Evaluation of Brassica Accessions as Potential Biocidal Green Manure to Control Tomato Bacterial Wilt

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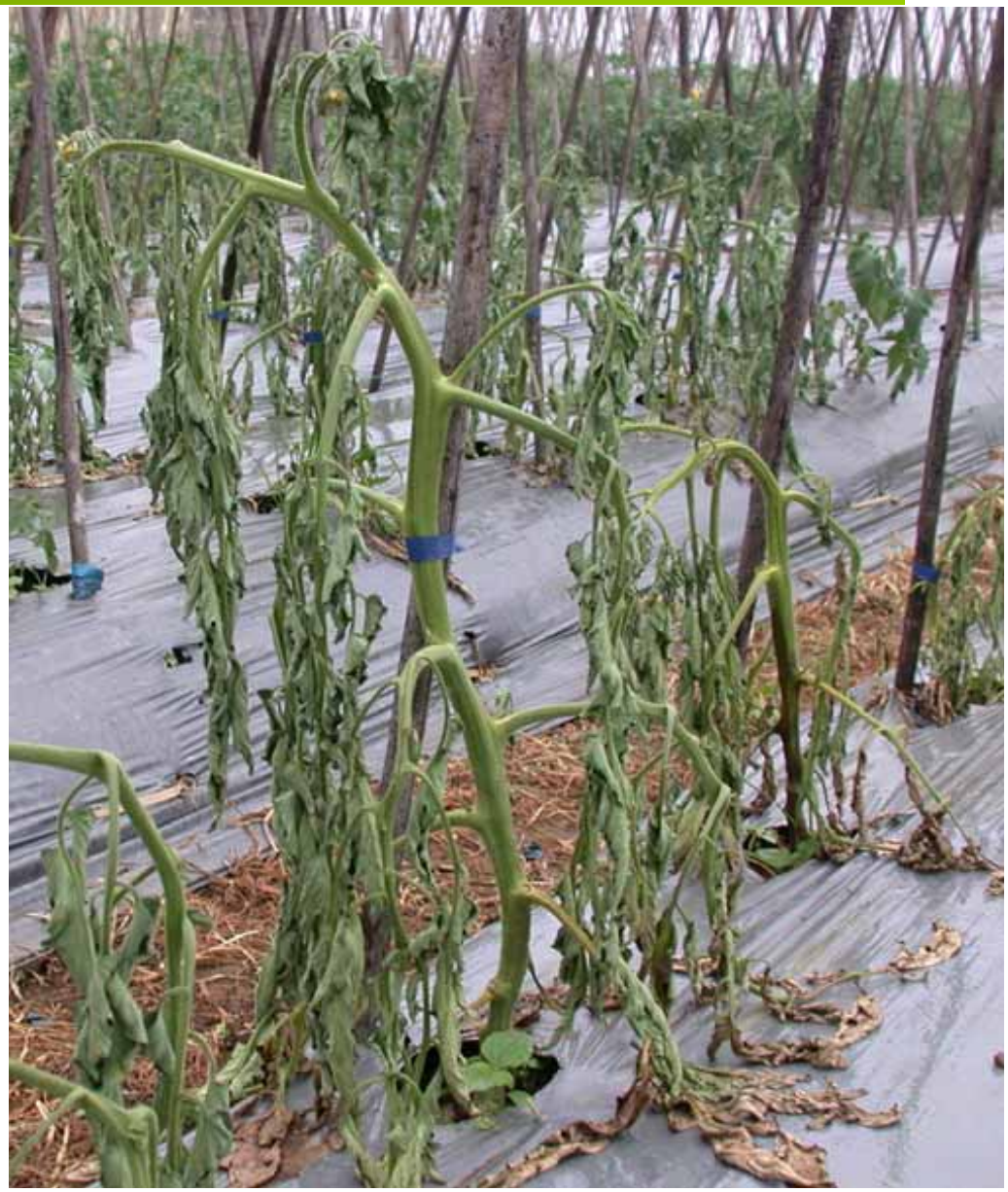
Tomato bacterial wilt

- A globally important disease
- Caused by a soil-borne bacterium, *Ralstonia solanacearum*
- Difficult to control due to wide host range and long term survival of the pathogen
- Host resistance base integrated disease management



Why biofumigation?

