach improvement
- Evenly distributed annotated R genes in *Spov3*, the reference genome

GENOMIC ANALYSIS APROACH



- Diversity analysis on our gene-bank accessions
- Separate domestication processes between Asia and Europe (Ribera, 2020)
- Accessions from central Asia performed generally well and were introduced to our breeding pool

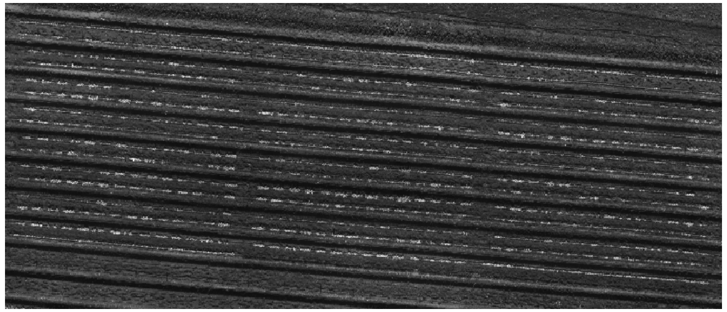
REMOTE SENSING

FIELD TRIALS

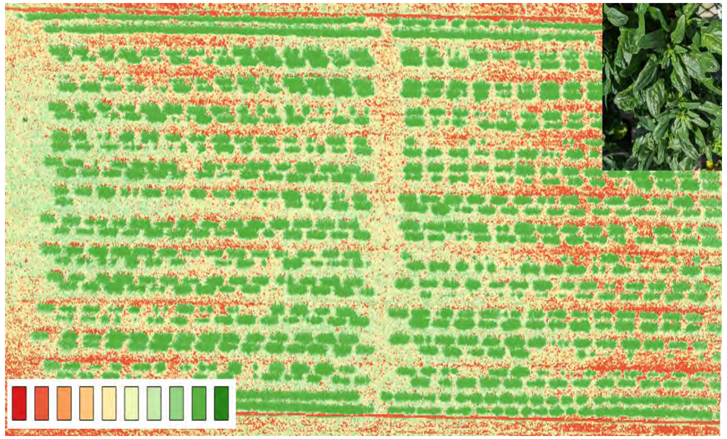
UAV Flights with multispectral sensor

Georeferenced image acquisition

Orthomosaics/ DSM



Reflectance data extraction by band

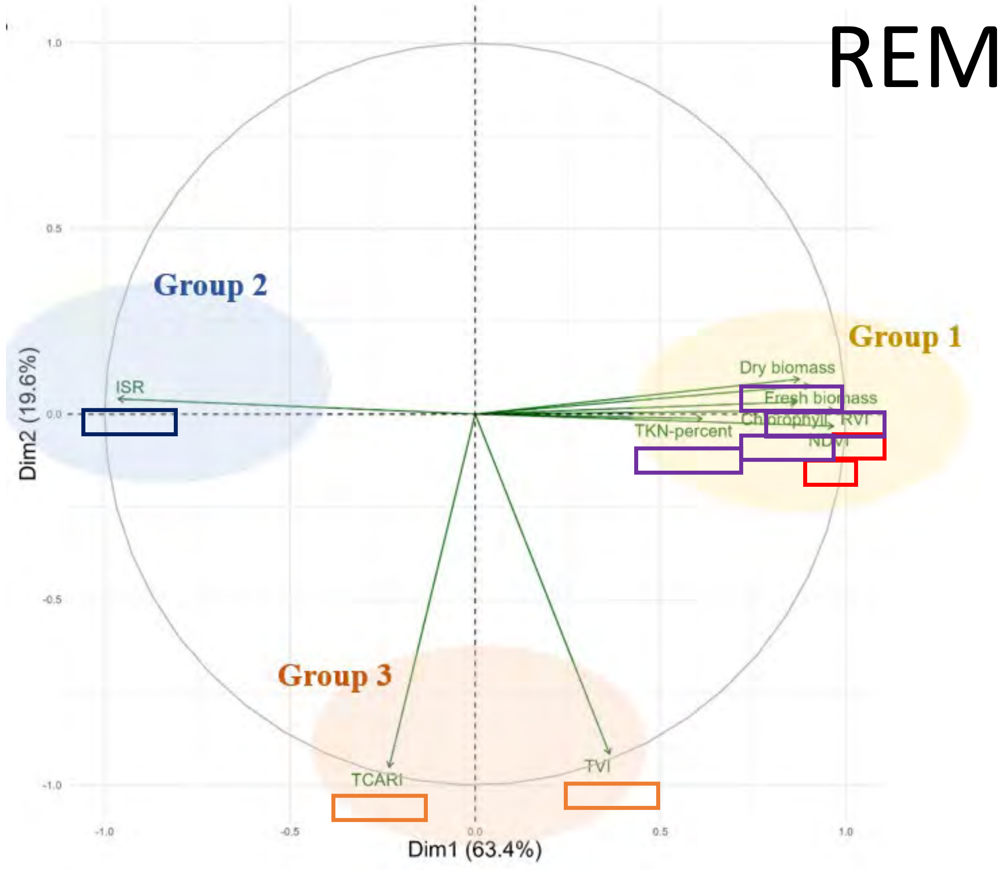


- Phenotyping can be a bottleneck in breeding program as it can time-consuming, expensive, and destructive

- Phenotyping by Remote sensing can contribute to:

1) Quick turn-around
2) Cost reduction
3) **No destruction of materials (important for transplanting from field to GH)**

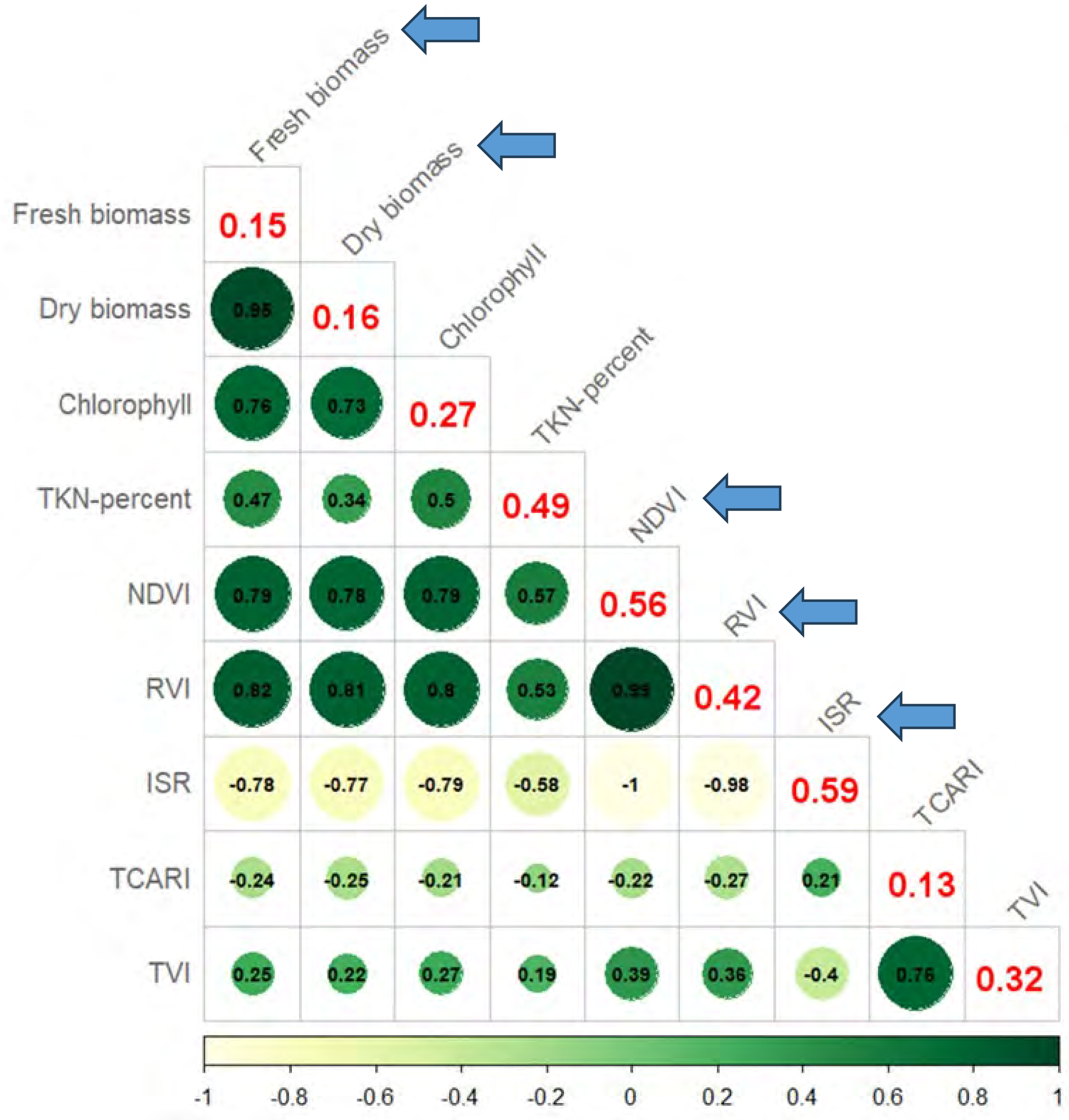
REMOTE SENSING



Features	Formula/explanation	Reference
Relative Vigor Index (RVI)	NIR/RED	(Jordan 1969)
Normalized Difference Vegetation Index (NDVI)	(NIR-RED)/(NIR+RED)	(Rouse et al. 1974)
Inverted Simple Ratio (ISR)	(RED/NIR)	(Fernandes et al. 2003)
Transformed Vegetation Index (TVI)	(120(NIR-GREEN) -200(RED-GREEN))	(Haboudane et al. 2004)
Transformed Chlorophyll Absorption in Reflectance Index (TCARI)	3(RE-RED) -0.2(RE-GREEN)*(RE/RED)	(Cui et al. 2019)

- Reflectance data from multispectral bands can be used to generate vegetative indices
- PCA analysis between physiological traits associated with NUE and vegetative indices from 344 gene-bank accessions
- Different vegetation indices showed distinct clustering patterns in relation to physiological traits

REMOTE SENSING



Pearson’s correlation coefficient (in black) and heritability (**in red**)

- Proof of concept: Using vegetative indices in genomic prediction framework
- Exploiting vegetative indices as auxiliary traits for their relative information to physiological traits (covariance)
- Especially, if target traits have low heritability and vegetative indices have high heritability

REMOTE SENSING

- Using vegetative indices as auxiliary traits in multi-trait framework

$$\mathbf{y} = \mathbf{1}\mu + \mathbf{Z}\mathbf{g} + \mathbf{e} \qquad \mathrm{var}(g) = \mathbf{G}\sigma_g^2$$

Physiological traits of interest

$$\begin{bmatrix} \mathbf{y}_1 \\ \mathbf{y}_2 \end{bmatrix} = \begin{bmatrix} \mathbf{I}_1 & \mathbf{0} \\ \mathbf{0} & \mathbf{I}_2 \end{bmatrix} \begin{bmatrix} \mu_1 \\ \mu_2 \end{bmatrix} + \begin{bmatrix} \mathbf{Z}_1 & \mathbf{0} \\ \mathbf{0} & \mathbf{Z}_2 \end{bmatrix} \begin{bmatrix} \mathbf{g}_1 \\ \mathbf{g}_2 \end{bmatrix} + \begin{bmatrix} \mathbf{e}_1 \\ \mathbf{e}_2 \end{bmatrix}$$

