Resistance Risk and Resistance Mechanism of *Ralstonia solanacearum* to SYP-14288

Mu Wenjun

### Zhengzhou Tobacco Research Institute of CNTC

October 19, 2023

Tobacco bacterial wilt

- Destructive soil-borne vascular disease

- *Ralstonia solanacearum*

- Chemical control: antibiotic, copper agents, sulfur preparations

- Lack of effective control bactericides

SYP-14288

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- Developed by Shenyang Chemical Research Institute, not commercialized
- Broad spectrum, great potential to control tobacco bacterial wilt
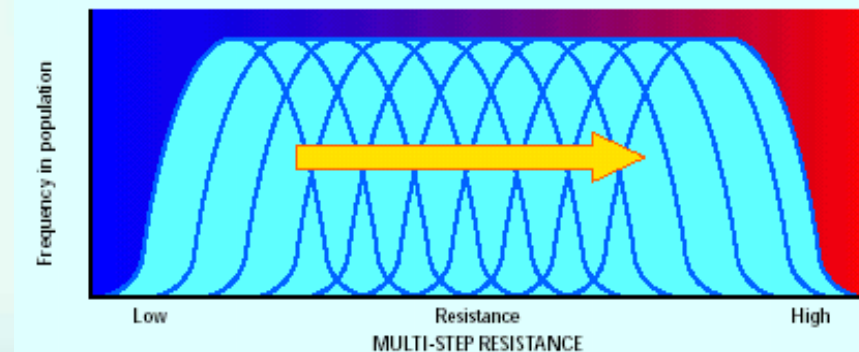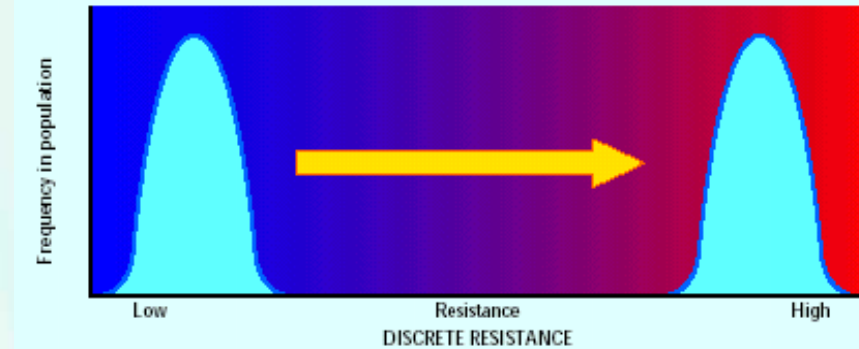- Action mechanism: uncoupling oxidative phosphorylation
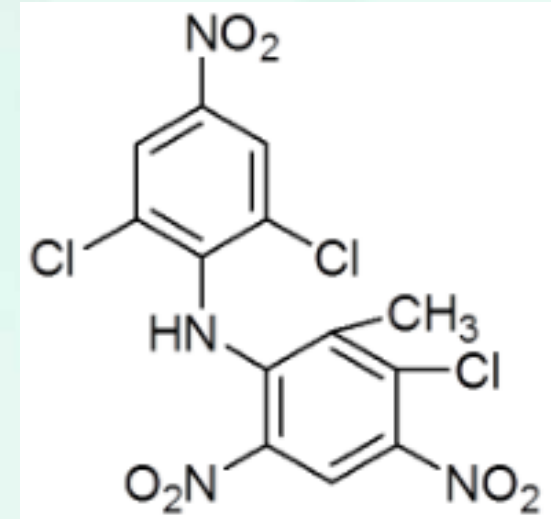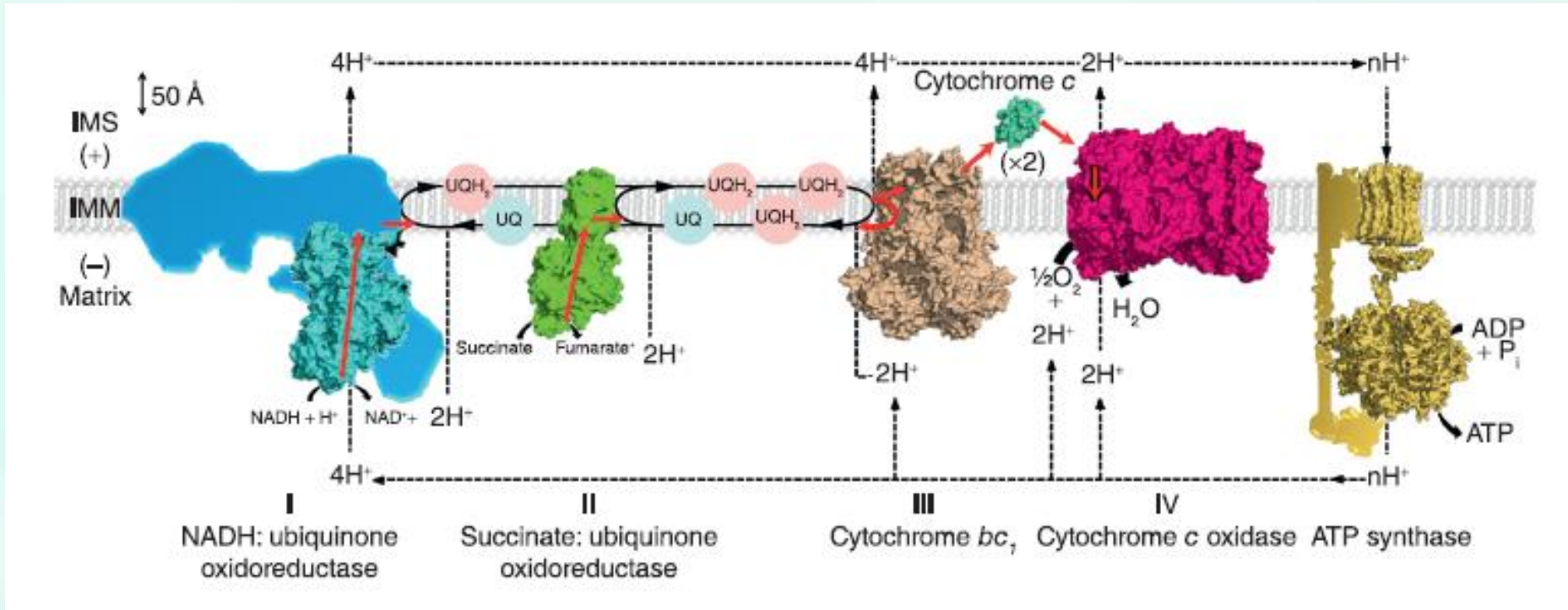
SYP-14288

