Transgenic expression of pattern recognition receptor EFR in tomato leads to effective field resistance to bacterial wilt

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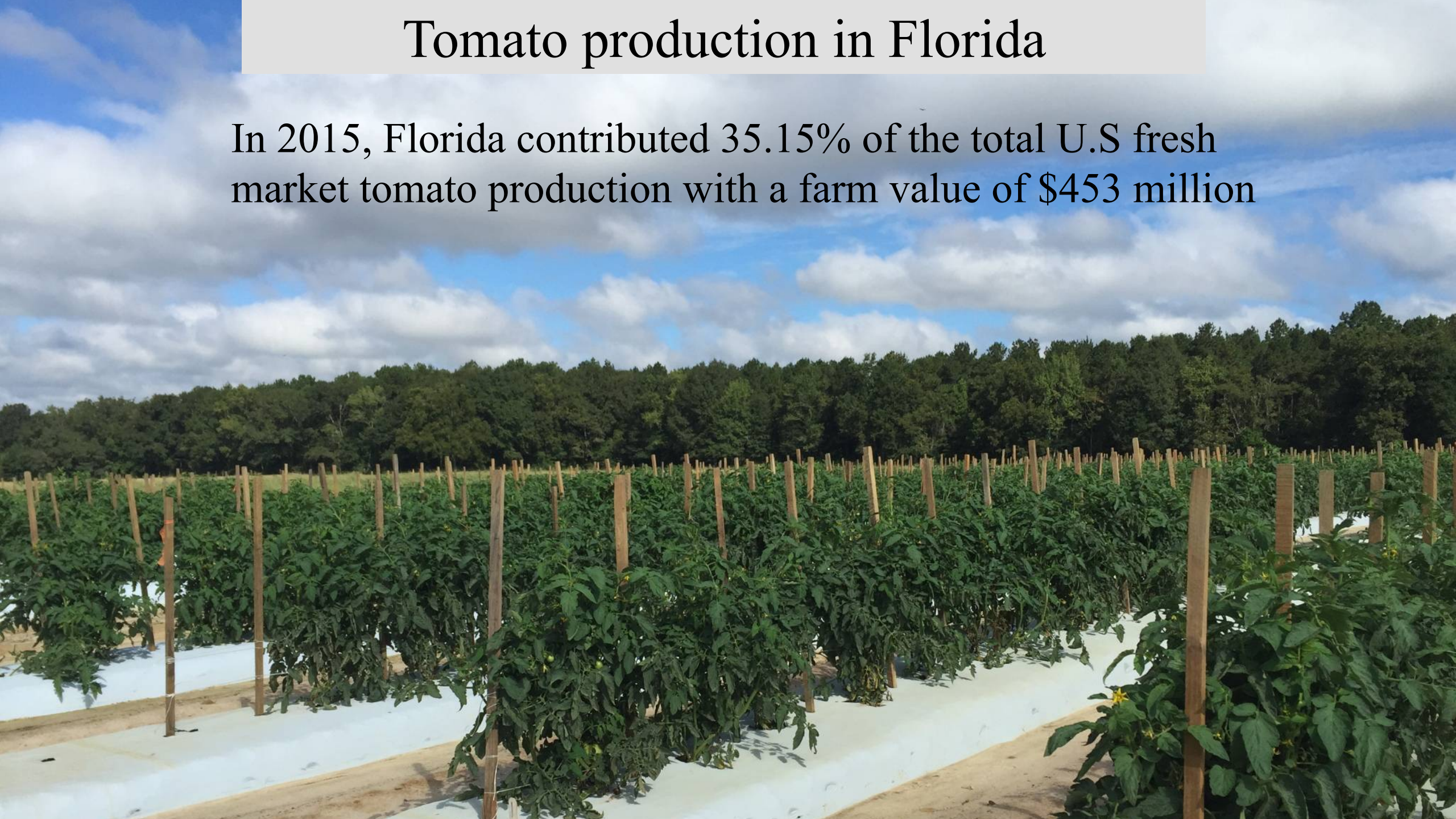
Tomato production in Florida

In 2015, Florida contributed 35.15% of the total U.S fresh market tomato production with a farm value of $453 million