Vegetative indices as auxiliary/non-target traits

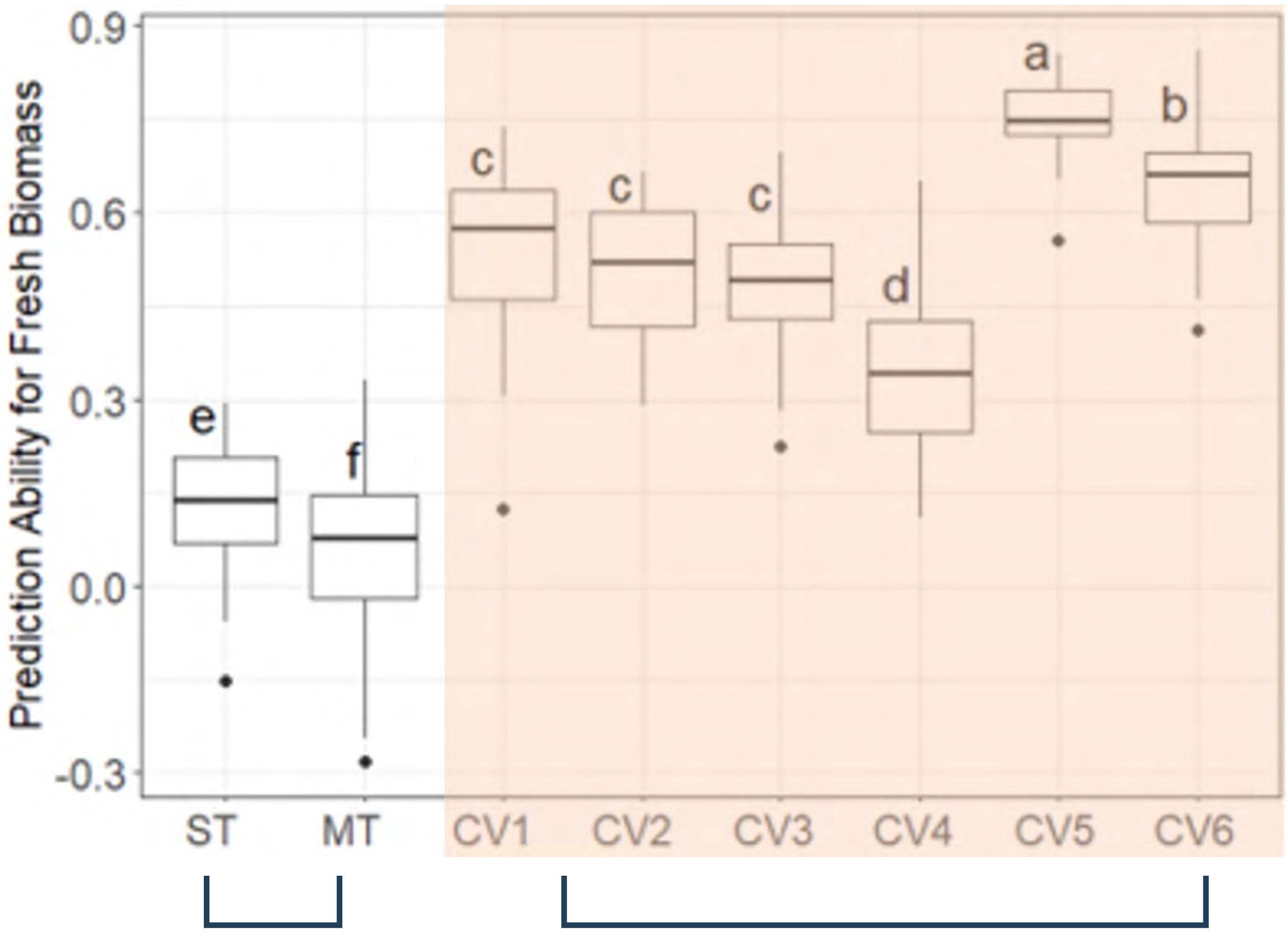
$$\mathrm{var}(g) = \mathrm{N}(\mathbf{o}, \mathbf{G} \otimes \mathbf{M})$$

(Jessica, 2016)

(Sun, 2017)

(Sandhu, 2021)

REMOTE SENSING



Prediction of biomass only using genomic data

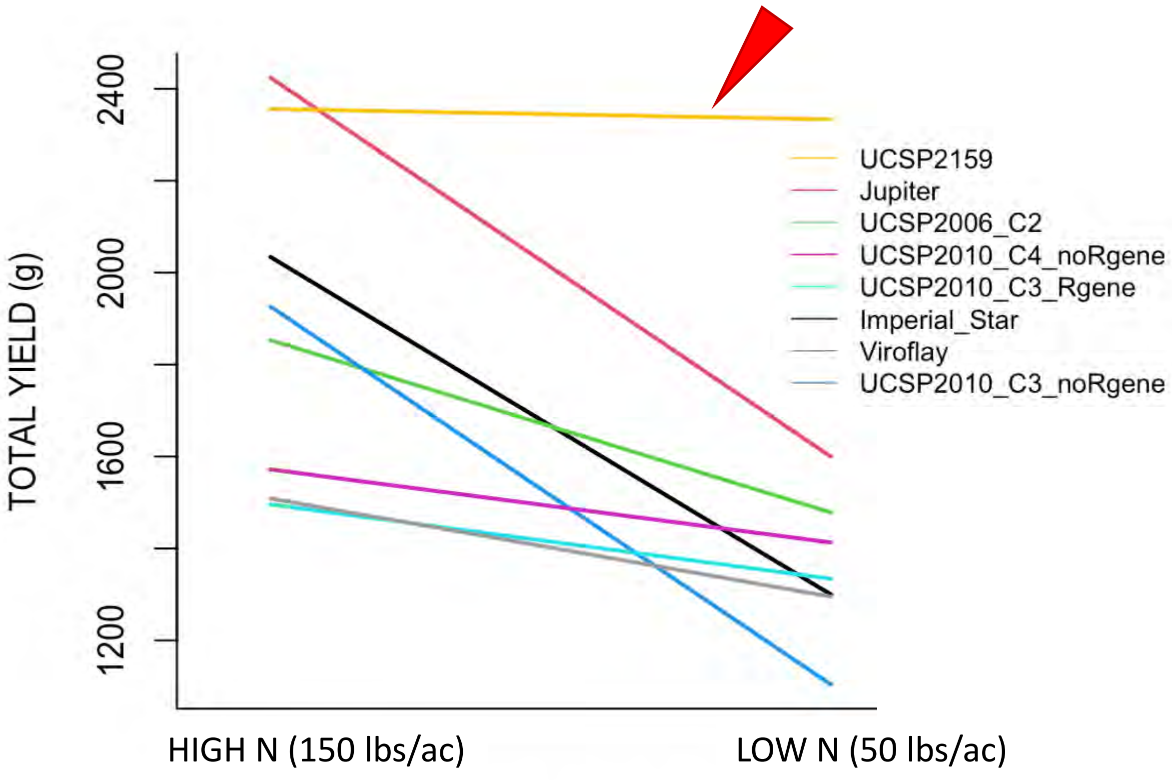
Prediction of biomass using both genomic data and vegetative indices in test dataset (unseen)

- Validation of using of vegetative indices as auxiliary traits in genomic prediction model
- Different clusters of vegetative indices (CV1 - CV6) tested
- As expected, the prediction accuracy using both genomic data and vegetative indices was higher than the prediction accuracy using only genomic data

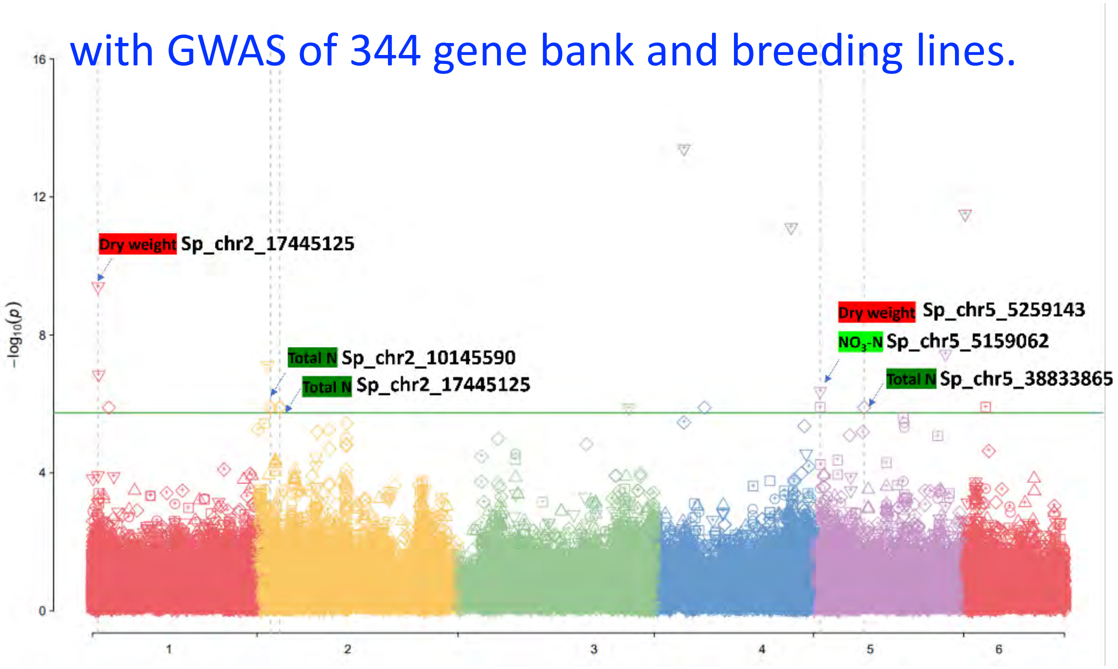
Testing Experimental Cultivars
UCSP2159 seems promising for NUE

Prediction model accuracy for: 0.30 Total Nitrogen, 0.60 for Chlorophyll and 0.75 for Biomass

UCSP2159 is promising (good NUE)



Found significant markers for dry weight, Total N, and NO_3-N with GWAS of 344 gene bank and breeding lines.



SUMMARY

- Evaluation of spinach germplasm accessions in low N environments for spinach breeding program
- GWAS on 344 germplasm accessions using target-enriched sequencing.
- Finding significant markers that are associated dry biomass, chlorophyll concentration, total N, and leaf shapes
- NUE breeding population developed from evaluated materials
- Explored combining vegetation indices in genomic prediction models to estimate NUE traits

Thank you

CALIFORNIA LEAFY GREENS RESEARCH BOARD

California Seed Association

USDA Agricultural Marketing Service
U.S. DEPARTMENT OF AGRICULTURE

cdfa CALIFORNIA DEPARTMENT OF FOOD & AGRICULTURE

Spinach Team
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USDA Agricultural Research Service

National Plant Germplasm System

Alex Cornwall
Collection Curator
Horticultural and *Beta* Crops
Western Regional Plant Introduction Station
Pullman Washington
USDA-ARS National Plant Germplasm System

USDA – ARS National Plant Germplasm System

243 families ~2,500 genera ~16,000 species

621,450 active accessions

Other Seed Banks

WAGENINGEN
UNIVERSITY & RESEARCH

Royal Botanic Gardens Kew

MILLENNIUM SEED BANK PARTNERSHIP
Kew

CGIAR
Science for a food-secure future

IRRI
International Rice Research Institute

CIMMYT MR
International Maize and Wheat Improvement Center
Centro Internacional de Mejoramiento de Maíz y Trigo

CIAT
International Center for Tropical Agriculture
Since 1967 Science to cultivate change

Centro Internacional de Agricultura Tropical

Horticulture and *Beta* Crops Collection:

57 Families
~315 genera
~1300 species

- Lettuce
- Beets
- Garlic

5 Missions of the NPGS

1. Acquire
2. Regenerate and Maintain
3. Evaluate and Characterize
4. Document
5. Distribute

Acquiring

- Domestic collecting trips
- Domestic donors
- Expiration of plant variety protection (PVP) certificates
- Foreign donors - often as an exchange
- Foreign plant collecting trips

    With consent from visited countries

Cultivated and Wild species

Regeneration and Maintenance

- Seed must be grown and harvested for reentry into storage
- Maintain genetic diversity by preventing crossing and contamination

Evaluating and Characterizing

- In-house evaluation trial
- Requestors who report their data
- Data collection during the normal regeneration cycle
  - Flower color, seed color, leaf and flower morphology, plant height
  - Seed nutrient content, disease resistances and many others.
  - Also, any genetic traits or data that have been reported.

Descriptors

- 82 phenotypic characters
  - Growth habit (2)
  - Cotyledon (5)
  - Stem (12)
  - Rosette leaves (18)
  - Cauline leaves (16)
  - Inflorescence and flower (16)
  - Fruit – cypselae (13)

Doležalová, I., Křístková, E., Lebeda, A., & Vinter, V. (2002). Description of morphological characters of wild Lactuca L. spp. Genetic resources (English-Czech version). Horticultural Science, 29(No. 2), 56–83. https://doi.org/10.17221/4461-HORTSCI

Phenotyping

Phenotyping

- Corrected and verify species designation
- Document morphological characteristics
- Create voucher specimens