Fungicide resistance

- Emergence of resistance: reduce the control effect, shorten the life of drugs.
- Resistance level and probability: pathogen biological characteristics, fungicide mode of action.

Objectives

I. Bactericidal spectrum of SYP-14288

II. Risk Assessment of *R. solanacearum* to SYP-14288

III. Molecular resictance mechanism of *R. solanacearum* to SYP-14288

✓ Guidance for SYP-14288 application and disease management strategy

Bactericidal spectrum
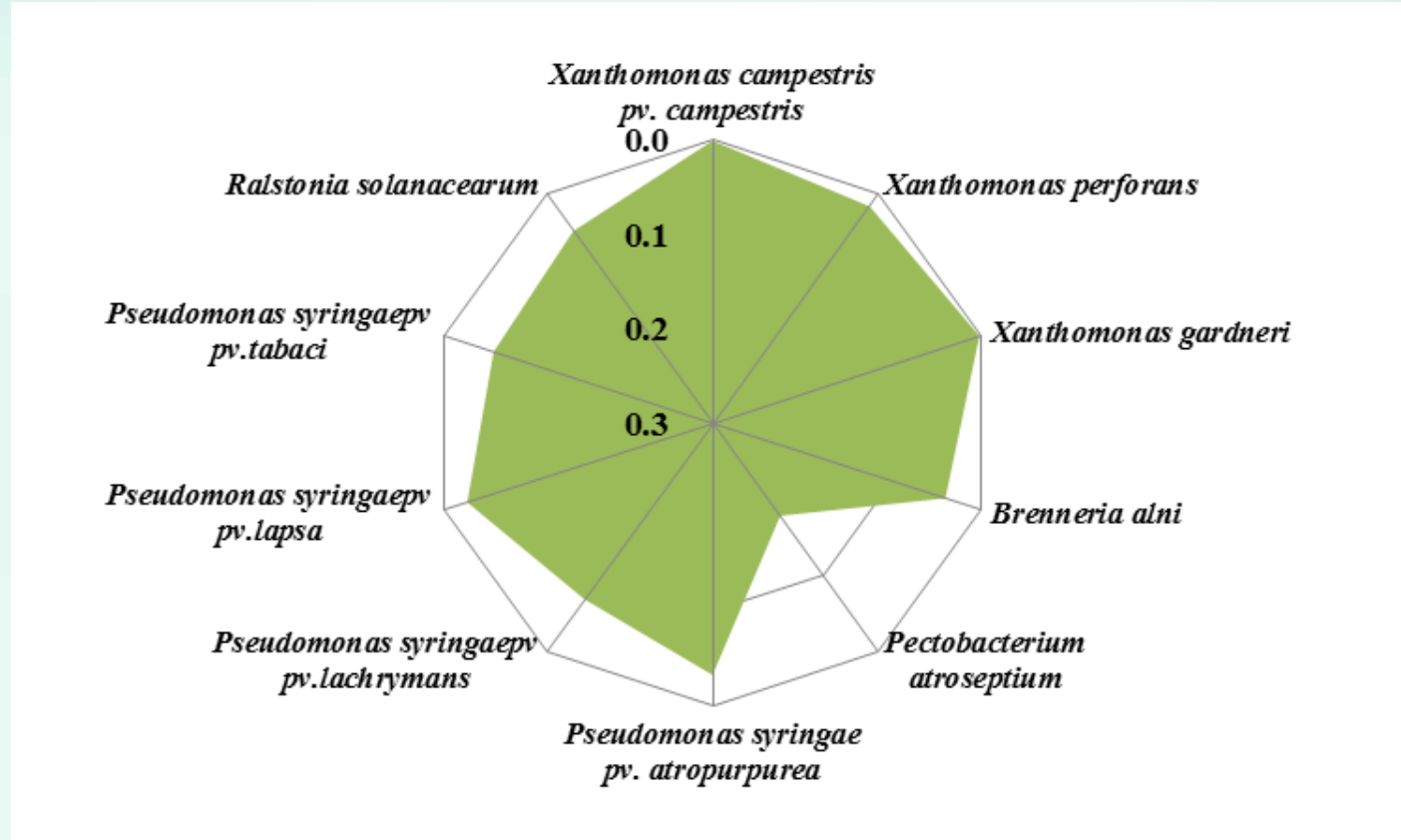
➢ SYP-14288 had good inhibitory effect on 10 plant pathogenic bacteria with EC$_{50}$ below 0.3 μg/mL.