Tomato varieties that are resistant to bacterial wilt

H7996

H7997

H7998

Limitations

- Resistance limited to locations, pathogen strain & temperature
- Unwanted linkage drag & negative correlation with fruit yield and quality (Chellemi et al, 1994; Grimault et al 1995)

Continuous need for alternatives to improve host resistance


Non-Grafted (BHN 602)
Grafted (BHN 602/BHN 998)

Application of Acibenzolar-*S*-Methyl Enhances Host Resistance in Tomato Against *Ralstonia solanacearum*

Table 3. Effect of acibenzolar-*S*-methyl on bacterial wilt incidence and yield of tomato cultivars BHN 466, Neptune, and Equinox under field conditions (Fall 2002, Quincy, FL)

Treatment[w,x]	Disease incidence (%)[y]	Yield (t/ha)[y,z]
BHN 466 + Actigard	21 c	30.1 a
BHN 466	46 b	22.6 c
Neptune + Actigard	25 c	27.9 ab
Neptune	54 b	24.7 bc
Equinox + Actigard	100 a	0 d
Equinox	100 a	0 d

Table 4. Effect of acibenzolar-*S*-methyl on bacterial wilt incidence and yield of tomato cultivars FL 7514, Neptune, and FL 47 under field conditions (Fall 2003, Quincy, FL)

Treatment[w,x]	Disease incidence (%)[y]	Yield (t/ha)[y,z]
FL 7514 + Actigard	18.6 c	41.7 a
FL 7514	40.2 b	31.5 b
Neptune + Actigard	10.8 c	44.0 a
Neptune	30.4 b	39.3 ab
FL 47 + Actigard	87.3 a	7.6 c
FL 47	96.1 a	3.0 c

Interfamily transfer of a plant pattern recognition receptor confers broad-spectrum bacterial resistance

