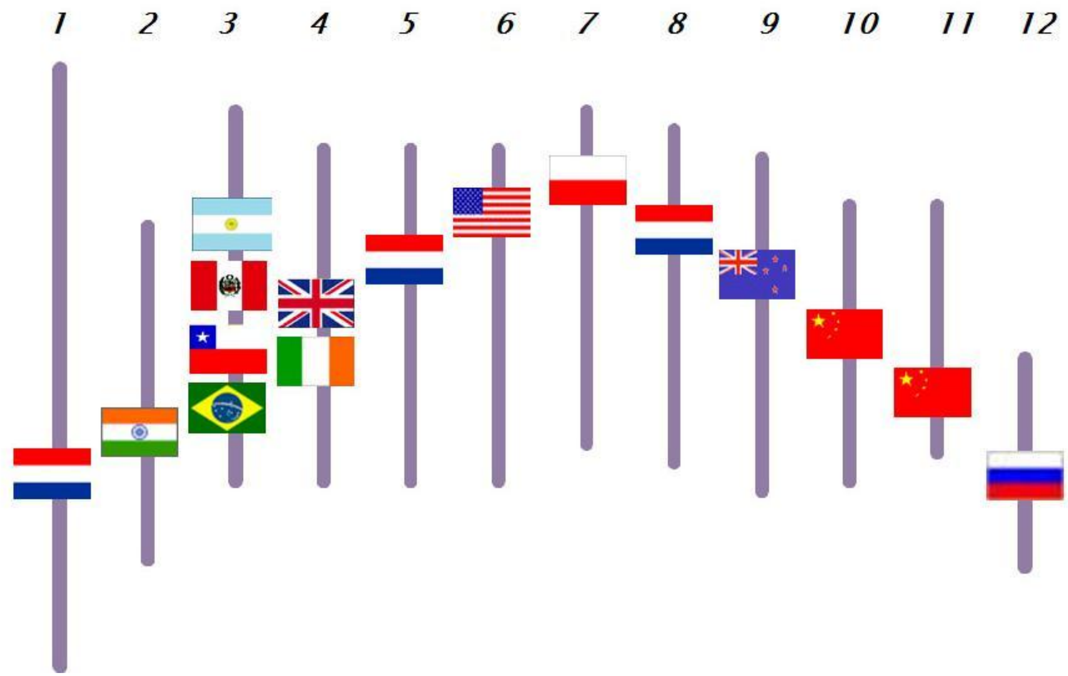
Use of Genome knowledge to feed the worlds poor

Potato genome sequence 2011



Case study – potato late blight
Indigenous community concerns

- Gota (late blight) causes significant losses
- We apply fungicides more than once a week
- Most of our profits go for fungicides
- Our children eat lots of pesticides and they get sick

Qualitative & Quantitative Resistance in plants/potato

MONOGENIC (*R*-genes)

POLYGENIC (*RR*-genes)

Sporulation surrounds lesion area in initial infection state

Sporulation is not on leaflets in advanced infection states

Sporulation is present on stem lesions

Qualitative Resistance
R genes reported = 21

R gene	*Solanum* species[b]	Origin[c]	Chr[d]
***R1* family**			
R1	*demissum*	Mexico	V
***R2* family**			
R2	*demissum*	Mexico	IV
Rpi-blb3	*bulbocastanum*	Mexico	IV
Rpi-abpt	Unknown[e]	Mexico	IV
R2-like	*edinense*	Mexico	IV
Rpi-edn1.1	*edinense*	Mexico	IV
Rpi-snk1.1	*schenckii*	Mexico	IV
Rpi-snk1.2	*schenckii*	Mexico	IV
Rpi-hjt1.1	*hjertingii*	Mexico	IV
Rpi-hjt1.2	*hjertingii*	Mexico	IV
Rpi-hjt1.3	*hjertingii*	Mexico	IV
Rpi-mcd1	*microdontum*	Argentina	IV
***R3a* family**			
R3a	*demissum*	Mexico	XI
Rpi-sto2	*stoloniferum*	Mexico	XI
***R4* family**			
R4	*demissum*	Mexico	XI
***Rpi-blb1* family**			
Rpi-blb1, *RB*	*bulbocastanum*	Mexico	VIII
Rpi-sto1	*stoloniferum*	Mexico	VIII
Rpi-pta1	*stoloniferum*[g]	Mexico	VIII
***Rpi-blb2* family**			
Rpi-blb2	*bulbocastanum*	Mexico	VI
***Rpi-vnt1* family**			
Rpi-vnt1.1	*venturii*	Argentina	IX
Rpi-vnt1.2	*venturii*	Argentina	IX
Rpi-vnt1.3	*venturii*	Argentina	IX