Baseline sensitivity
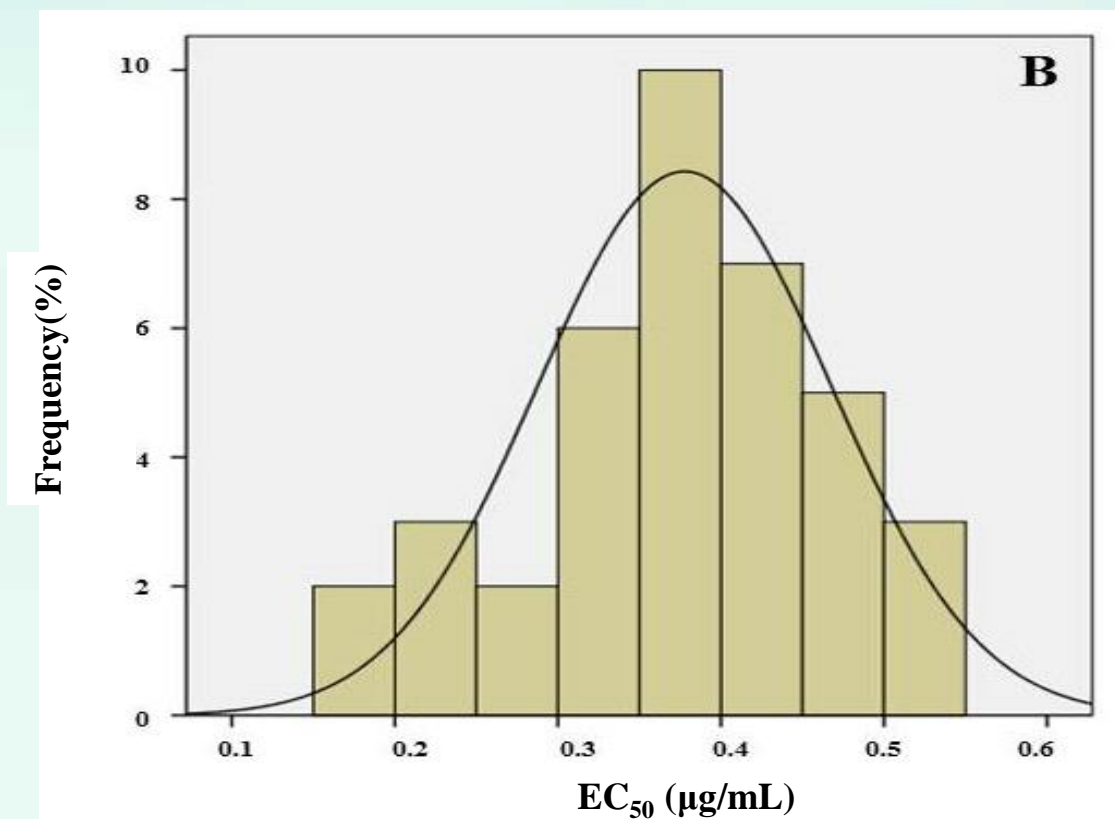
- The EC$_{50}$ value ranged from 0.0534 to 0.0187 μg/mL with a mean of 0.0378 μg/mL.
- The baseline was a normal distribution and presented as continuous single peak curve.

Resistance factors and stability of resistant mutants

Resistance type	Isolates	EC$_{50}$（μg/mL）					Resistance level					Resistance change factor
		1st	10th	20th	30th	40th	1st	10th	20th	30th	40th	
S	3	0.0610	0.0535	0.0506	0.0482	0.0399	--	--	--	--	-	--
MR	SB100-3	1.0017	2.7005	2.9907	2.1962	1.8360	16.42	50.44	59.14	45.52	45.99	2.80
S	5	0.0517	0.0511	0.0440	0.0490	0.0420	--	--	--	--	-	--
LR	SB100-5	4.2572	2.5794	1.6965	2.1207	1.1228	82.37	50.51	38.60	43.25	26.74	0.32
S	17	0.0535	0.0492	0.0418	0.0451	0.0414	--	--	--	--	-	--
LR	SB100-17	3.8546	1.4544	1.0786	1.3721	1.0360	72.00	29.56	25.83	30.45	25.05	0.35
S	20	0.0371	0.0540	0.0539	0.0489	0.0640	--	--	--	--	-	--
MR	SB100-20	1.0828	2.4937	1.0978	3.3760	2.4243	29.15	46.22	20.35	68.99	37.87	1.30
S	22	0.0341	0.0480	0.0364	0.0389	0.0512	--	--	--	--	-	--
MR	SB100-22	4.0352	2.3587	3.4508	3.0491	2.0297	118.30	49.13	94.82	78.44	39.61	0.33
S	26	0.0370	0.0417	0.0411	0.0471	0.0480	--	--	--	--	-	--
MR	SB100-26	3.4078	2.4826	1.1852	2.8799	1.7592	92.07	59.54	28.86	61.15	36.61	0.40
S	28	0.0507	0.0663	0.0448	0.0475	0.0565	--	--	--	--	-	--
MR	SB100-28	1.5711	1.8281	2.2266	1.5330	2.1427	30.97	27.59	49.72	32.25	37.92	1.22
S	31	0.0324	0.0453	0.0362	0.0666	0.0472	--	--	--	--	-	--
HR	SB100-31	2.4371	1.0601	1.4787	3.3915	3.3729	75.29	23.42	40.86	50.92	71.47	0.95
S	35	0.0403	0.0464	0.0453	0.0542	0.0628	--	--	--	--	-	--
LR	SB100-35	2.9715	1.8911	1.1892	1.7860	1.3642	73.73	40.77	26.23	32.93	21.73	0.29
S	38	0.0489	0.0484	0.0419	0.0486	0.0550	--	--	--	--	-	--
LR	SB100-38	1.3687	1.2978	1.3816	1.0790	1.2826	28.01	26.81	32.94	22.20	23.33	0.83
S	42	0.0465	0.0423	0.0450	0.0330	0.0483	--	--	--	--	-	--
HR	SB100-42	1.1183	1.0183	2.0088	2.3637	3.2793	24.03	24.08	44.61	71.56	67.90	2.83
S	44	0.0487	0.0507	0.0487	0.0611	0.0647	--	--	--	--	-	--
LR	SB100-44	1.0451	1.2408	1.2115	1.4454	1.4941	21.47	24.45	24.89	23.65	23.09	1.08