Lacombe et al. 2010

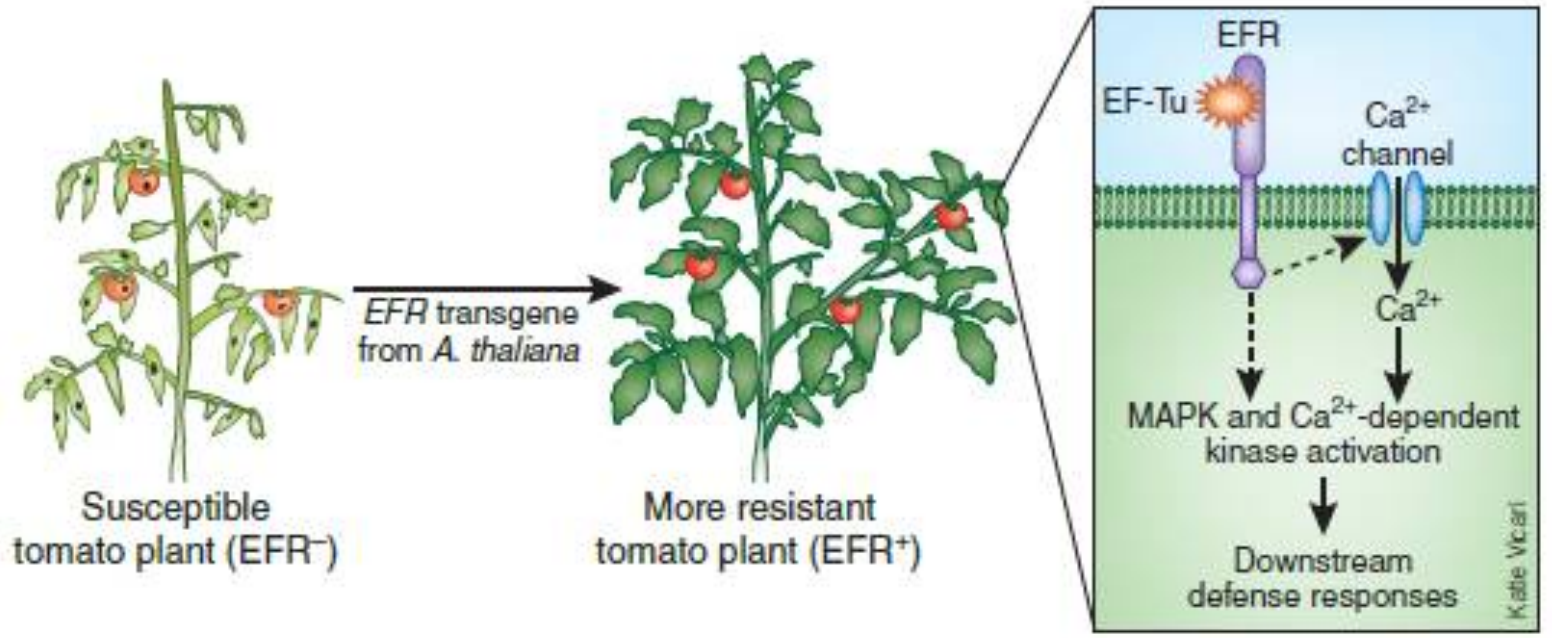


Figure 1 Engineering broad-spectrum resistance in tomato, a member of the Solanaceae, by transferring a pattern-recognition receptor from the wild species *A. thaliana* (family Brassicaceae). Elongation factor Tu receptor (EFR; purple) is absent from the solanaceous species *N. benthamiana* and tomato, and these plants are normally susceptible to infection by *Agrobacterium tumefaciens*, *Pseudomonas syringae*, *Ralstonia solanacearum* and *Xanthomonas perforans*. Transgenic expression of *A. thaliana* EFR increases their resistance to all of these bacterial pathogens, presumably by activating a signaling cascade that confers resistance to a range of bacteria expressing its cognate PAMP, elongation factor Tu (EF-Tu; orange). The successful inter-family transfer of EFR-mediated disease resistance suggests that *N. benthamiana* and tomato contain all components necessary for EFR signaling other than the receptor. MAPK, mitogen-activated protein kinase.

Brutus et al. 2010

To test the effect of *EFR* gene on bacterial wilt disease and yield of tomatoes in open field conditions

BW Field trial: Quincy, FL, Fall 2015

Entry[w]	Fruit yield (kg/ha[y])						BW[x] incidence (%)	BW[y] severity (%)
	Small	Medium	Large	Extra Large	Total marketable	Total yield		
Fl8000	5,488 c[z]	6,786 b	7,673 a	4,758 a	19,217 a	24,705 b	82.49 a	71.29 a
Fl8000+Bs2	8,607 c	6,509 b	6,570 a	4,176 a	17,254 a	25,861 b	69.94 a	58.33 a
Fl8000+EFR	24,300 a	13,453 a	10,874 a	3,574 a	27,901 a	52,201 a	15.99 b	12.65 b
Fl8000+EFR+ BS2	13,218 b	9,640 ab	9,744 a	6,548	25,753 a	40,653 a	35.23 b	30.17 b

[w]Each entry consisted of 4 reps with 14 plants/reps, and the expt. was arranged as a RCBD.
[x]Percentage bacterial wilt incidence before harvest.
[y]Percentage bacterial wilt severity before harvest.
[x, y]Field was inoculated with 50 ml of 10^5 CFU/ml of *R. solanacearum* Rs5 strain in each planting hole. The effect of the treatments on the BW incidence and severity data were analyzed separately with one-way ANOVA at P = 0.05
[y]Marketable yield of tomato fruits recorded at harvest and graded according to USDA specifications. The effect of the treatments on the marketable yield were analyzed separately with one-way ANOVA at P = 0.05.
[z]Column means followed by the same letter do not differ significantly at P = 0.05 based on Least Significant Difference (LSD).

To test the effect of *EFR* **gene** on bacterial wilt disease of tomatoes in open field conditions

BW Field trial: Quincy, FL, Fall 2016

Entry[w]	BW[x] incidence (%)	BW[y] severity (%)
Fl8000	22.92 A[z]	17.19 a
Fl8000+Bs2	33.33 A	27.08 a
Fl8000+EFR	0.00 B	0.00 b
Fl8000+EFR+ BS2	0.00 B	0.00 b

[w]Each entry consisted of 4 reps with 12 plants/reps, and the expt. was arranged as a RCBD.
[x]Percentage bacterial wilt incidence (as of 28th Oct, 2016).
[y]Percentage bacterial wilt severity (as of 28th Oct, 2016).
[x, y]Field was inoculated with 50 ml of 10^5 CFU/ml of *R. solanacearum* Rs5 strain in each planting hole. The effect of the treatments on the BW incidence and severity data were analyzed separately with one-way ANOVA at P = 0.05
[z]Column means followed by the same letter do not differ significantly at P = 0.05 based on Least Significant Difference (LSD).