Phenotypic Descriptors

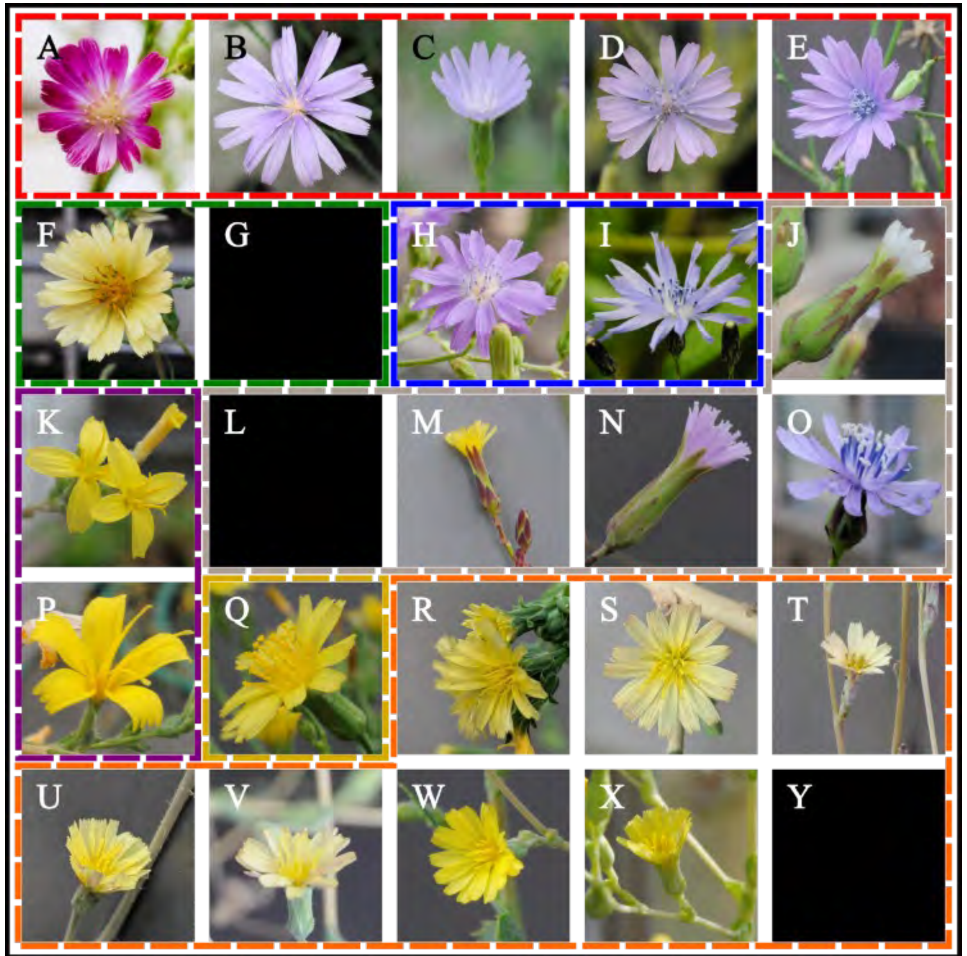


Figure 2. Flowers of 25 species from the genus *Lactuca*. **A.** *L. inermis* (W6-34016), B. *L. tenerrima* (CGN 09388), **C.** *L. dissecta* (W6-28049), **D.** *L. undulata* (W6-47236), **E.** *L. perennis* (CGN09319), **F.** *L. indica* (CGN11322), **G.** *L. raddeana* (CGN19018), **H.** *L. tatarica* (W6 10792), **I.** *L. siberica* (CGN23446), **J.** *L. biennis* (CGN22025), **K.** *L. orientalis* (W6-47256), **L.** *L. ludoviciana* (W6-50704), **M.** *L. canadensis* (CGN14308), **N.** *L. graminifolia* (AC_FL_2022_LAC_3) **O.** *L. floridana* (W6-50740), **P.** *L. viminea* (CGN14301), **Q.** *L. quercina* (CGN25495), **R.** *L. virosa* (CGN13338), **S.** *L. georgica* (PI-274375), **T.** *L. saligna* (CGN16244), **U.** *L. aculeata* (W6-34010), **V.** *L. altaica* (CGN25414), **W.** *L. serriola* (W6-23180), **X.** *L. sativa* (W6-34004), **Y.** *L. dregeana* (PI 273574).

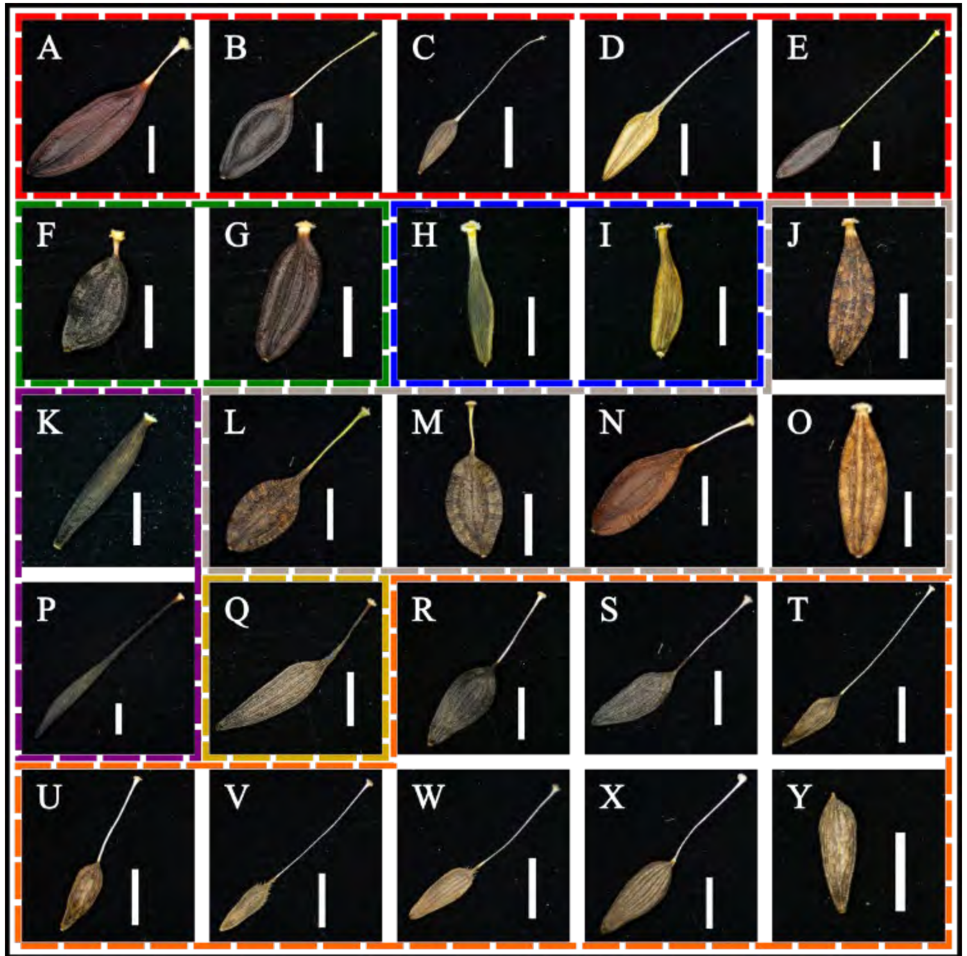
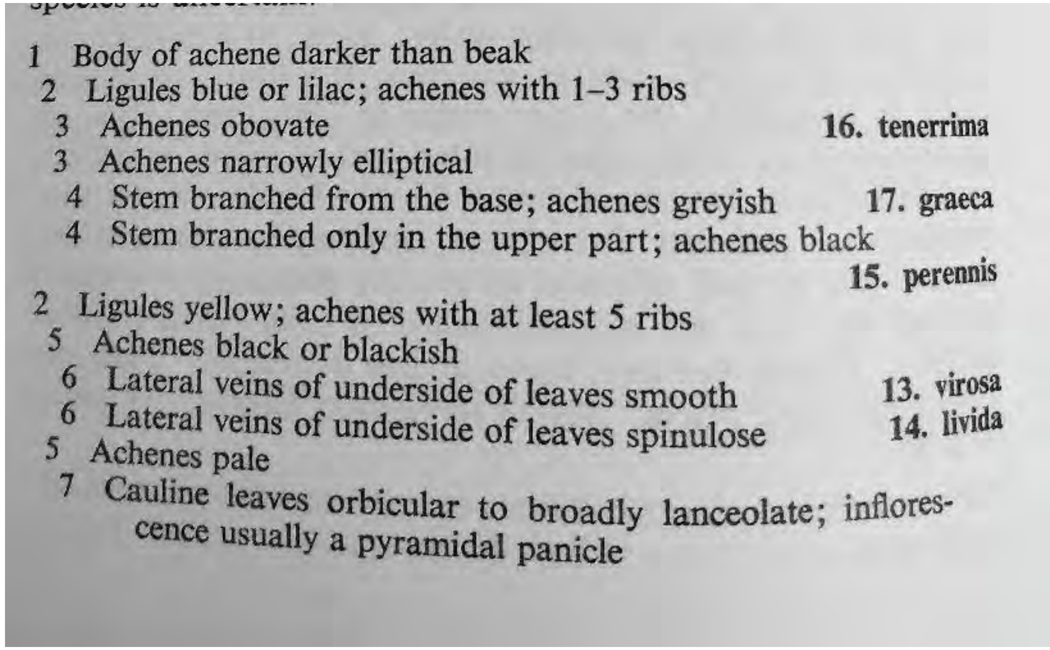


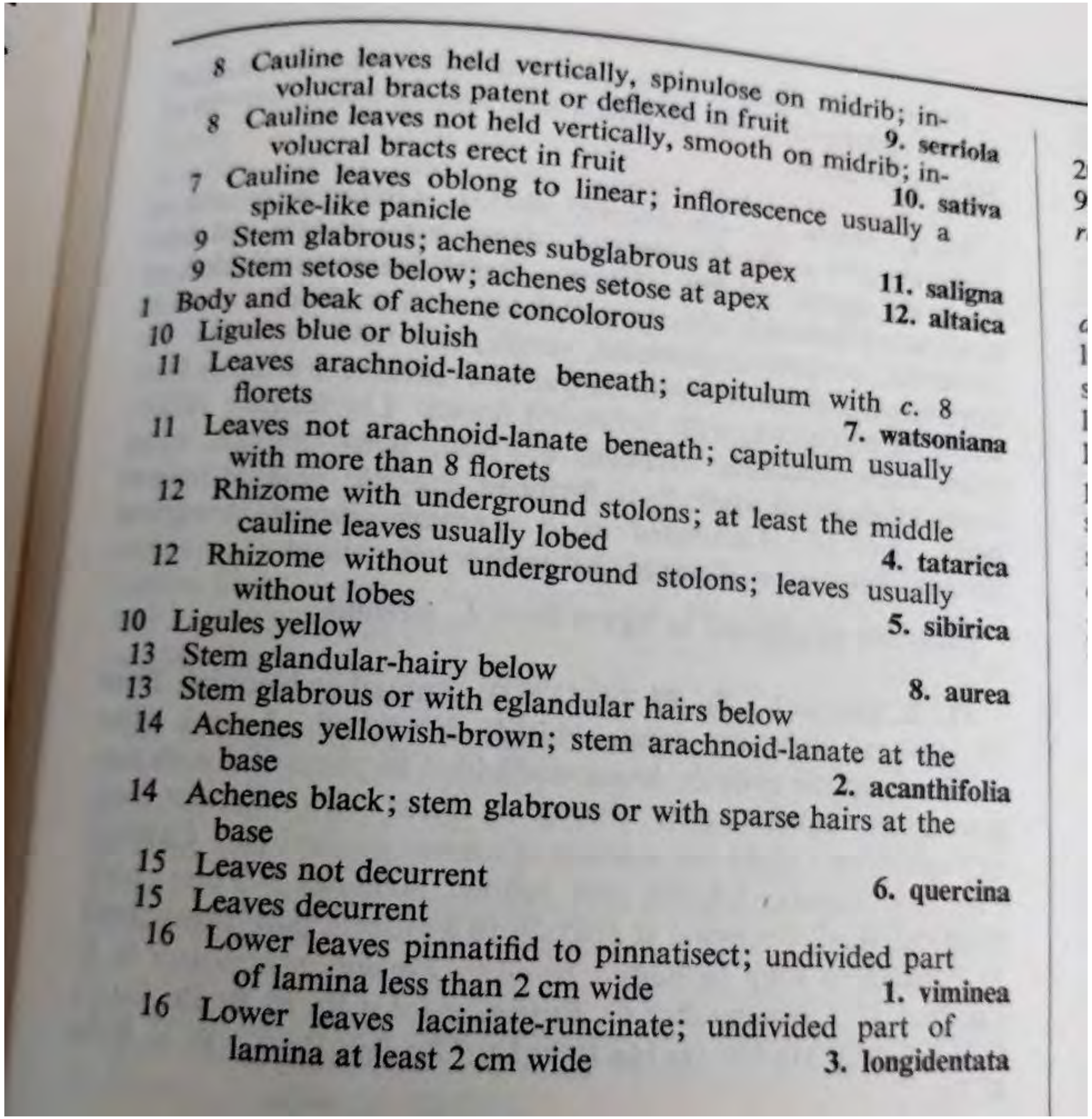
Figure 3. Cypselae of 25 species from the genus *Lactuca*. All scale bars = 2 mm. **A.** *L. inermis* (W6-34016), B. *L. tenerrima* (CGN 09388), **C.** *L. dissecta* (W6-28049), **D.** *L. undulata* (W6-47236), **E.** *L. perennis* (CGN09319), **F.** *L. indica* (CGN14316), **G.** *L. raddeana* (CGN19018), **H.** *L. tatarica* (CGN9390), **I.** *L. siberica* (CGN23446), **J.** *L. biennis* (CGN22025), **K.** *L. orientalis* (W6-47256), **L.** *L. ludoviciana* (W6-50704), **M.** *L. canadensis* (PI 674747), **N.** *L. graminifolia* (AC_FL_2022_LAC_2) **O.** *L. floridana* (W6-50712), **P.** *L. viminea* (W6-40648), **Q.** *L. quercina* (CGN25495), **R.** *L. virosa* (CGN09316), **S.** *L. georgica* (W6-50803), **T.** *L. saligna* (W6-34007), **U.** *L. aculeata* (W6-34010), **V.** *L. altaica* (W6-47263), **W.** *L. serriola* (W6-6329), **X.** *L. sativa* (W6-34153), **Y.** *L. dregeana* (PI 273574).