S:sensitive isolate, LR: low resistant mutant, MR: moderate resistant mutant, HR: high resistant mutant

Fitness of *R. solanacearum* to SYP-14288

Resistance type	Isolates	Temperature sensitivity（cm）					Lesion length (cm)	Disease index
		7℃	15℃	25℃	30℃	37℃		
S	3	0.118	0.188	0.499	0.684	0.603	4.2	48.15
MR	SB100-3	0.110	0.204	0.434	0.620	0.655	2.4	25.93
S	5	0.104	0.159	0.482	0.737	0.585	4.3	85.19
LR	SB100-5	0.113	0.223	0.406	0.550	0.488	1.7	33.33
S	17	0.106	0.154	0.492	0.667	0.510	3.4	70.37
LR	SB100-17	0.107	0.190	0.434	0.695	0.473	4.4	62.96
S	20	0.106	0.156	0.492	1.060	0.643	3.7	100.00
MR	SB100-20	0.108	0.177	0.485	1.185	0.610	4.7	92.59
S	22	0.100	0.129	0.488	1.184	0.575	4.2	77.78
MR	SB100-22	0.101	0.176	0.397	1.113	0.413	3.2	48.15
S	26	0.104	0.161	0.373	0.829	0.465	1.7	33.33
MR	SB100-26	0.103	0.183	0.399	0.827	0.398	4.2	48.15
S	28	0.097	0.159	0.432	0.513	0.693	2.6	48.15
MR	SB100-28	0.111	0.201	0.443	0.713	0.460	3.3	70.37
S	31	0.103	0.174	0.436	0.777	0.658	1.9	40.74
HR	SB100-31	0.106	0.200	0.437	0.625	0.508	4.7	100.00
S	35	0.098	0.092	0.439	0.759	0.650	2.5	48.15
LR	SB100-35	0.105	0.169	0.457	0.744	0.648	1.3	25.93
S	38	0.115	0.153	0.462	0.735	0.568	4.8	100.00
LR	SB100-38	0.106	0.197	0.453	0.717	0.550	3.2	85.19
S	42	0.110	0.146	0.451	0.669	0.535	2.3	100.00
HR	SB100-42	0.105	0.186	0.402	0.744	0.553	3.3	100.00
S	44	0.100	0.102	0.399	0.838	0.595	1.5	40.74
LR	SB100-44	0.106	0.193	0.447	0.728	0.610	3.2	77.78

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Resistance mechanism - Transcriptome

Isolates	Sensitive isolate 31		Resistant mutant SB100-31	
Treatments	Without SYP-14288	Add SYP-14288 0.25 µg/mL	Without SYP-14288	Add SYP-14288 30 µg/mL
Mark	S-CK	S-T0.25	M-CK	M-T30
Group	A	B	C	D