FL8000 (Control)

Fl 8000 + BS2

Fl 8000 + EFR

Fl 8000 + BS2+ EFR


fisherbra
GLASS

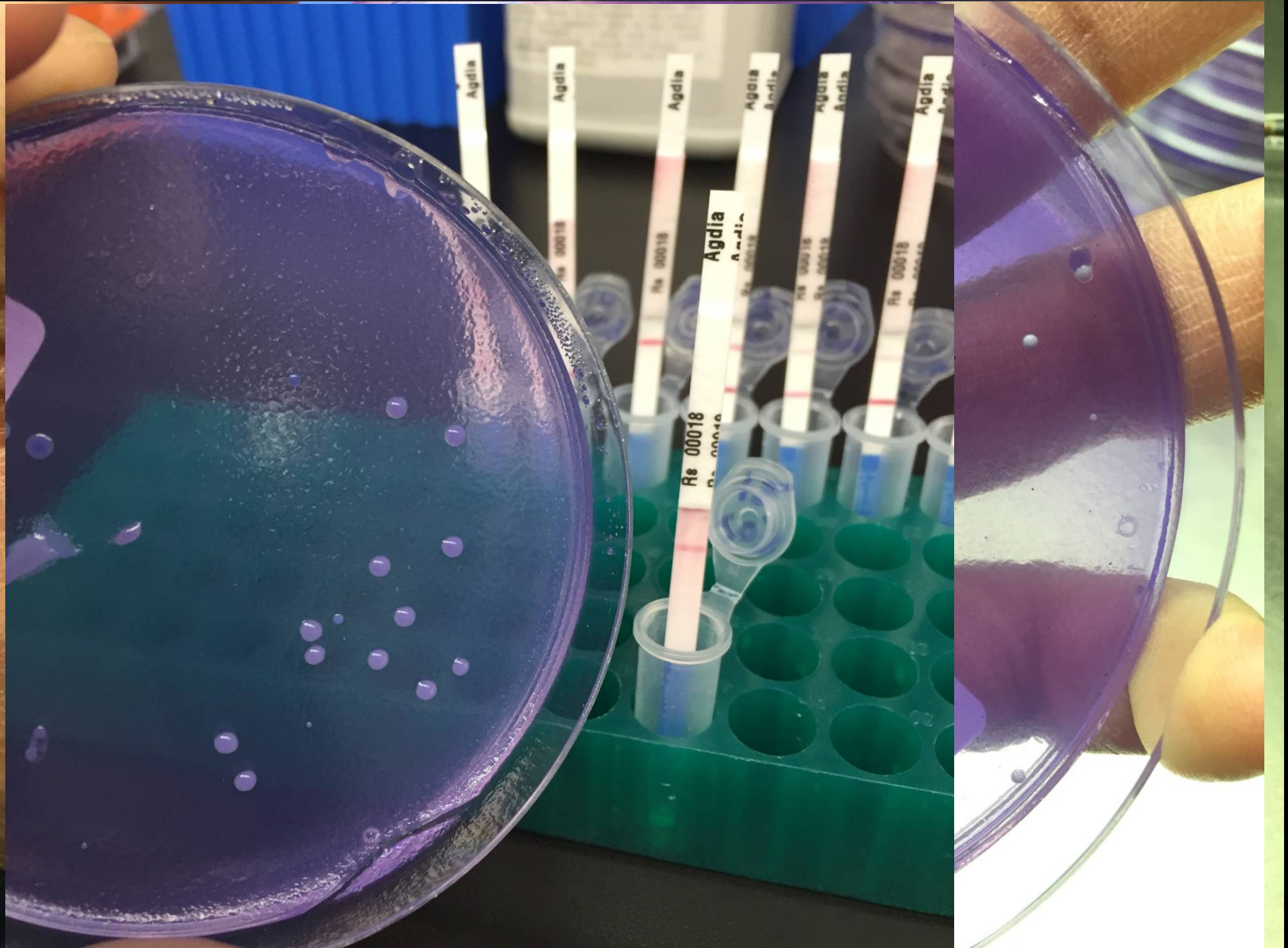
Percentage of plants from which bacteria were recovered in SMSA media after harvest (includes both symptomatic and non-symptomatic plants).