- Suppression of pathogen population in the soil could contribute to sustainable management of soil-borne diseases.
- Use of soil amendments like urea / lime, compost etc. to control bacterial wilt has been reported.
- Biofumigation: Use of plants with biologically active compound to suppress soil-borne pathogens
- Brassicaceae contain glucosinolate (GSL). Hydrolysis of GSL by myrosinase produces isothiocyanate (ITC).
- ITC is volatile, possess biocidal effect against *Ralstonia solanacearum* and other organisms.

Objectives

- Evaluate the potential of Brassica spp. as a biocidal green manure to manage tomato bacterial wilt

1. Screening of *Brassica* spp. for biofumigation

- Materials

  26 varieties of *B. campestris* (8), *B. juncea* (13), *B. napus* (3), *B. rapa* (2)

- Conditions

  - using 5% (w/w) leaf tissue in AVRDC soil
  - soil moisture at 64% field capacity
  - negative control with no leaf addition
  - positive control: *B. juncea* var. Nemfix (with high GSL)

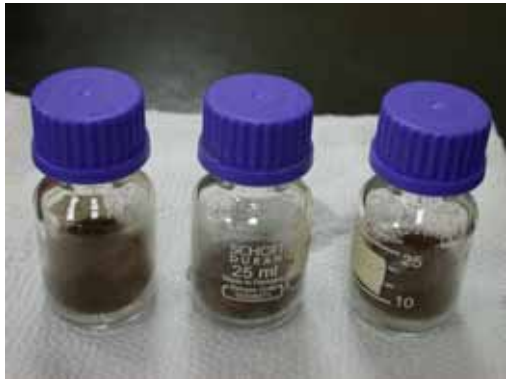
Freeze and thaw bioassay

1. Cut *Brassica*'s leaves into pieces

2. Put weighed leaves (5% w/w) into bottle

3. Put bottle in -20^0 C until frozen

4. Take out bottle from freezer, put on ice chips; pour 10g infested soil into bottle



5. Incubate bottle at 30^{0}C for 3 days.

6. Pour soil-leaf mixture into 90ml water for enumeration process

Freeze and thaw assay results

Variety	Log (CFU/g soil)	
	Bioassay 1	Bioassay 2
Daulat	0.0	0.0
Rai 5	4.7	2.9
Shree	3.8	1.5
Sharisha 11	3.5	0.0
Toria	4.6	0.0
TB574	0.0	0.0
Varanasi brown	4.2	1.8
Ankit	3.2	1.1
CK	7.6 – 7.7	7.8

2. Evaluation of *B. juncea* as a potential host

- Mustards have been reported as a host of *R. solanacearum* in India in 1988. It is a concern that growing Indian mustard could maintain or increase the pathogen population in soil.
- Inoculation method: drenching Pss4 suspension (10^8 cell/ml) with or without root wounding.
- Rating by wilting incidence (W%) and disease index (DI).

Scale 0

Scale 1

Scale 2

Scale 3

Scale 4

Scale 5

Disease Index (DI) = $[(N_0x0+N_1x1+N_2x2+N_3x3+N_4x4+N_5x5) / (N_Tx5)] x100\%$

N_0-N_5: number of plants with 0-5 rating; N_T: number of total plants

B. juncea inoculation results (21 DAI)

Variety	W %		DI	
	Wounded	Not wounded	Wounded	Not wounded
Ankit	75.0 b	0.0 c	44.2 b	0.0 c
Nemfix	100.0 a	8.3 c	95.0 a	5.0 c
Sharisha-11	87.5 ab	8.3 c	41.7 b	5.0 c
TB574	91.7 ab	0.0 c	48.3 b	0.0 c
Rai-5	95.8 ab	12.5 c	50.8 b	10.0 c
Toria	91.6 ab	0.0 c	53.3 b	0.0 c
Varanasi- brown	95.8 ab	4.2 c	58.3 b	0.8 c
Daulat	95.8 ab	4.2 c	50.8 b	0.8 c
Shree	91.7 ab	4.2 c	60.0 b	0.8 c