C vs A   D vs B

Up-regulated: 139

Down-regulated: 373

512   475   983
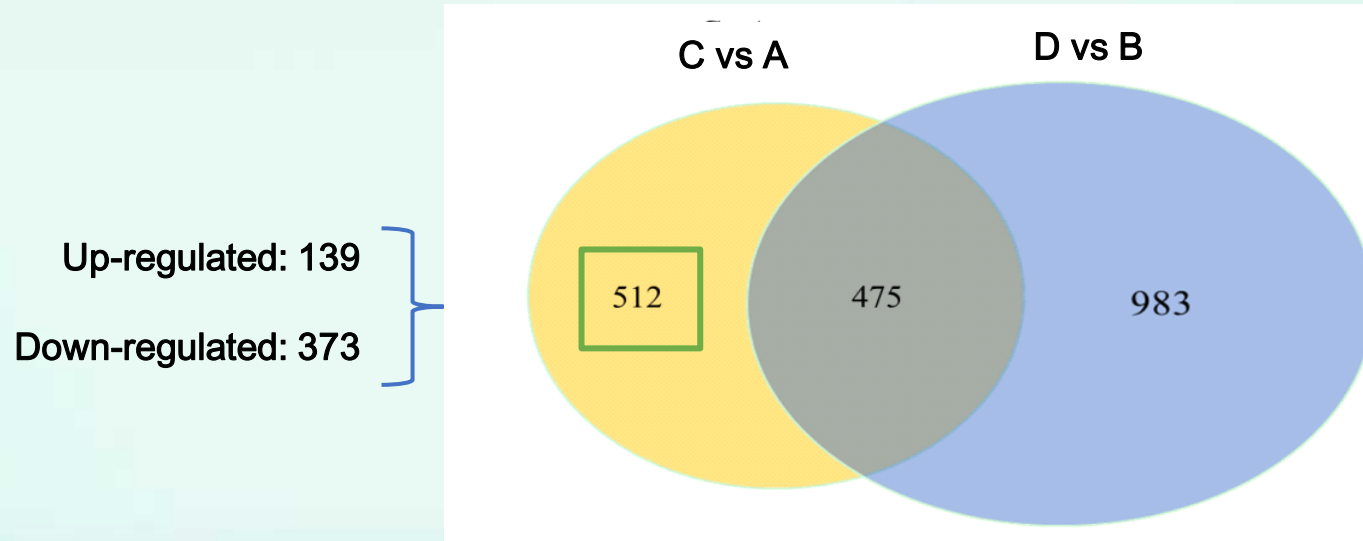