$$\frac{\text{No of symptomatic and non-symptomatic plants from which bacteria was recovered in SMSA}}{\text{Total number of plants in that plot}} * 100\ \%$$

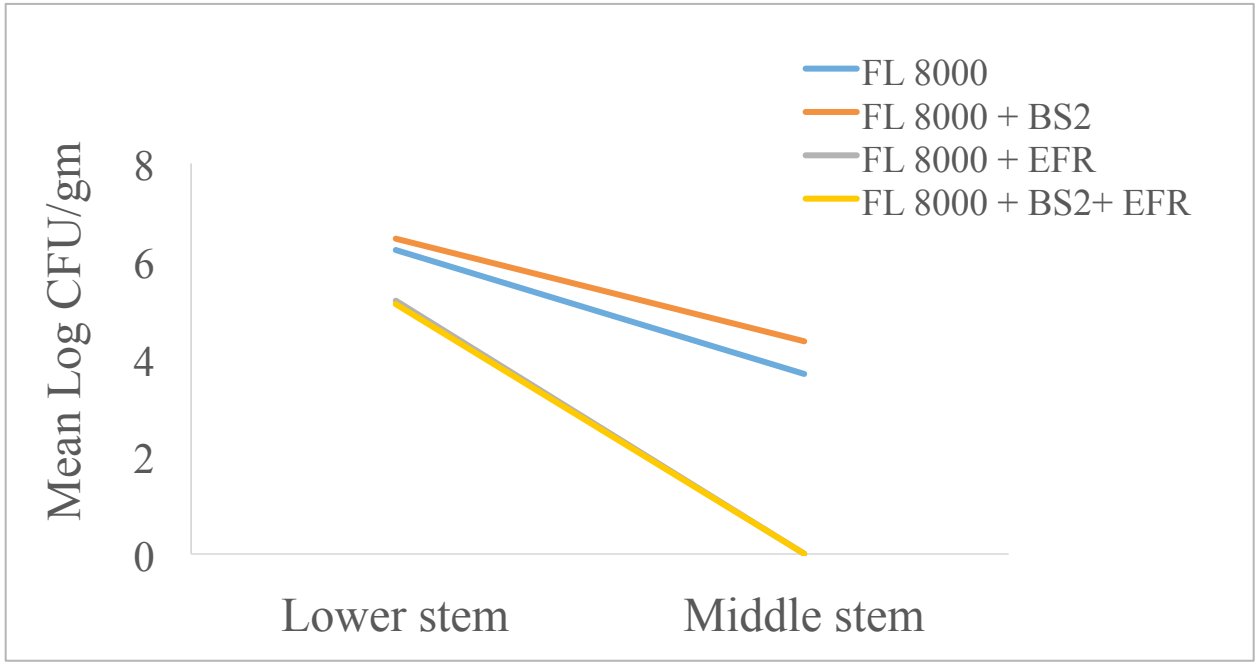
Entry[w]	% of plants from which bacteria was recovered in SMSA media*	
	Basal stem	Middle stem
Fl8000	85.12 A[z]	66.07 a
Fl8000+Bs2	75.04 A	57.85 a
Fl8000+EFR	26.45 B	0.00 b
Fl8000+EFR+ BS2	13.38 B	0.00 b

*Middle stem and lower stems were anayzed separately.

[z]The columns with the same letter do not differ significantly based on SNK at P = 0.05