Identifying

- Verification of species designation
  - Using dichotomous keys

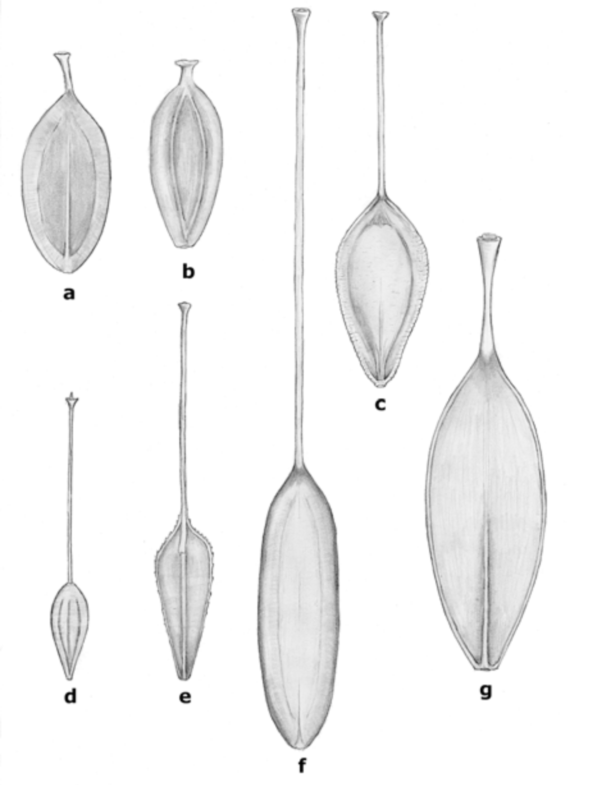


1 Body of achene darker than beak
2 Ligules blue or lilac; achenes with 1–3 ribs
3 Achenes obovate **16. tenerrima**
3 Achenes narrowly elliptical
4 Stem branched from the base; achenes greyish **17. graeca**
4 Stem branched only in the upper part; achenes black **15. perennis**
2 Ligules yellow; achenes with at least 5 ribs
5 Achenes black or blackish
6 Lateral veins of underside of leaves smooth **13. virosa**
6 Lateral veins of underside of leaves spinulose **14. livida**
5 Achenes pale
7 Cauline leaves orbicular to broadly lanceolate; inflorescence usually a pyramidal panicle



8 Cauline leaves held vertically, spinulose on midrib; involucral bracts patent or deflexed in fruit **9. serriola**
8 Cauline leaves not held vertically, smooth on midrib; involucral bracts erect in fruit **10. sativa**
7 Cauline leaves oblong to linear; inflorescence usually a spike-like panicle
9 Stem glabrous; achenes subglabrous at apex **11. saligna**
9 Stem setose below; achenes setose at apex **12. altaica**
1 Body and beak of achene concolorous
10 Ligules blue or bluish
11 Leaves arachnoid-lanate beneath; capitulum with *c.* 8 florets **7. watsoniana**
11 Leaves not arachnoid-lanate beneath; capitulum usually with more than 8 florets
12 Rhizome with underground stolons; at least the middle cauline leaves usually lobed **4. tatarica**
12 Rhizome without underground stolons; leaves usually without lobes **5. sibirica**
10 Ligules yellow
13 Stem glandular-hairy below **8. aurea**
13 Stem glabrous or with eglandular hairs below
14 Achenes yellowish-brown; stem arachnoid-lanate at the base **2. acanthifolia**
14 Achenes black; stem glabrous or with sparse hairs at the base
15 Leaves not decurrent **6. quercina**
15 Leaves decurrent
16 Lower leaves pinnatifid to pinnatisect; undivided part of lamina less than 2 cm wide **1. viminea**
16 Lower leaves laciniate-runcinate; undivided part of lamina at least 2 cm wide **3. longidentata**

Flora Europaea: Plantaginaceae to Compositae (and Rubiaceae). Vol. 4. Cambridge university press, 1976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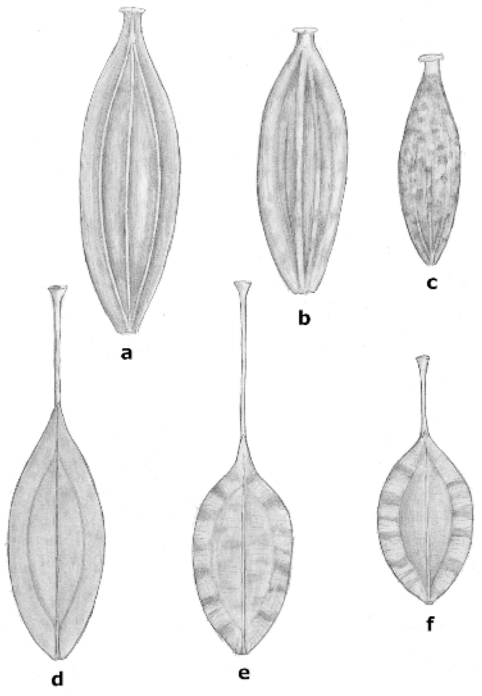
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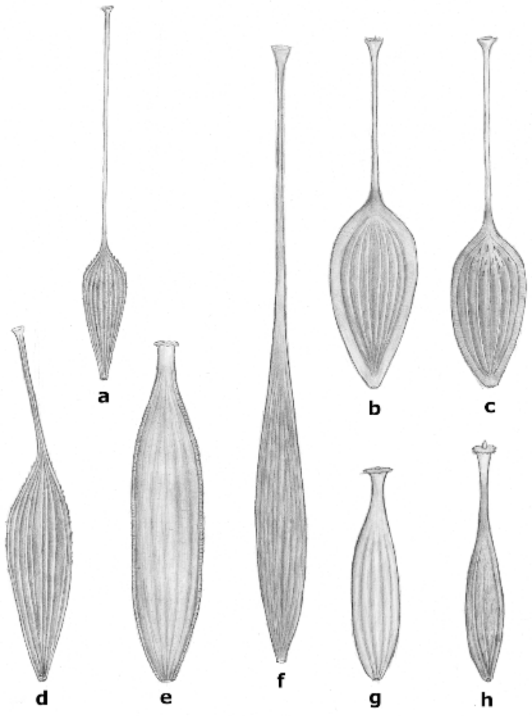
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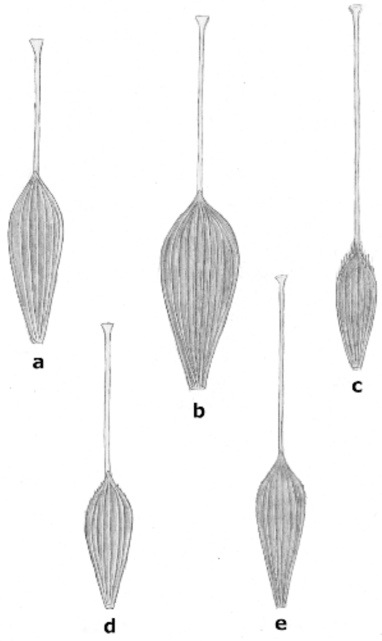
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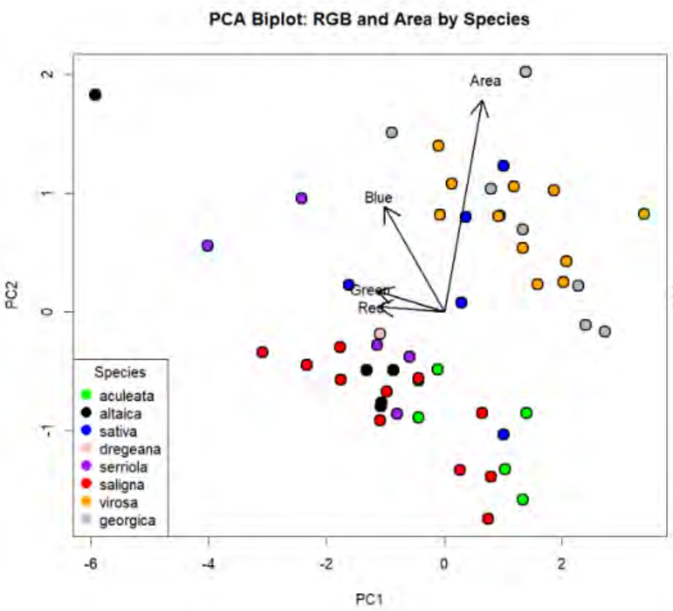
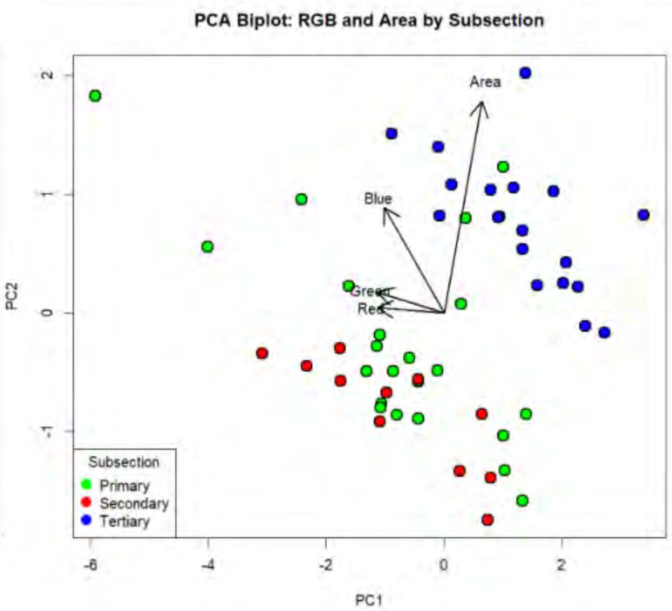
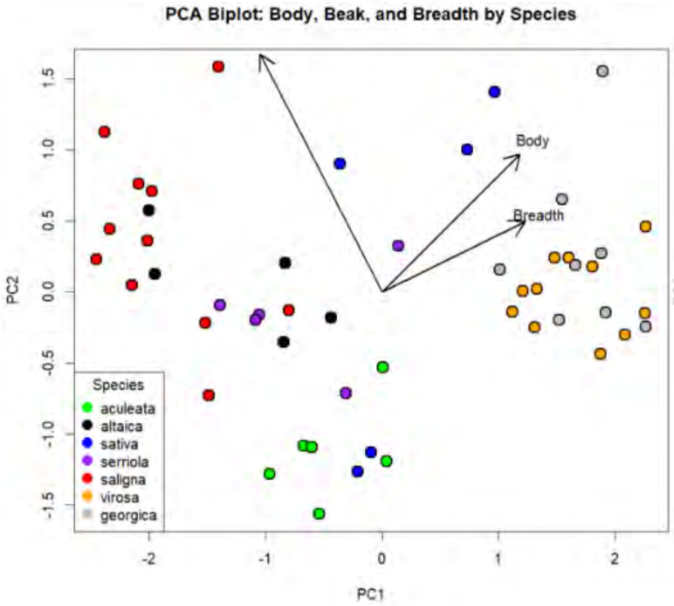
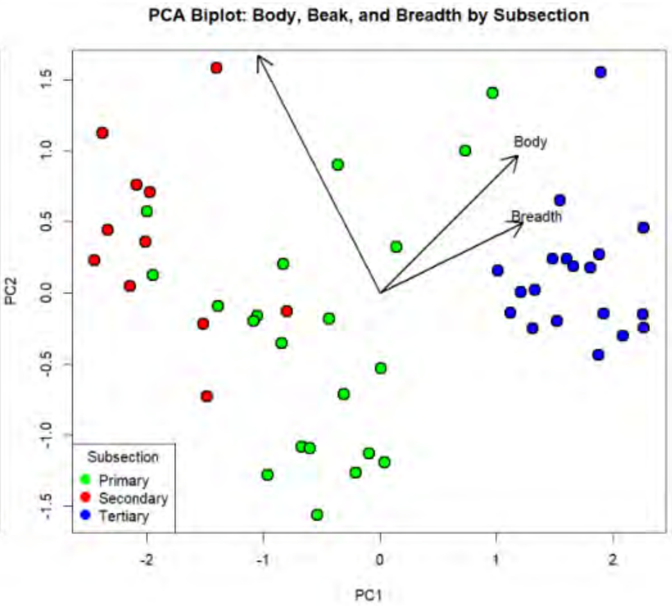