3. Hoticultural traits of selected *B. juncea*

Variety	Days to flowering	Number of leaves	Leaf length (cm)	Leaf width (cm)	Pubescence
Daulat	34	9	12.0	8.5	intermediate
Rai 5	28	8	9.5	7.0	intermediate
Shree	50	10	14.0	9.5	sparse
Sharisha 11	38	7	9.5	6.5	intermediate
Toria	45	8	15.0	10.5	intermediate
TB574	50	10	14.5	9.5	abundant
Varanasi brown	-	7	14.0	9.0	abundant
Ankit	42	8	17.5	11.5	sparse

(Evaluated in March – May, 2006)

4. Pot trial with TB574 (*B. juncea*)

- Experiment design
  - 4 factors RCBD; 3 replications
  - Soil (3): TSS, AVRDC, Puli
  - Infestation: Pss4 (10^7 cell/g soil)
  - Application (3): macerated, chopped, control (5% w/w)
  - Incubation (2): 3 or 21 days
  - Tomato variety (2): AVRDC10 (S), L180 (MR)
- Measurements
  - Pathogen density before transplanting
  - Soil microbial community assay (DGGE) before transplanting
  - Disease incidence and progress

Basic soil properties

Soil	Texture	Sand (%)	Silt (%)	Clay (%)	OM (%)	CEC (cmol/kg)	pH
AVRDC	L	32.1	45.9	22	0.85	5.58	7.2
Puli	CL	43.0	19.7	37.3	2.21	8.17	5.3
TSS	C	16.5	33.2	50.4	3.02	14.00	6.5