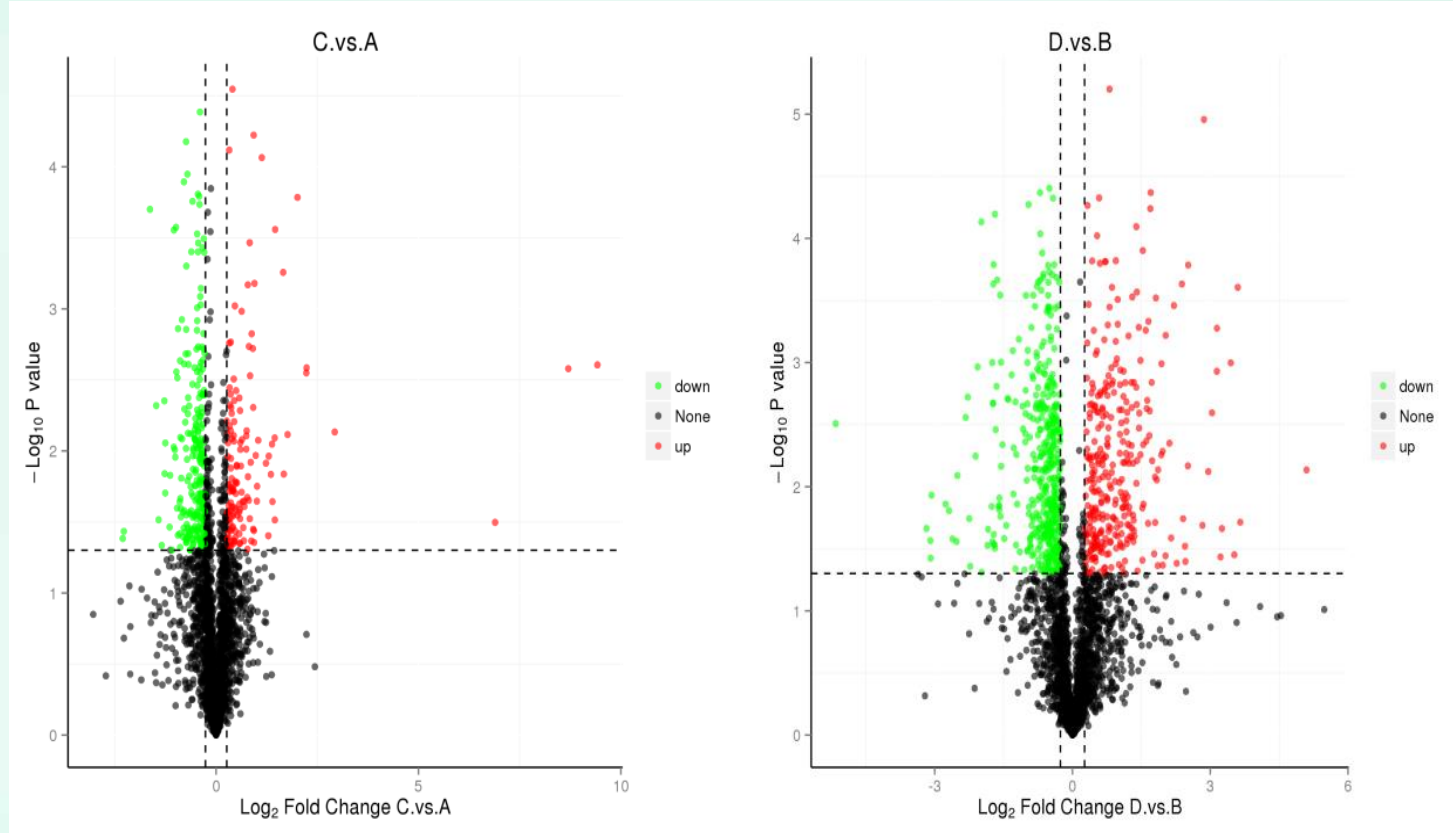
KEGG analysis of transcriptome
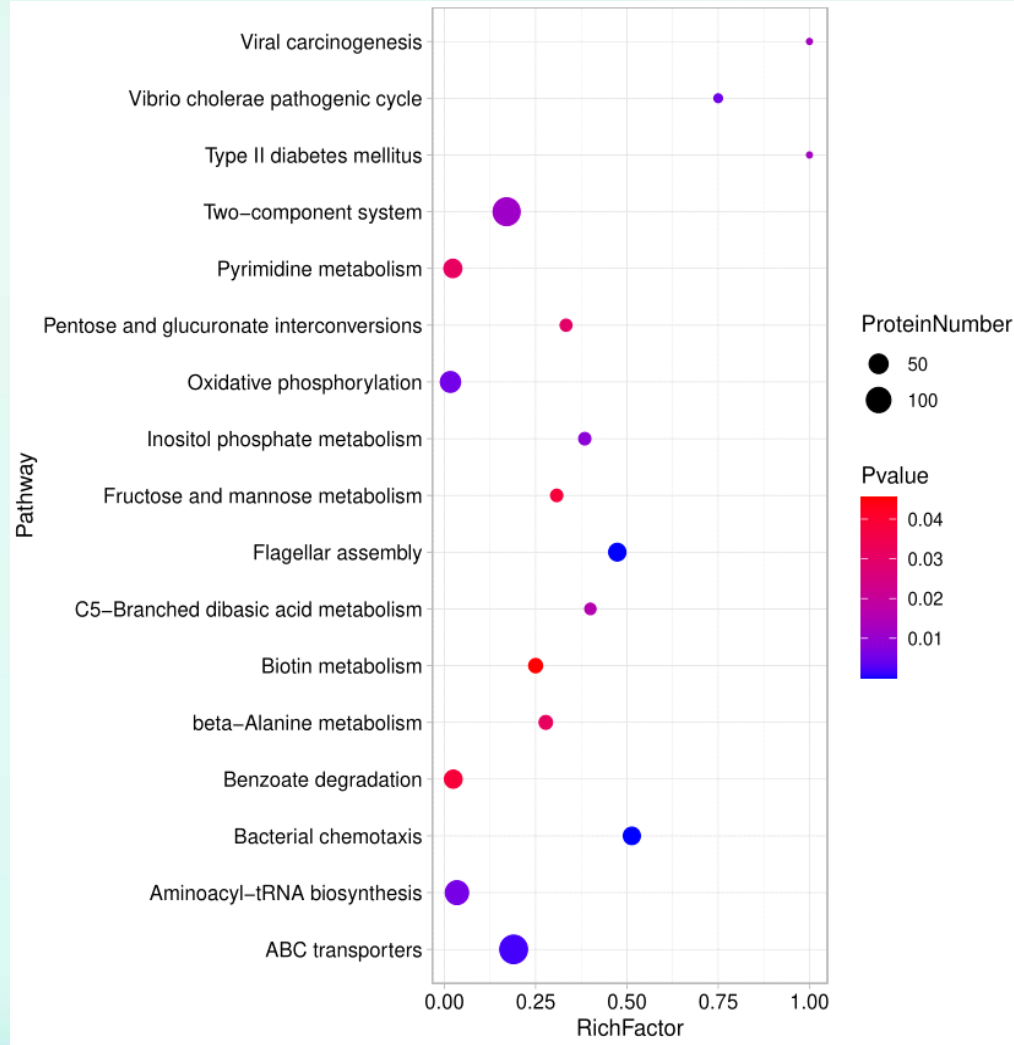
- ➢ ABC transporters