PCA Biplot: RGB and Area by Species

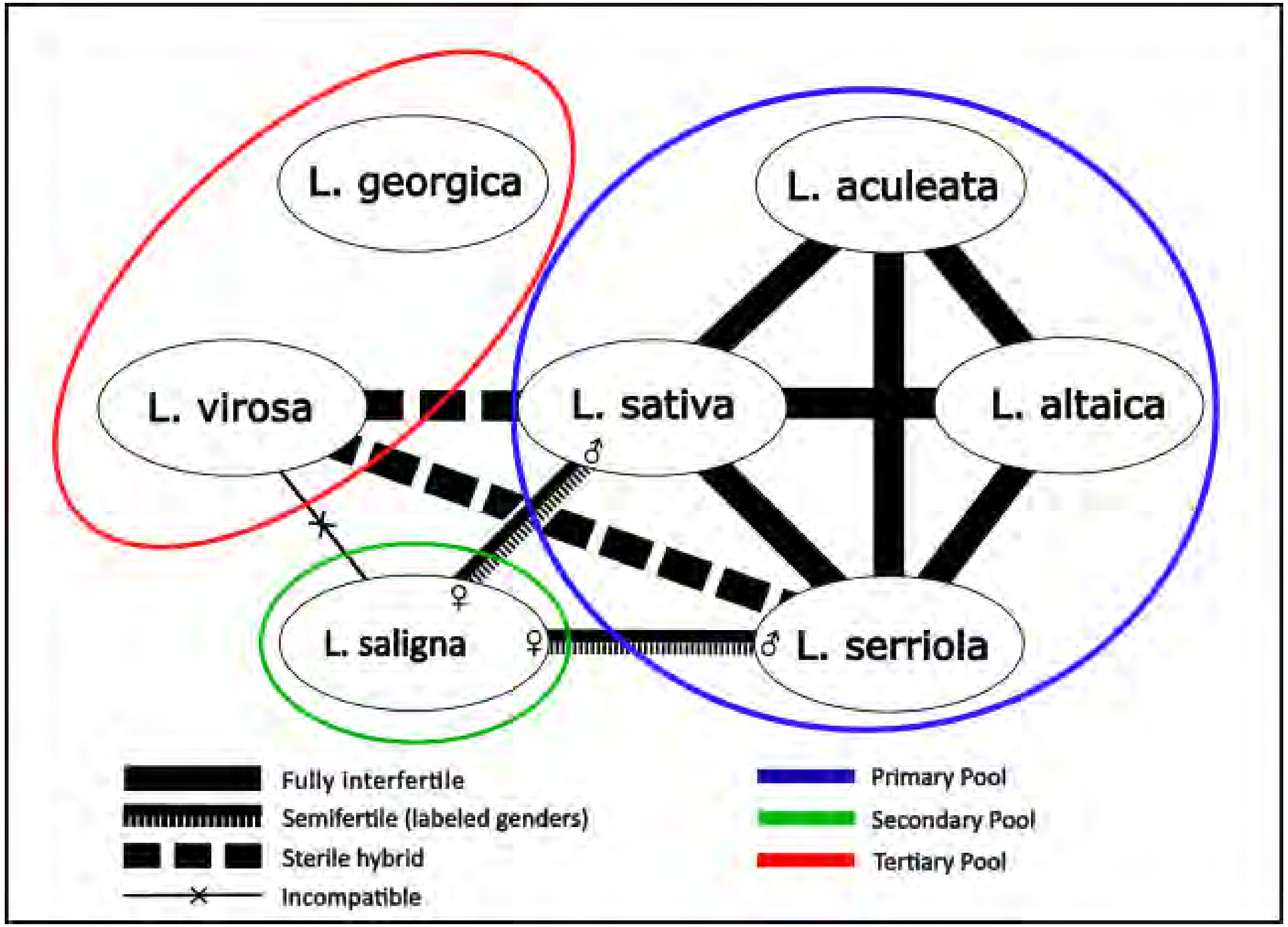

PCA Biplot: RGB and Area by Subsection

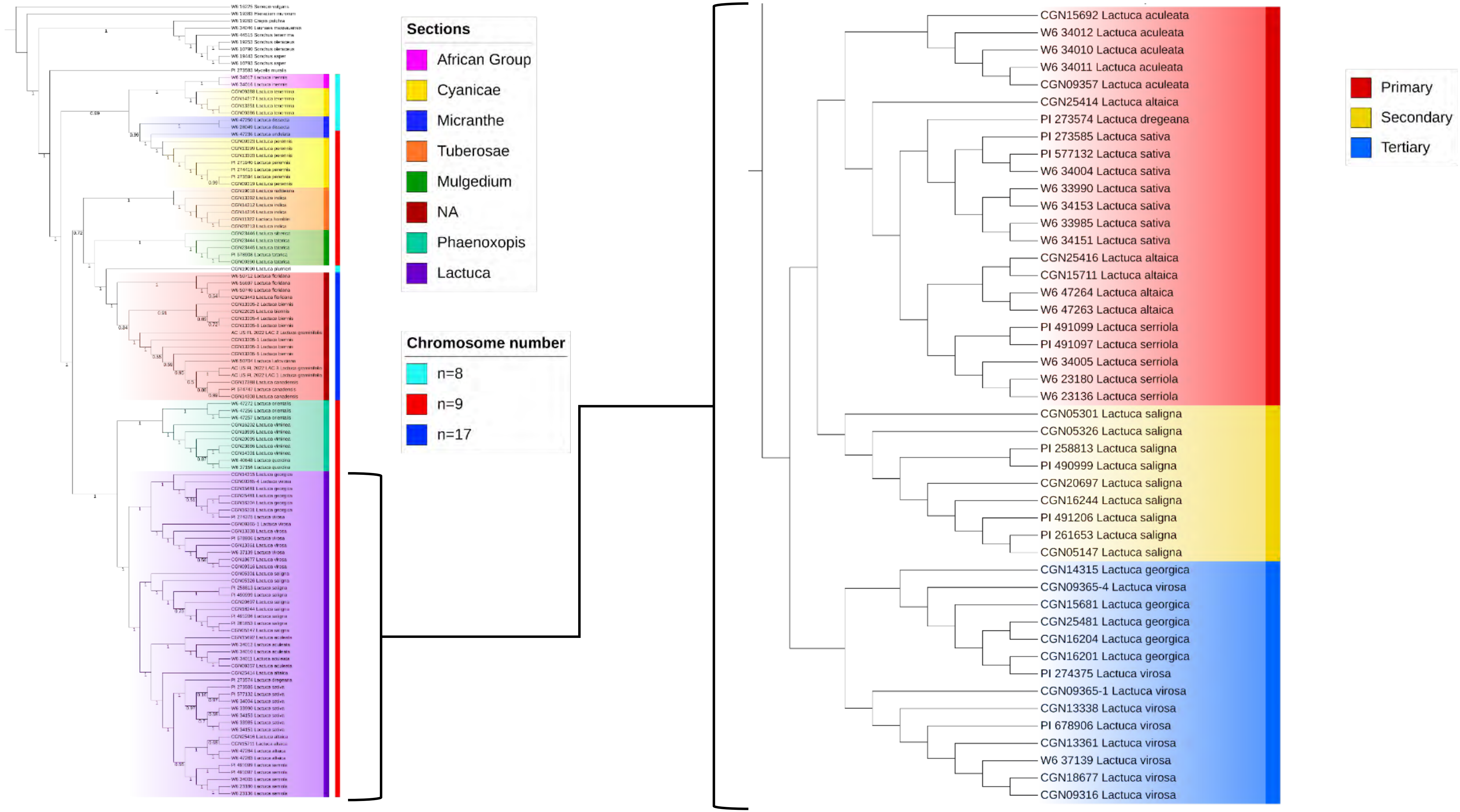

PCA Biplot: Body, Beak, and Breadth by Species


PCA Biplot: Body, Beak, and Breadth by Subsection

Genotyping

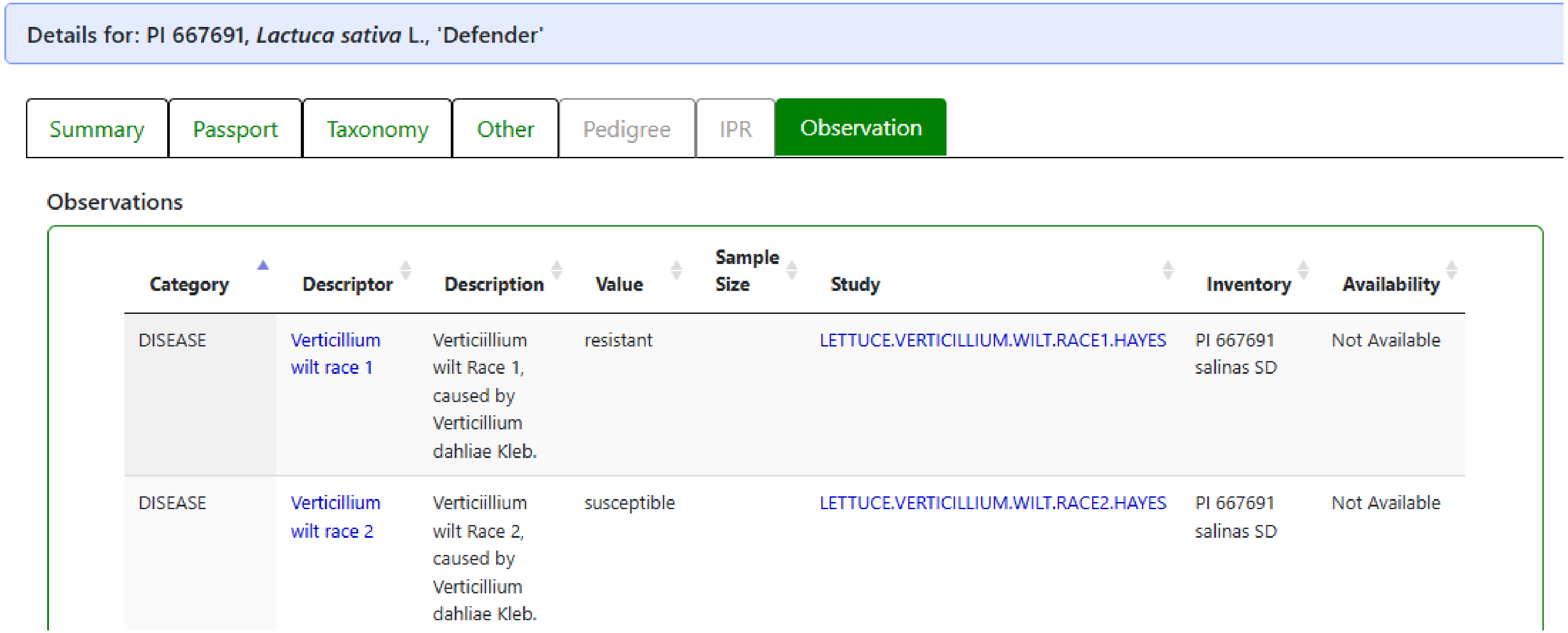
- Phylogenetic studies
- Taxonomic studies
- Species genetic barcode exploration




Sections
African Group
Cyanicae
Micranthe
Tuberosae
Mulgedium
NA
Phaenoxopis
Lactuca
Chromosome number
n=8
n=9
n=17
Primary
Secondary
Tertiary
CGN15692 Lactuca aculeata
W6 34012 Lactuca aculeata
W6 34010 Lactuca aculeata
W6 34011 Lactuca aculeata
CGN09357 Lactuca aculeata
CGN25414 Lactuca altaica
PI 273574 Lactuca dregeana
PI 273585 Lactuca sativa
PI 577132 Lactuca sativa
W6 34004 Lactuca sativa
W6 33990 Lactuca sativa
W6 34153 Lactuca sativa
W6 33985 Lactuca sativa
W6 34151 Lactuca sativa
CGN25416 Lactuca altaica
CGN15711 Lactuca altaica
W6 47264 Lactuca altaica
W6 47263 Lactuca altaica
PI 491099 Lactuca serriola
PI 491097 Lactuca serriola
W6 34005 Lactuca serriola
W6 23180 Lactuca serriola
W6 23136 Lactuca serriola
CGN05301 Lactuca saligna
CGN05326 Lactuca saligna
PI 258813 Lactuca saligna
PI 490999 Lactuca saligna
CGN20697 Lactuca saligna
CGN16244 Lactuca saligna
PI 491206 Lactuca saligna
PI 261653 Lactuca saligna
CGN05147 Lactuca saligna
CGN14315 Lactuca georgica
CGN09365-4 Lactuca virosa
CGN15681 Lactuca georgica
CGN25481 Lactuca georgica
CGN16204 Lactuca georgica
CGN16201 Lactuca georgica
PI 274375 Lactuca virosa
CGN09365-1 Lactuca virosa
CGN13338 Lactuca virosa
PI 678906 Lactuca virosa
CGN13361 Lactuca virosa
W6 37139 Lactuca virosa
CGN18677 Lactuca virosa
CGN09316 Lactuca virosa

Documenting

- Compiled into one spot for ease of access
- For the NPGS, the USDA-ARS created a database called The Germplasm Resource Information Network (GRIN)-global
  - 16 other gene banks around the world have adopted.