Not durable

Pathogen produces
Effector protein (AVR)

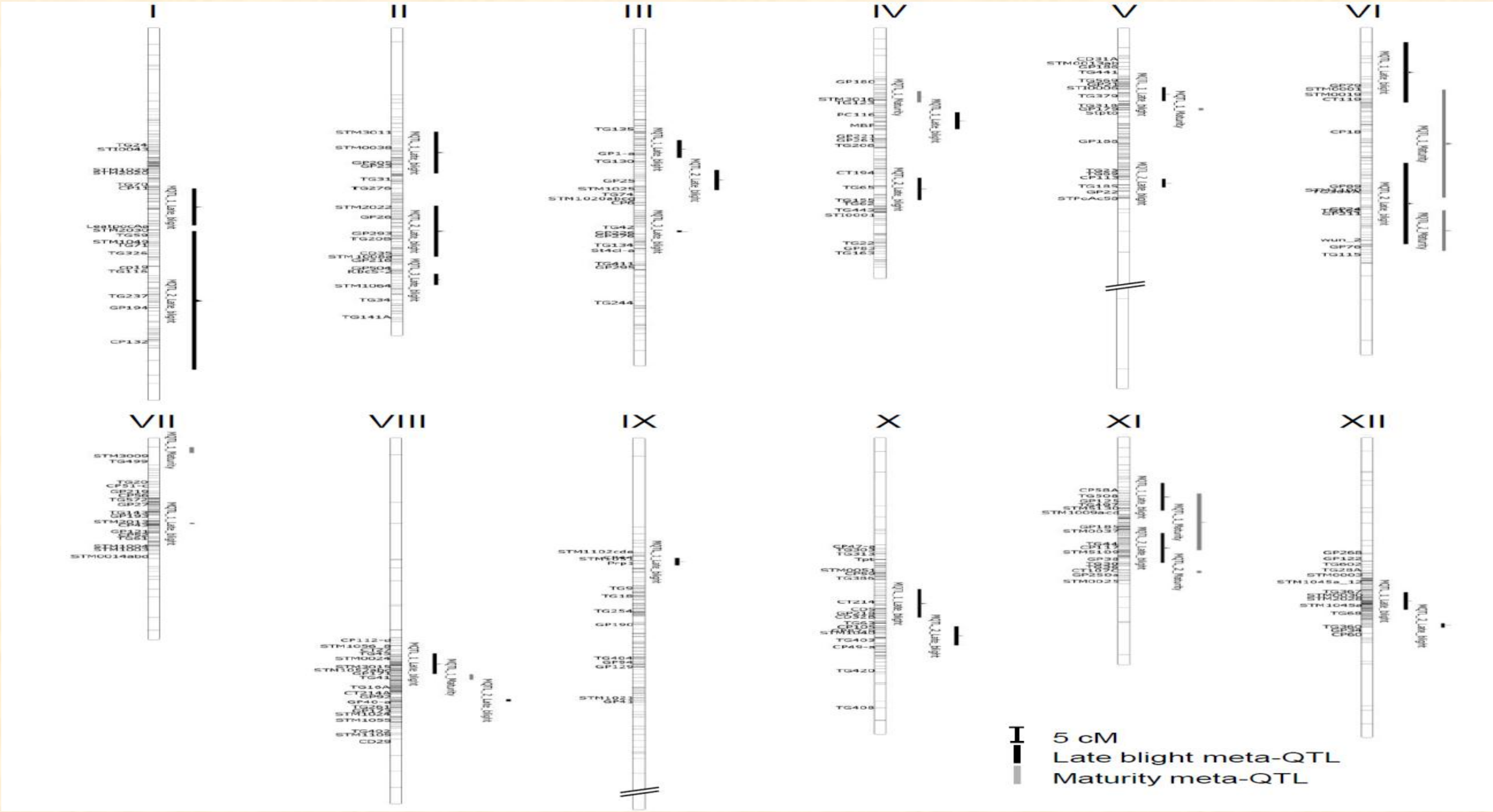
Host produces
R proteins by
R-genes

Effecter triggered immunity
Qualitative resistance
Hypersensitive reaction

(Vleeshouwers et al., 2011)

Quantitative Resistance: QTLs = 211

Resistance mechanisms unknown (Black Box)



So far, **211 QTLs** have been identified, based on 29 QTL maps for resistance to foliage, stem and tubers, with phenotypic variance of 4-63%.