- ➢ Oxidative phosphorylation

- ➢ Bicomponent systems

- ➢ Aminoacyl tRNA synthesis

Resistance mechanism - Proteome

CvsA group:
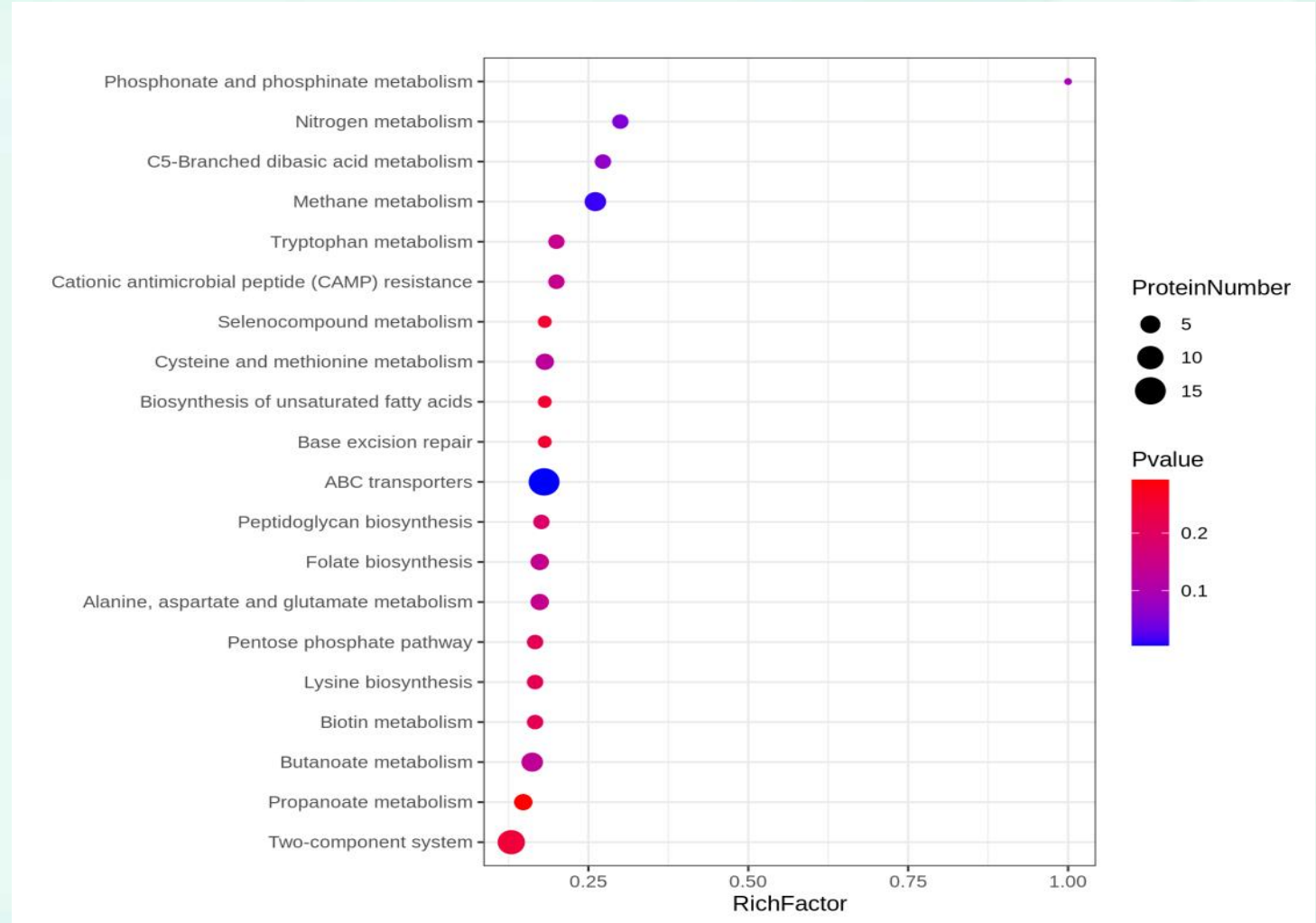
- DEPs: 2445

- Up-regulated: 147

- Down-regulated: 257

DvsB group:

- DEPs: 2494

- Up-regulated: 335

- Down-regulated: 462

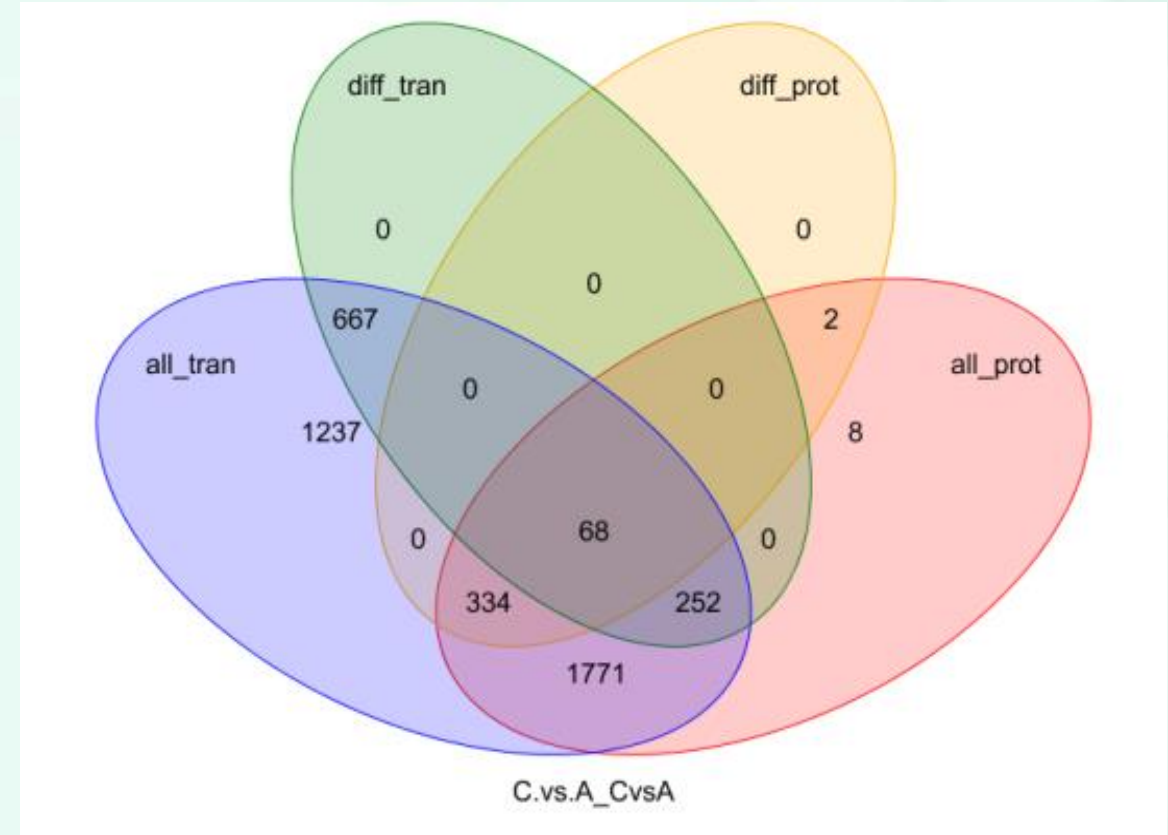2023_AP50_MuWenjun.pdf

Resistance mechanism - Proteome

KEGG analysis:

➢ ABC transporters

➢ Methanol metabolic

Omics joint analysis

➢ A total of 2425 transcriptional genes and corresponding proteins were identified.

➢ DEGs:987, DEPs:404.

➢ 36 proteins and transcriptional genes are expressed consistently.

➢ The difference times of 20 genes were more than 0.5(log2FC).