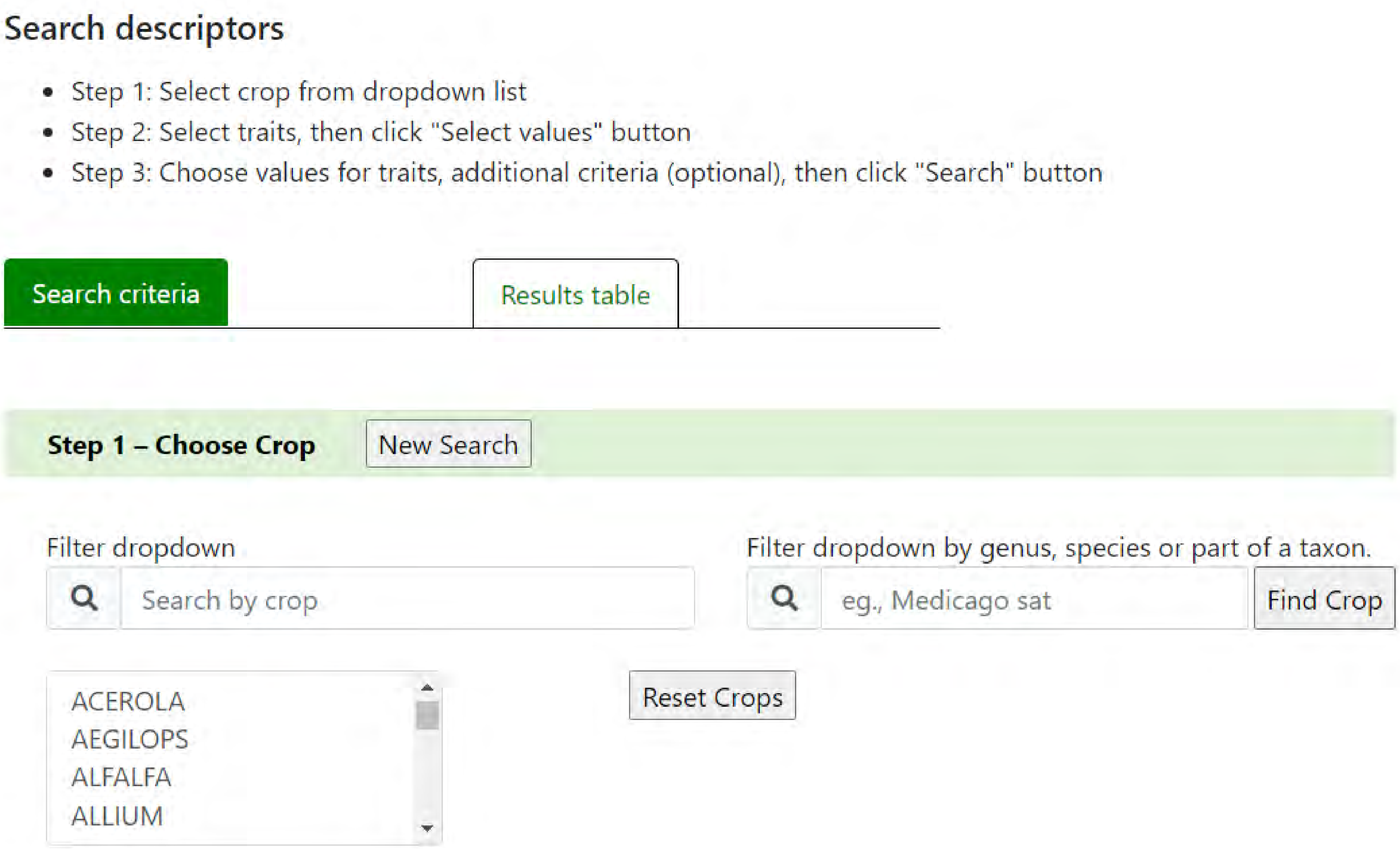


Details for: PI 667691, *Lactuca sativa* L., 'Defender'

Observations

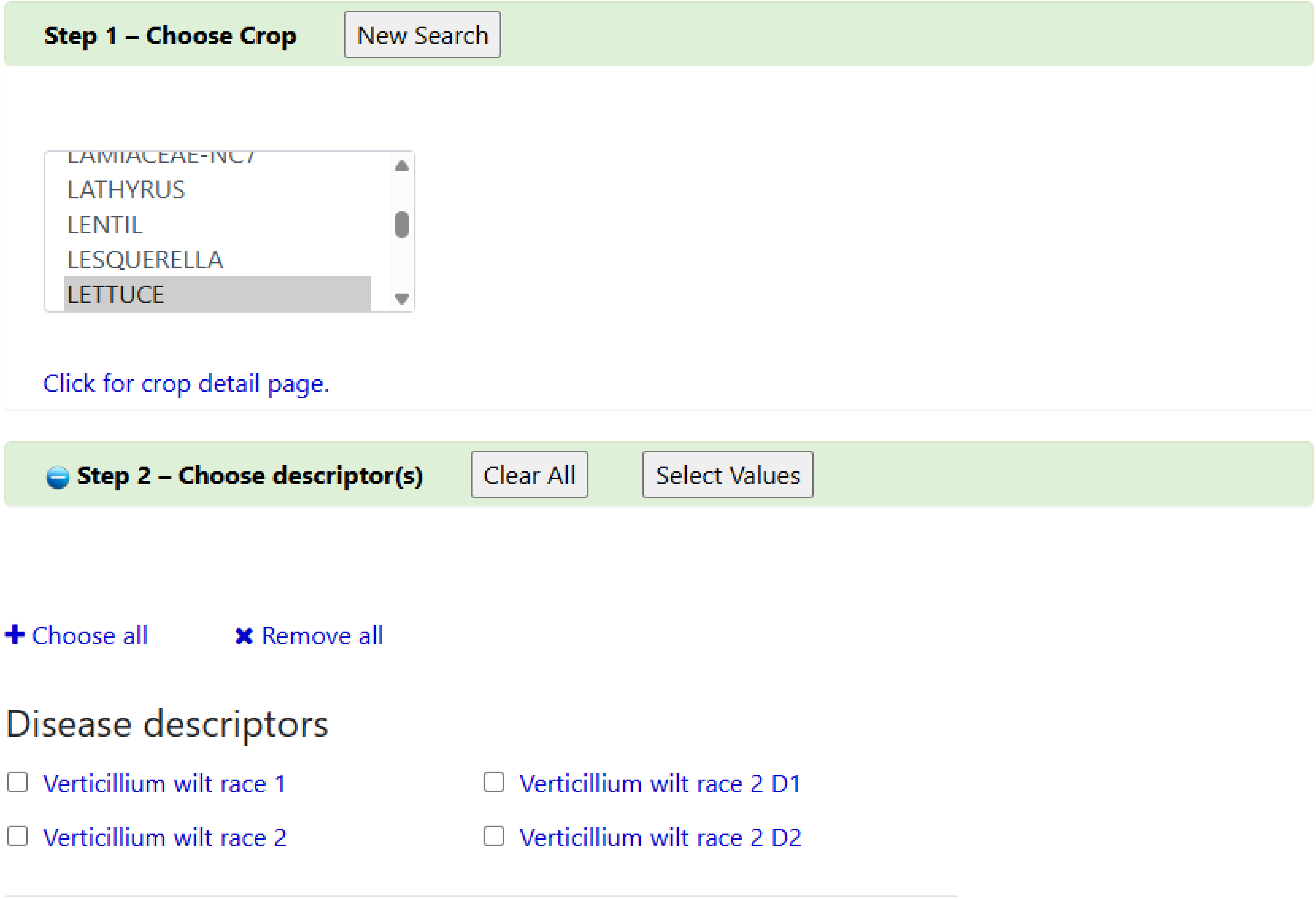
Category	Descriptor	Description	Value	Sample Size	Study	Inventory	Availability
DISEASE	Verticillium wilt race 1	Verticiillium wilt Race 1, caused by Verticillium dahliae Kleb.	resistant		LETTUCE.VERTICILLIUM.WILT.RACE1.HAYES	PI 667691 salinas SD	Not Available
DISEASE	Verticillium wilt race 2	Verticiillium wilt Race 2, caused by Verticillium dahliae Kleb.	susceptible		LETTUCE.VERTICILLIUM.WILT.RACE2.HAYES	PI 667691 salinas SD	Not Available

https://npgsweb.ars-grin.gov/gringlobal/descriptors



Search descriptors

- Step 1: Select crop from dropdown list
- Step 2: Select traits, then click "Select values" button
- Step 3: Choose values for traits, additional criteria (optional), then click "Search" button



Step 1 – Choose Crop New Search

LAMIACEAE-NC7
LATHYRUS
LENTIL
LESQUERELLA
LETTUCE

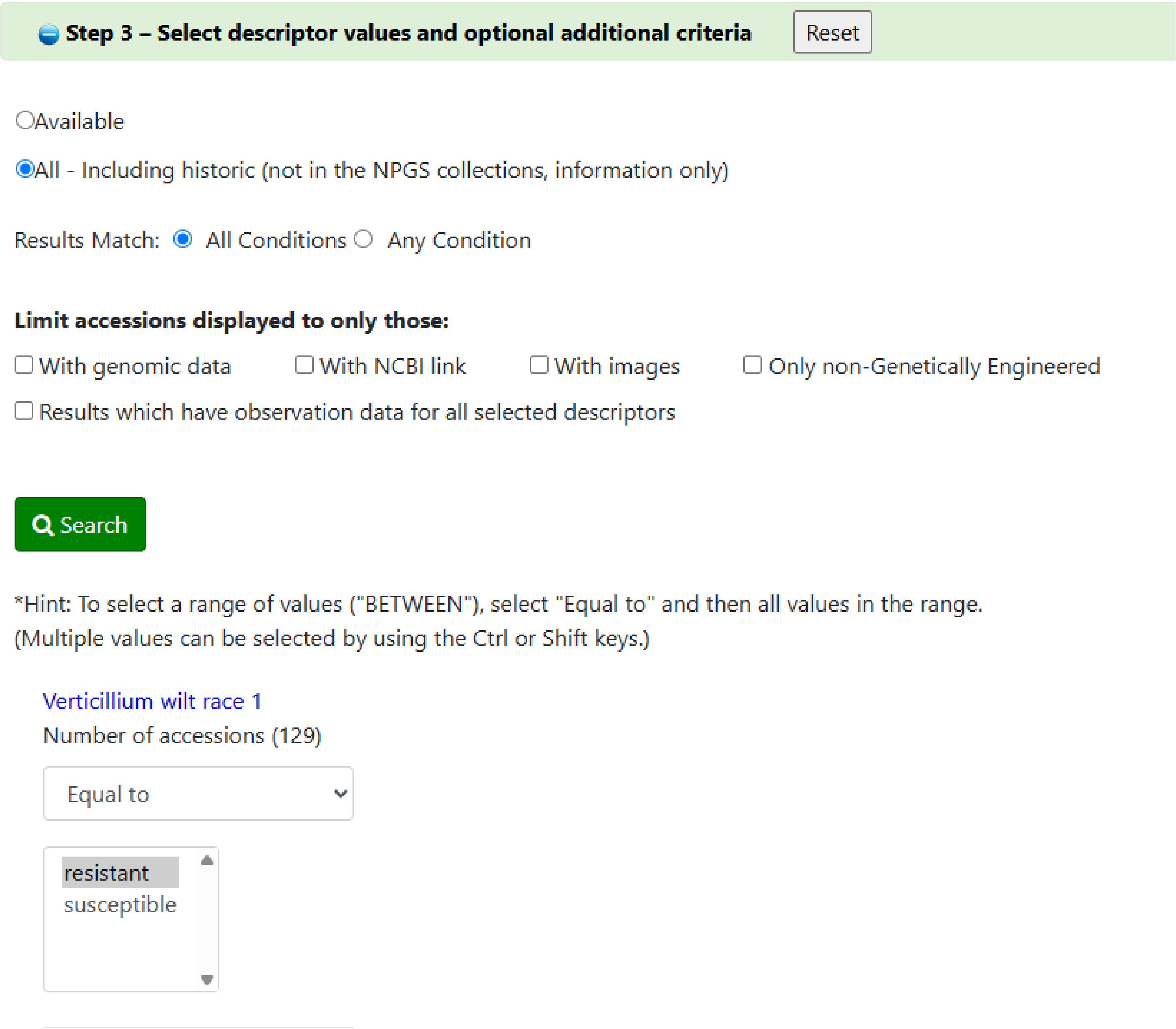
Click for crop detail page.