(Danan et al. 2011)

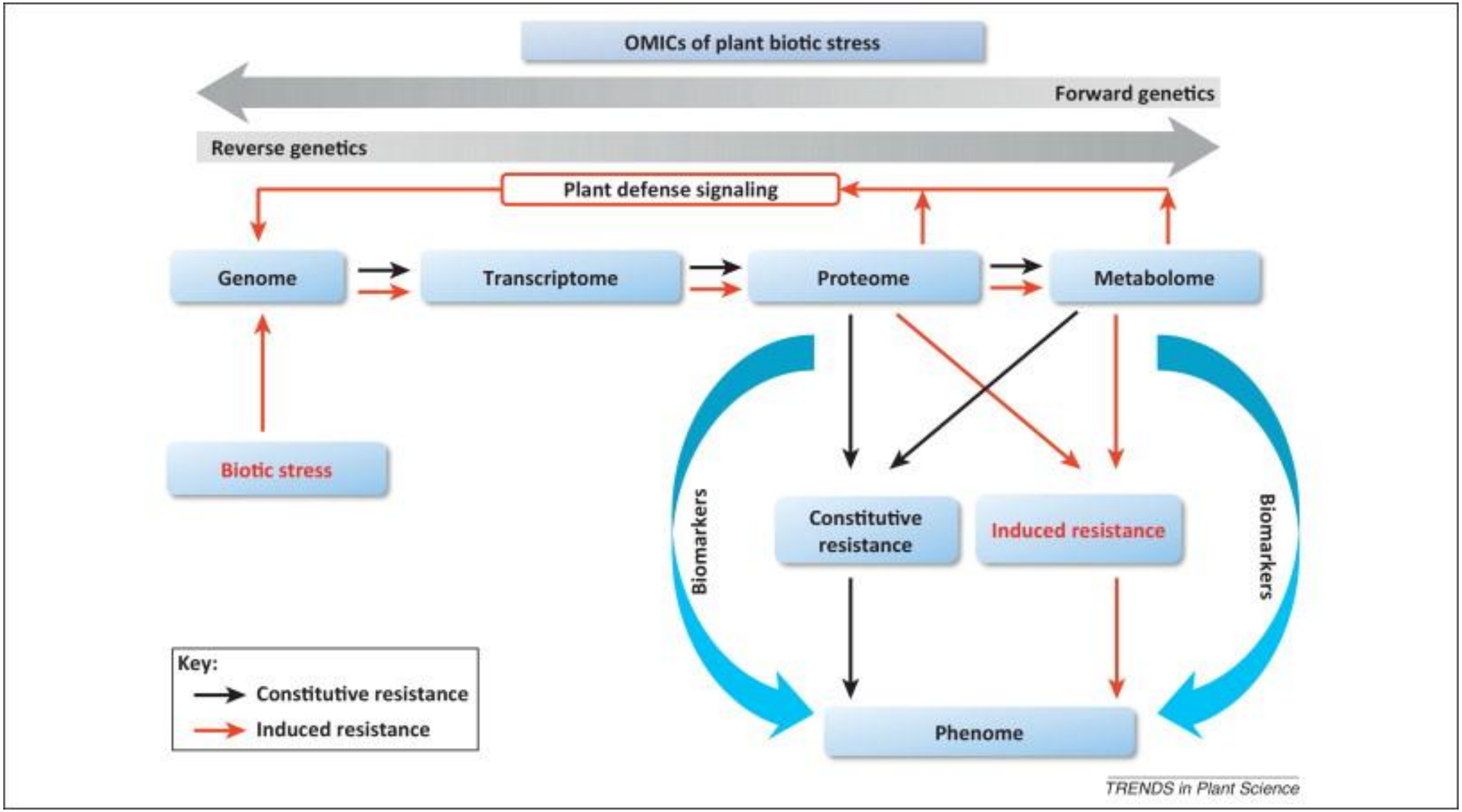
Quantitative Resistance Improvement (durable)