Agdia
Rs 00018

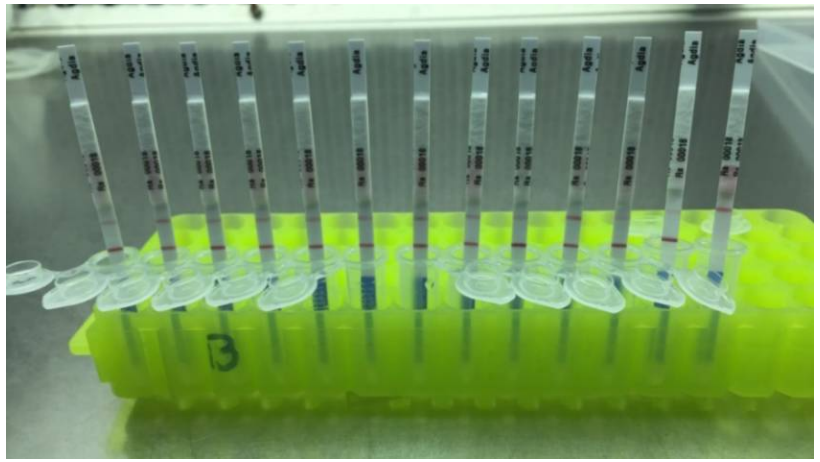
Bacterial colonization in the basal and middle stem tissue of the infected plants after harvest (considering only those plants from which bacteria was recovered)



Entry	Bacterial population (Log CFU/gm tissue)	
	Basal stem	Middle stem
Fl8000	6.23 ± 1.02	3.68 ± 0.44
Fl8000+Bs2	6.47 ± 0.76	4.36 ± 0.90
Fl8000+EFR	5.20 ± 0.63	No colony
Fl8000+EFR+ BS2	5.11 ± 1.82	No colony

To test the transgenic *EFR* tomato line against various strains of *R. solanacearum* collected from southeast U.S.

S. No.	ID	Iso Date	Source	Location	Notes	Isolator	former ID	Multiplex/ phylotype	R3B2 630/631	HR on tobacco 24hrs	HR on tobacco 48hrs	Biovar test
1	**UF101***	1999	Tomato	FL	Quincy	T. Momol	RS5	2	-	+	+	1
2	UF219	1999	Tomato	FL	Imok.	P. Robers	P576	2	-	+	+	1
3	UF220	2000	Tomato	FL	Central	D. Norman	P594	2	-	-	-	1
4	UF242		Tomato	NC		C. Allen	P 691	2	-	+	+	1
5	UF401*	1981	Tomato	GA	Rabun Co	R. Gitaitis	Rso 81-5	2	-	-	-	1
6	UF528*		Tomato	AL		M. Schel	AW1	2	-	+	+	1
7	UF205*	1996	Tomato	FL	Central	D. Norman	P507	2	-	+	+	1
8	UF343*	2005	Tomato	FL	Sun City Field 3	P. Ji	RS136	2	-	+	+	1
9	UF344*	2005	Tomato	FL	Sun City Field 4	P. Ji	RS137	2	-	+	+	1
10	UF526*	2008	Tomato	NC			K136	2	-	-	-	1
11	UF525	2008	Tomato	NC			K74	2	-	-	-	1
12	**UF101***	1999	Tomato	FL	Quincy	T. Momol	RS5	2	-	+	+	1