Resistance related genes

Tran_ID	Prot_ID	Protein(log2FC)	Tran(log2FC)	Gene_description
AC251_17300	Q8Y318	9.42	6.56	secretion protein HlyD
AC251_17295	Q8Y317	8.70	6.39	multidrug ABC transporter ATP-binding protein
AC251_17285	Q8Y315	6.89	6.50	ABC transporter permease
AC251_17310	Q8Y320	2.01	2.70	aldehyde dehydrogenase
AC251_09915	Q8XYA6	1.77	2.43	signal peptidase
AC251_09285	Q8XYN2	1.67	1.90	hypothetical protein
AC251_17305	Q8Y319	1.35	5.44	TetR family transcriptional regulator
AC251_25235	Q8XQA3	-0.84	-1.57	sulfonate ABC transporter substrate-binding protein
AC251_20910	Q8XSE7	-0.99	-2.44	efflux transporter periplasmic adaptor subunit
AC251_22640	Q8XRL7	-1.26	-2.04	malate:quinone oxidoreductase
AC251_09540	Q8XYI5	-2.31	-1.25	MFS transporter

qRT-PCR rapid detection method
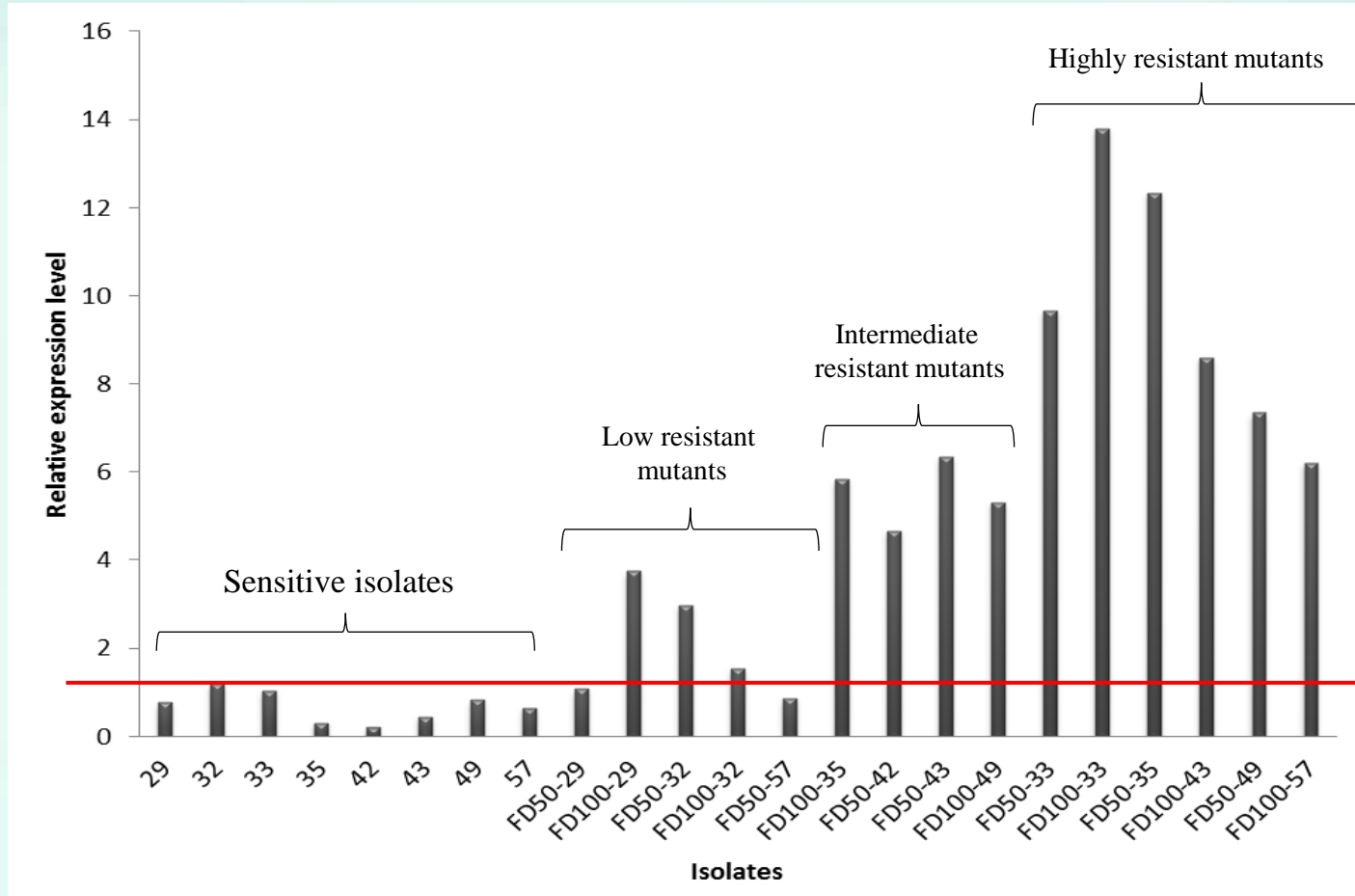
➢When the expression level of AC251_17295 gene was more than 1.5 times, all strains showed resistance.

Conclusion

- ✓ The inhibition spectrum of SYP-14288 showed that it had a good inhibitory effect on 10 plant pathogenic bacteria.

- ✓ The sensitive baseline of tobacco bacterial wilt to SYP-14288 was established, based on the survival suitability of sensitive isolates and resistance mutants, the resistance risk of tobacco bacterial wilt to SYP-14288 could be moderate.

- ✓ Through the joint analysis of transcriptome and proteome, the key metabolic pathways and main differential genes were cleared. The drug efflux mediated by ABC transporter was one of the main reasons for the resistance of tobacco bacterial wilt to SYP-14288.

Thank you for your attention!

E-mail: muwenjun@126.com