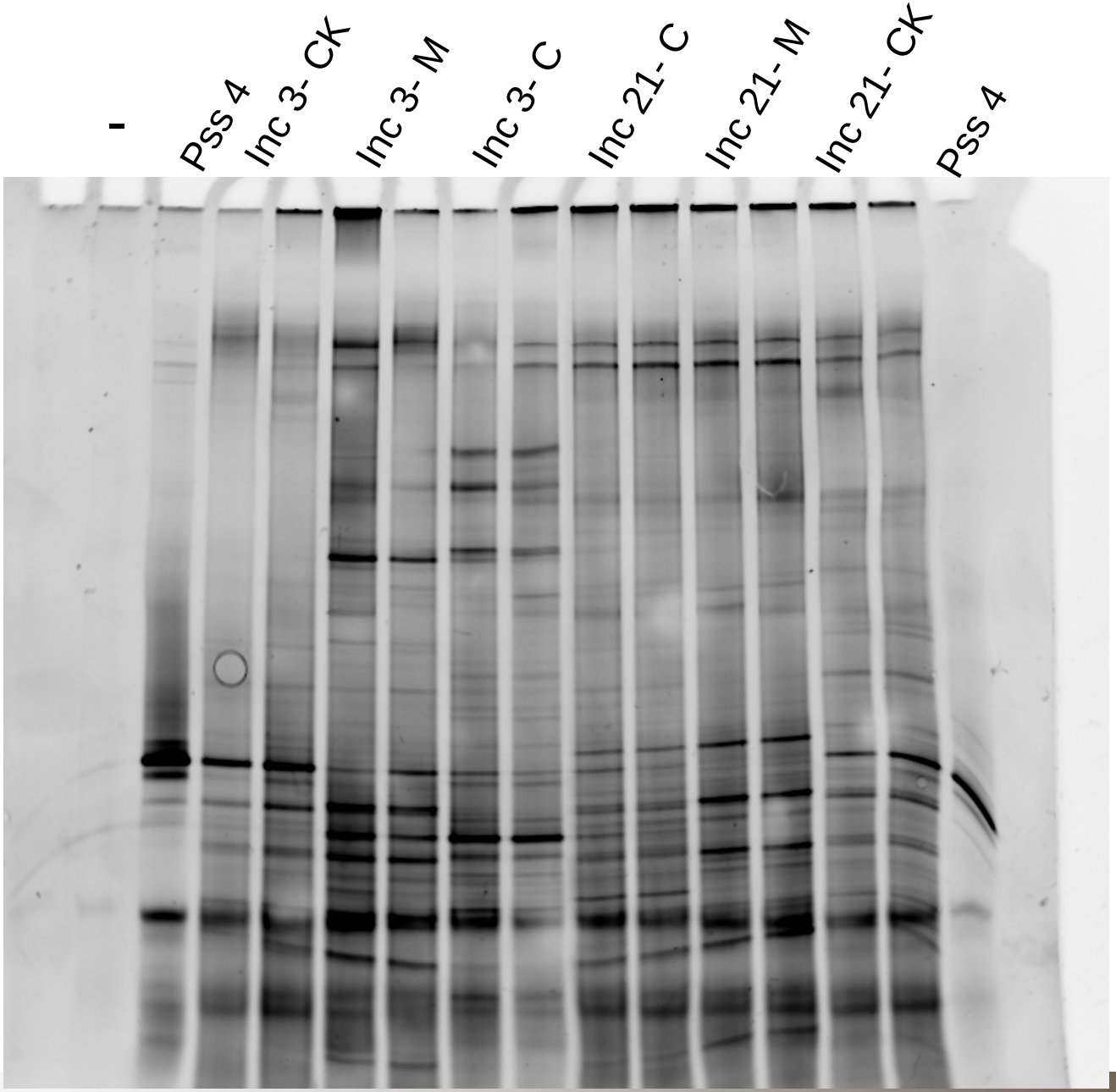
Biocidal effect is soil dependent

		Incubation 3 days			Incubation 21 days	
Soil	**Chopped**	**Macerated**	**CK**	**Chopped**	**Macerated**	**CK**
AVRDC	7.44 a A	7.52 a A	7.41 a A	5.81 b B	5.41 c B	6.48 a A
Puli	7.09 a B	6.77 b B	7.04 a B	5.15 b C	3.53 c C	6.37 a A
TSS	7.47 a A	7.49 a A	7.30 a A	6.60 a A	6.66 a A	6.54 a A
LSD = 0.2056 ($P < 0.0001$)						

Means with the same letter are not significantly different at P < 0.05 according to Fisher's LSD t test (lowercase for application comparison and uppercase for soil) by incubation.

Changes on soil microbial community is observed

Puli soil



Control efficacy is more obvious on susceptible variety

Mean disease incidence

Application	AVRDC10	L180
Chopped	31.5 a C	20.4 b A
Macerated	39.8 a B	8.8 b B
CK	60.6 a A	26.9 b A
LSD = 7.1544 (*P* = 0.0002)		

Disease suppression effect is observed in all soils

Mean AUDPC

Soil	Chopped	Macerated	CK
AVRDC	260.2 b B	213.7 b B	731.5 a B
Puli	177.3 a B	85.2 b B	286.7 a C
TSS	461.8 c A	685.5 b A	1040.4 a A
LSD = 168.8 (*P* = 0.0008)			

5. On-farm trial (Summer 2007; Ankit)

- Trial details
  - Nantou, Taiwan (ca. 700 masl)
  - Indian mustard Ankit sowed on 6 Jan. 07 and plowed on 13 Mar. 07
  - Plant materials: AVRDC18 or grafted on eggplant rootstocks
  - Transplanting on 17 Apr. 07

On-fram trial results

Treatment	Mean percent wilted plants (%)				
	21 DAT	42 DAT	63 DAT	84 DAT	105 DAT
Green manure					
AVRDC18	10.0 a	67.9 a	93.6 a	100.0 a	100.0 a
AVRDC18/EG203	0.0 b	0.0 b	13.1 b	23.4 b	23.7 b
AVRDC18/EG219	0.0 b	0.3 b	7.1 b	17.4 b	20.0 b
AVRDC18/TS90	0.0 b	0.3 b	11.0 b	24.7 b	27.3 b
No green manure					
AVRDC18	15.4 a	98.0 a	99.7 a	100.0 a	100.0 a
AVRDC18/EG203	0.0 b	0.3 b	11.3 b	25.8 b	27.3 b
AVRDC18/EG219	0.0 b	1.0 b	10.7 b	28.6 b	30.0 b
AVRDC18/TS90	0.0 b	0.3 b	4.1 b	12.8 b	16.5 b

Future research

- Confirmation on the efficacy of Indian mustard as a biocidal green manure
  - Confirm soil effect on the control efficacy
  - Determine effect on yield
- Understand control mechanisms
  - Confirm ITC effect
  - Soil and rhizosphere microbial community
  - Promotion of antagonistic bacteria or PGPR