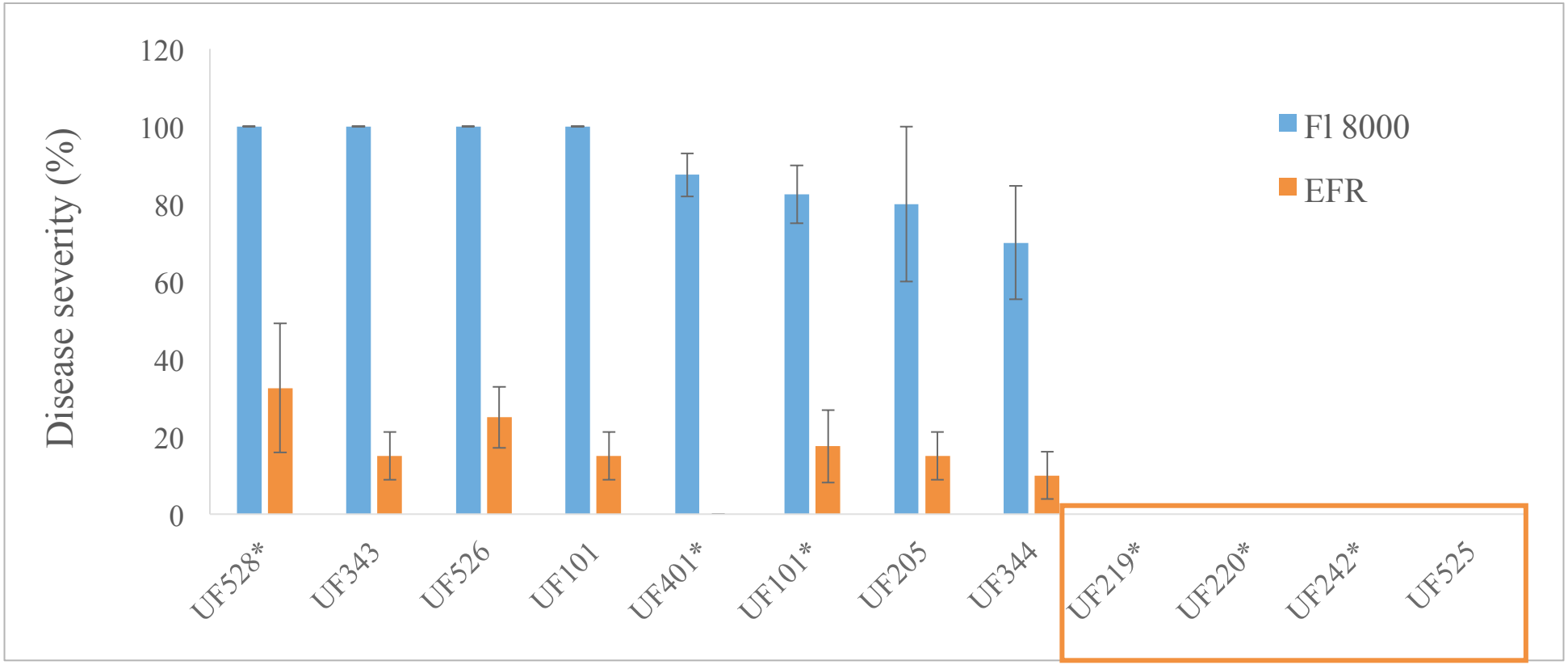
To test the transgenic *EFR* tomato line against various strains of *R. solanacearum* collected from southeast U.S.