Step 2 – Choose descriptor(s) Clear All Select Values

✚ Choose all ✖ Remove all

Disease descriptors

☐ Verticillium wilt race 1
☐ Verticillium wilt race 2
☐ Verticillium wilt race 2 D1
☐ Verticillium wilt race 2 D2



Step 3 – Select descriptor values and optional additional criteria Reset

○ Available

● All - Including historic (not in the NPGS collections, information only)

Results Match: ● All Conditions ○ Any Condition

Limit accessions displayed to only those:

☐ With genomic data ☐ With NCBI link ☐ With images ☐ Only non-Genetically Engineered

☐ Results which have observation data for all selected descriptors

Search

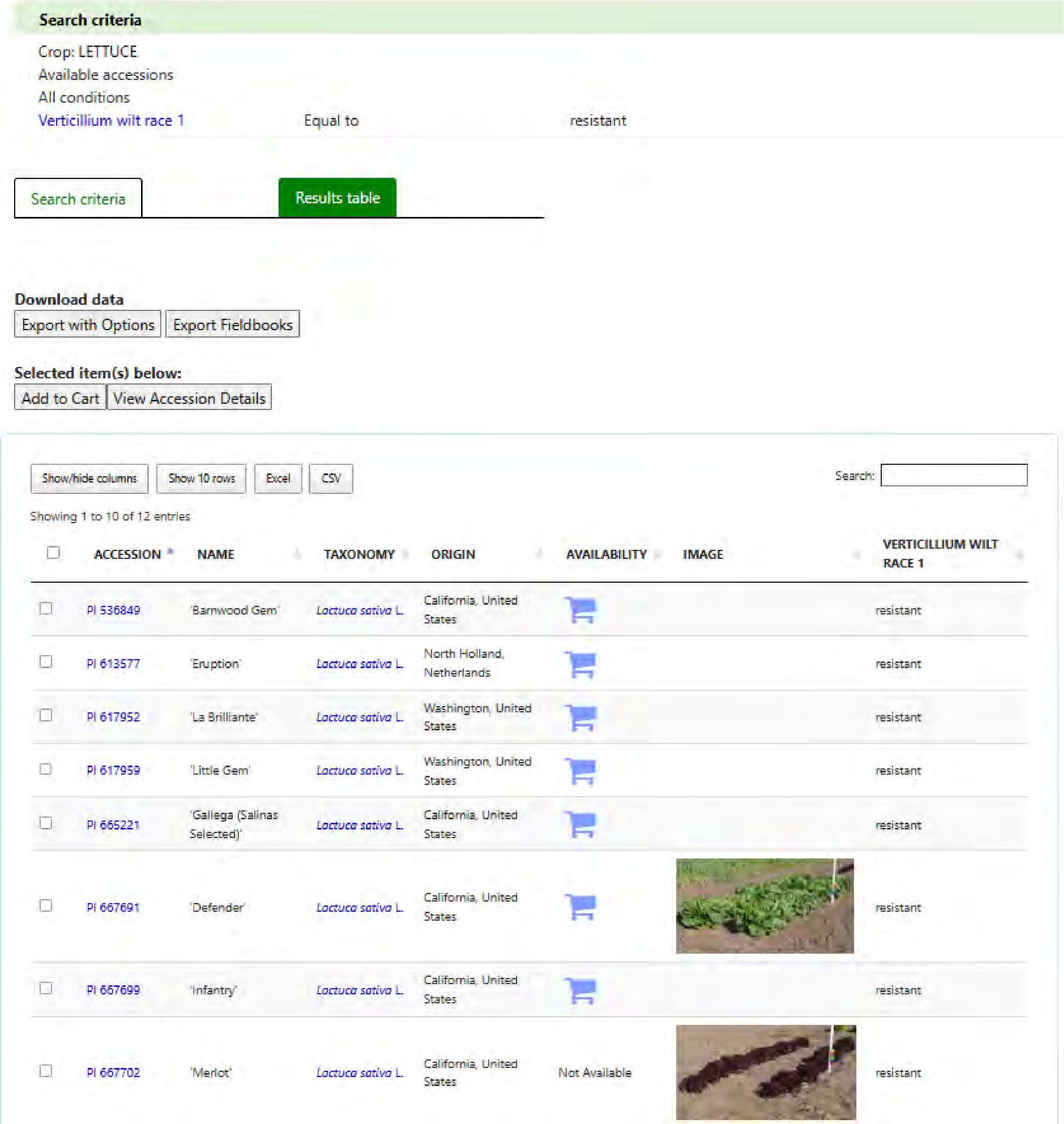
*Hint: To select a range of values ("BETWEEN"), select "Equal to" and then all values in the range.
(Multiple values can be selected by using the Ctrl or Shift keys.)

Verticillium wilt race 1

Number of accessions (129)

Equal to

resistant
susceptible



Search criteria

Crop: LETTUCE

Available accessions

All conditions

Verticillium wilt race 1 Equal to resistant

Download data

Selected item(s) below:

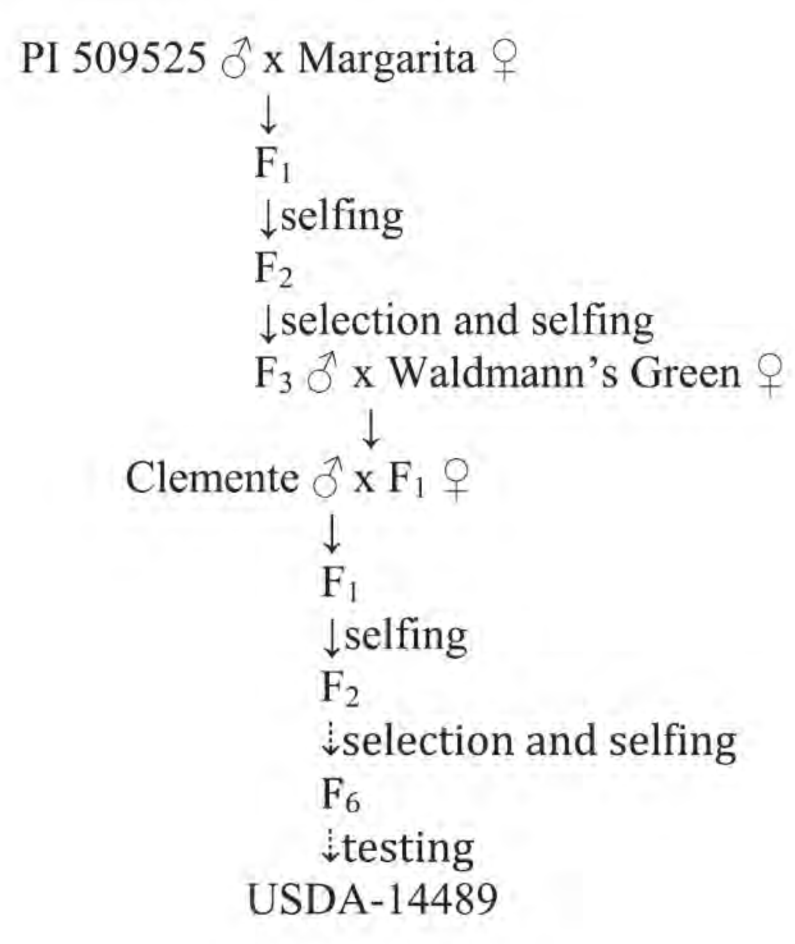
Showing 1 to 10 of 12 entries

☐	ACCESSION	NAME	TAXONOMY	ORIGIN	AVAILABILITY	IMAGE	VERTICILLIUM WILT RACE 1
☐	PI 536849	'Barnwood Gem'	*Lactuca sativa* L.	California, United States	🛒		resistant
☐	PI 613577	'Eruption'	*Lactuca sativa* L.	North Holland, Netherlands	🛒		resistant
☐	PI 617952	'La Brilliante'	*Lactuca sativa* L.	Washington, United States	🛒		resistant
☐	PI 617959	'Little Gem'	*Lactuca sativa* L.	Washington, United States	🛒		resistant
☐	PI 665221	'Gallega (Salinas Selected)'	*Lactuca sativa* L.	California, United States	🛒		resistant
☐	PI 667691	'Defender'	*Lactuca sativa* L.	California, United States	🛒		resistant
☐	PI 667699	'Infantry'	*Lactuca sativa* L.	California, United States	🛒		resistant
☐	PI 667702	'Merlot'	*Lactuca sativa* L.	California, United States	Not Available		resistant

Merlot – PI 667702

Defender - PI 667692

Lettuce Corky Root Virus



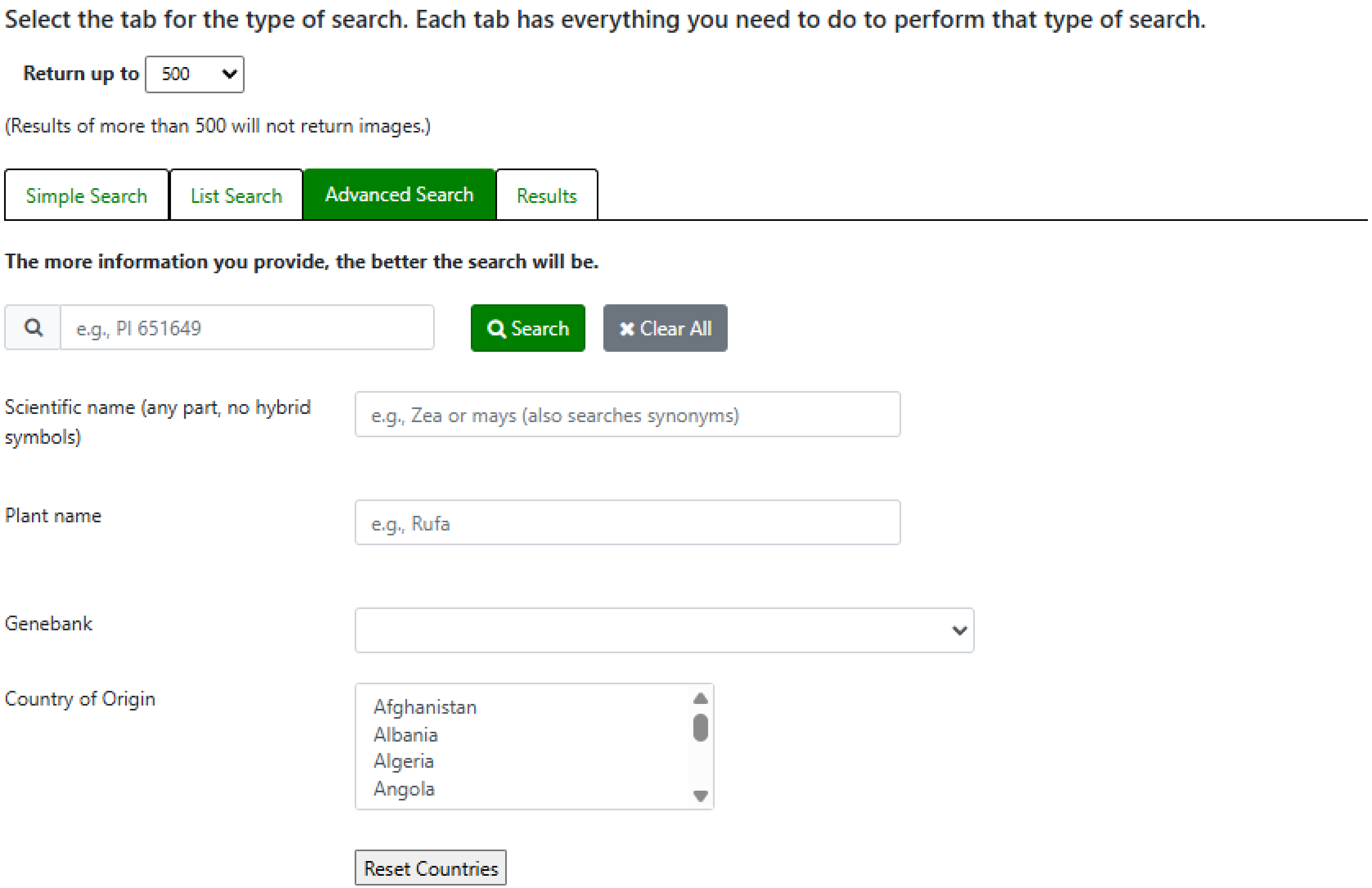
PI 509525 ♂ x Margarita ♀
↓
F_1
↓selfing
F_2
↓selection and selfing
F_3 ♂ x Waldmann’s Green ♀
↓
Clemente ♂ x F_1 ♀
↓
F_1
↓selfing
F_2
↓selection and selfing
F_6
↓testing
USDA-14489

Mou, B. (2023). *Green Leaf, Red Leaf, and Romaine Lettuce Breeding Lines with Resistance to Leafminer, Corky Root, and Downy Mildew*. https://doi.org/10.21273/HORTSCI17069-22