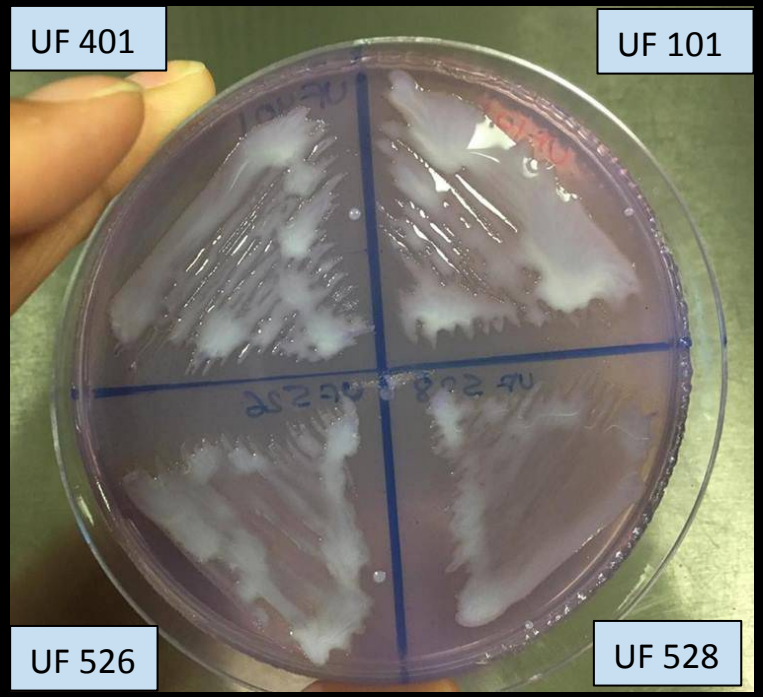
Seed germination →

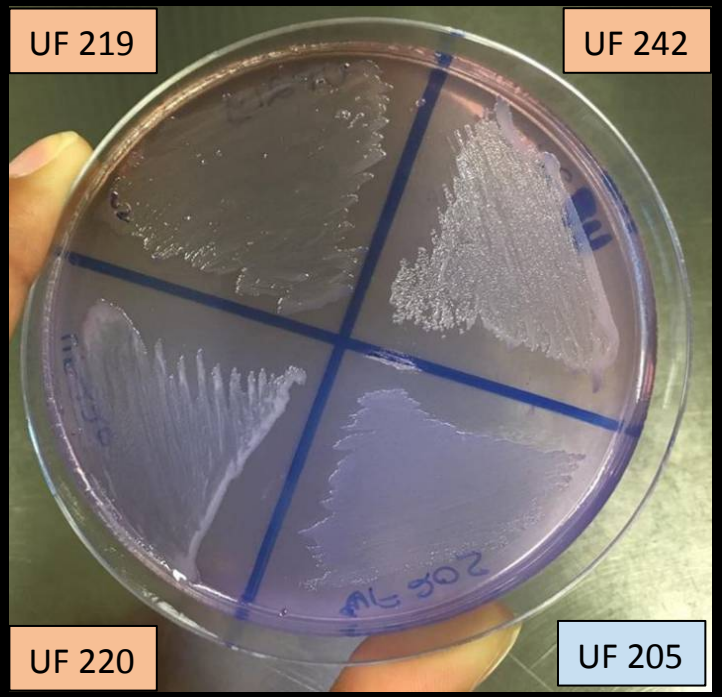
→ Inoculation:
-11 strains
-10^7 CFU/ml;
-50 ml/pot

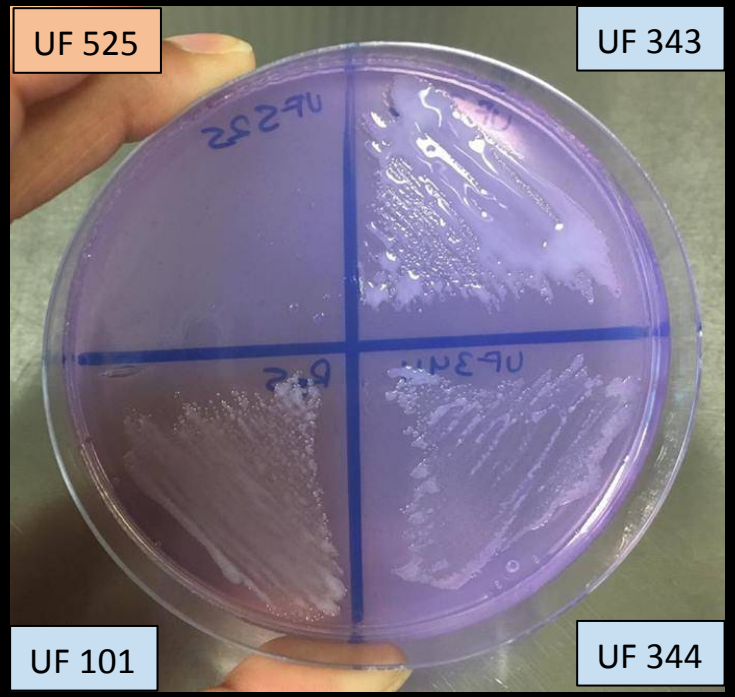
Plant Genotypes
FL 8000
FL 8000 + EFR

To test the transgenic *EFR* tomato line against various strains of *R. solanacearum* collected from southeast U.S.




UF 401
UF 101
UF 526
UF 528


UF 219
UF 242
UF 220
UF 205


UF 525
UF 343
UF 101
UF 344

UF 101/ N. FL

UF 401/GA

UF528/ AL

UF 242/ NC

UF 219/ S. FL

UF 220/ C. FL

UF 526/ NC

UF 205/ C. FL

UF 343/ C. FL

UF 344/ C. FL

UF 101/ N. FL

UF 525/ NC

Potential impacts

- *EFR* transgenic tomato lines: rapid gene transfer, no negative effect on yield, minimal risk of transgene escape.

- PRR proteins like EFR work by recognizing PAMPs (highly conserved in microbes; less prone to mutation) offering more durable resistance in the field compared to R-gene based resistance which can break down rapidly in the field due to frequent mutation in effectors. Therefore, it offers possibility for universal applicability against bacterial wilt of tomato worldwide.

- The transgenic line containing both *EFR* and *Bs2* genes together may provide durable field resistance against bacterial wilt and bacterial spot of tomato, two of the important tomato disease in Florida and worldwide.

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THE STORKAN-HANES-McCASLIN RESEARCH FOUNDATION


Thank you


FL8000


EFR


EFR+Bs2