Distribution

- Ordered using GRIN-Global
- Shipped to researchers across the world

https://npgsweb.ars-grin.gov/gringlobal/search

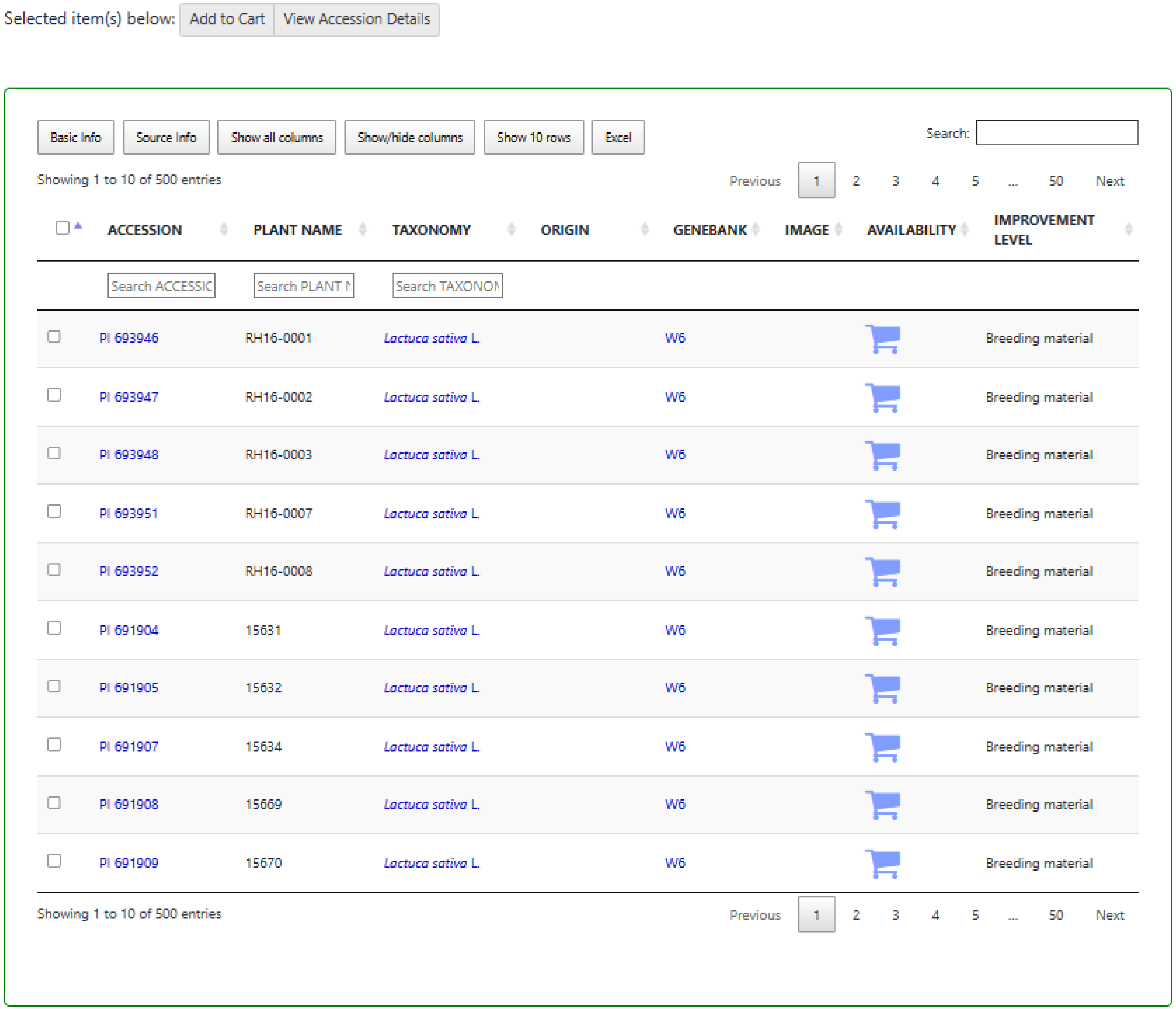


Select the tab for the type of search. Each tab has everything you need to do to perform that type of search.

Return up to 500

(Results of more than 500 will not return images.)

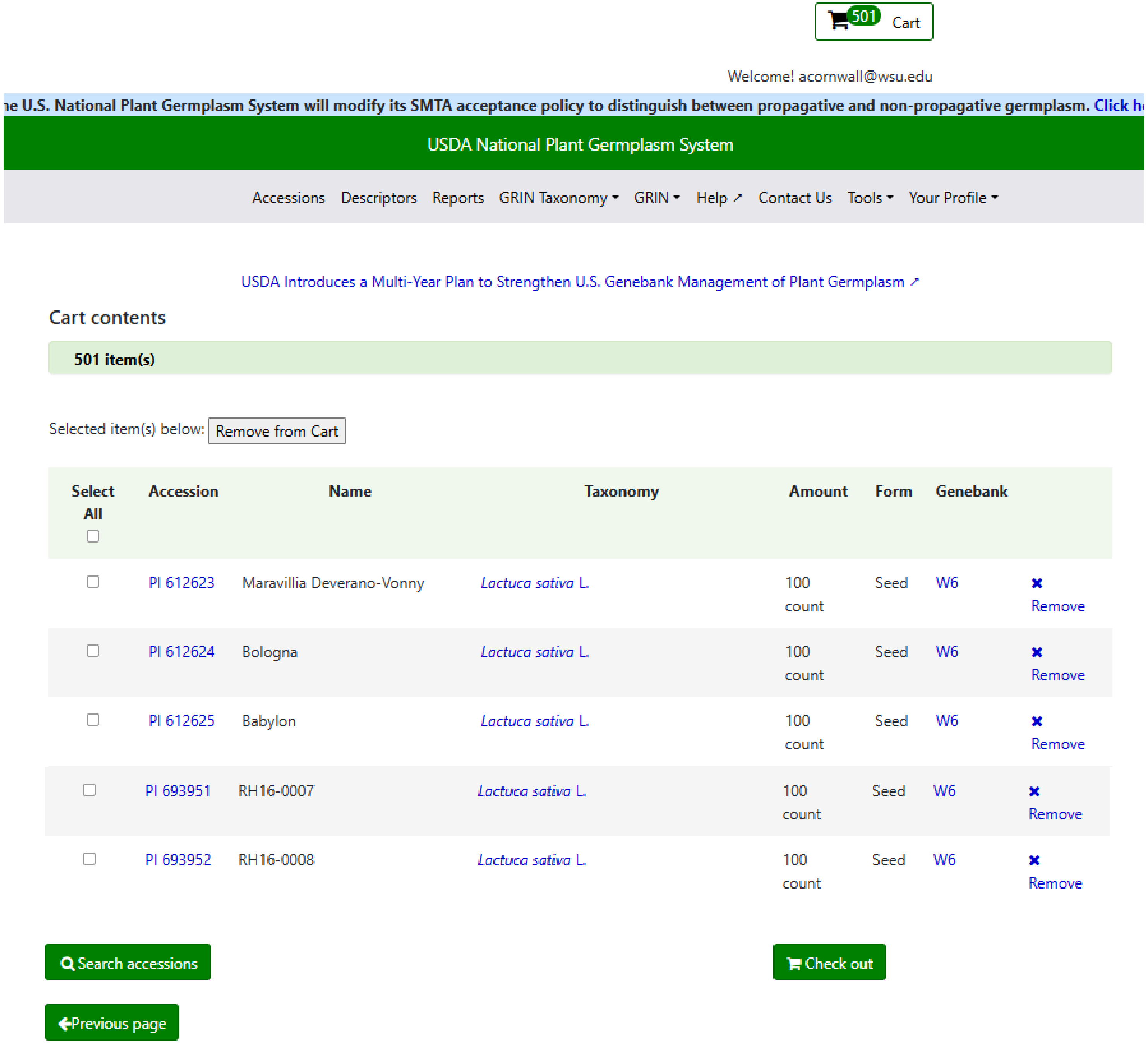
The more information you provide, the better the search will be.



Showing 1 to 10 of 500 entries

☐	ACCESSION	PLANT NAME	TAXONOMY	ORIGIN	GENEBANK	IMAGE	AVAILABILITY	IMPROVEMENT LEVEL
☐	PI 693946	RH16-0001	*Lactuca sativa* L.		W6		🛒	Breeding material
☐	PI 693947	RH16-0002	*Lactuca sativa* L.		W6		🛒	Breeding material
☐	PI 693948	RH16-0003	*Lactuca sativa* L.		W6		🛒	Breeding material
☐	PI 693951	RH16-0007	*Lactuca sativa* L.		W6		🛒	Breeding material
☐	PI 693952	RH16-0008	*Lactuca sativa* L.		W6		🛒	Breeding material
☐	PI 691904	15631	*Lactuca sativa* L.		W6		🛒	Breeding material
☐	PI 691905	15632	*Lactuca sativa* L.		W6		🛒	Breeding material
☐	PI 691907	15634	*Lactuca sativa* L.		W6		🛒	Breeding material
☐	PI 691908	15669	*Lactuca sativa* L.		W6		🛒	Breeding material
☐	PI 691909	15670	*Lactuca sativa* L.		W6		🛒	Breeding material

Showing 1 to 10 of 500 entries



Welcome! acornwall@wsu.edu

e U.S. National Plant Germplasm System will modify its SMTA acceptance policy to distinguish between propagative and non-propagative germplasm. Click h

USDA National Plant Germplasm System

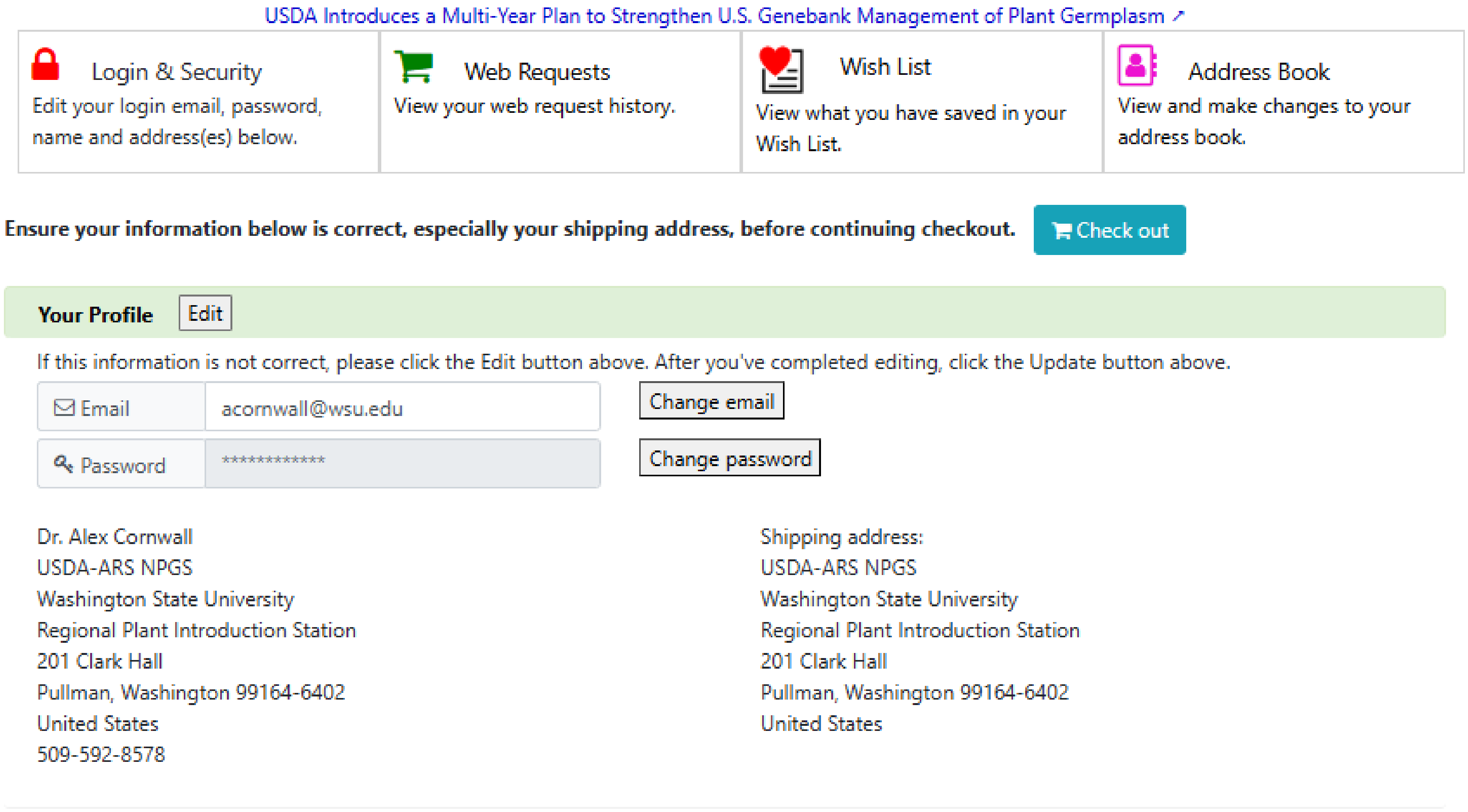
USDA Introduces a Multi-Year Plan to Strengthen U.S. Genebank Management of Plant Germplasm

Cart contents

501 item(s)

Selected item(s) below:

Select All ☐	Accession	Name	Taxonomy	Amount	Form	Genebank	
☐	PI 612623	Maravillia Deverano-Vonny	*Lactuca sativa* L.	100 count	Seed	W6	✖ Remove
☐	PI 612624	Bologna	*Lactuca sativa* L.	100 count	Seed	W6	✖ Remove
☐	PI 612625	Babylon	*Lactuca sativa* L.	100 count	Seed	W6	✖ Remove
☐	PI 693951	RH16-0007	*Lactuca sativa* L.	100 count	Seed	W6	✖ Remove
☐	PI 693952	RH16-0008	*Lactuca sativa* L.	100 count	Seed	W6	✖ Remove



Edit your login email, password, name and address(es) below.

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View and make changes to your address book.

Ensure your information below is correct, especially your shipping address, before continuing checkout.

Your Profile

If this information is not correct, please click the Edit button above. After you've completed editing, click the Update button above.

Email	acornwall@wsu.edu
Password	************

Dr. Alex Cornwall
USDA-ARS NPGS
Washington State University
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United States
509-592-8578

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TAKII

Vilmorin
MIKADO