- Step I: Identification of disease resistance QTLs and genes
  - Molecular marker assisted selection (MAS)
  - Genome wide association studies (GWAS)
  - Metabolo-genomics of potato genotypes
- Step II: Gene function elucidation - OMICs
- Step III: Transfer to elite cultivars - Cisgenics

Project Philosophy

We have our own way of life, but we welcome your help to improve our food security

OMICs: Forward and Reverse genetics



Kushalappa and Gunnaiah 2013

Inoculation and incubation

(spot inoculation to reduce expt. error)

Resistance phenotype: Disease severity assessment

Lesion diameter
Area

Lesion length
Area

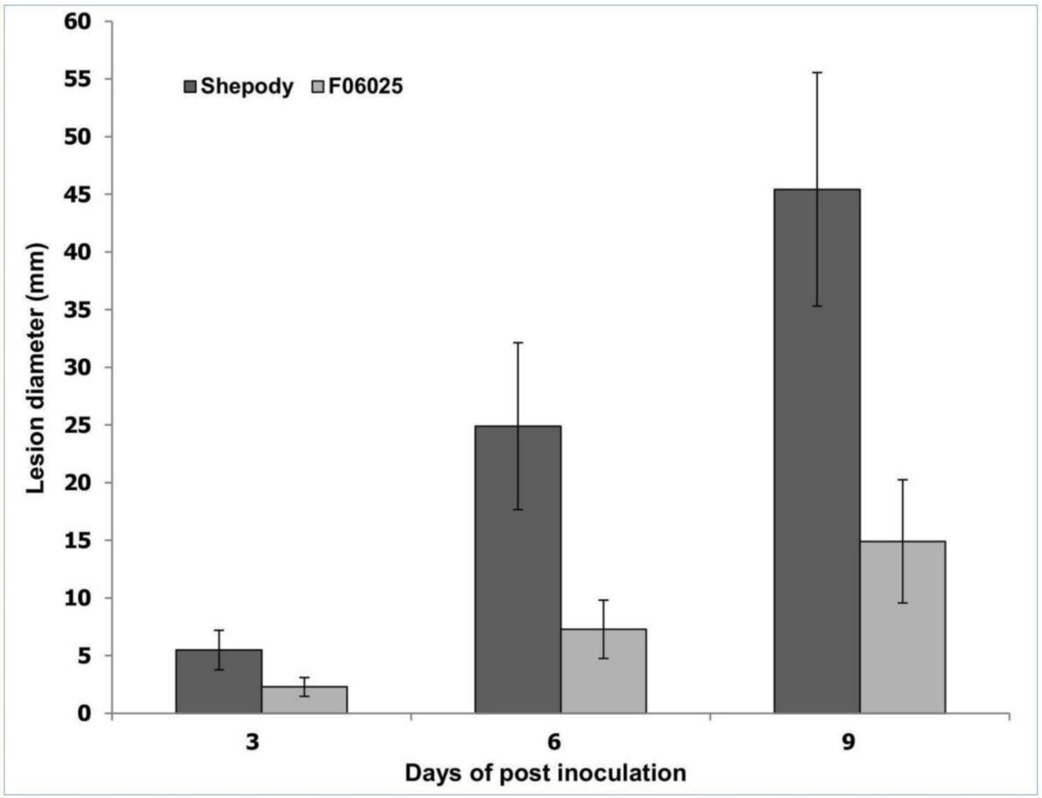
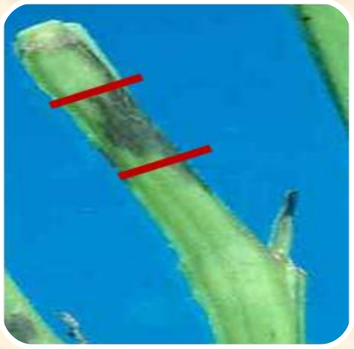


Figure 1. Late blight disease severity progress on resistant (F06025) and susceptible (Shepody) potato genotypes. Disease severity was quantified as lesion diameter (mm), at three day intervals.

Sample collection Metabolomics & proteomics

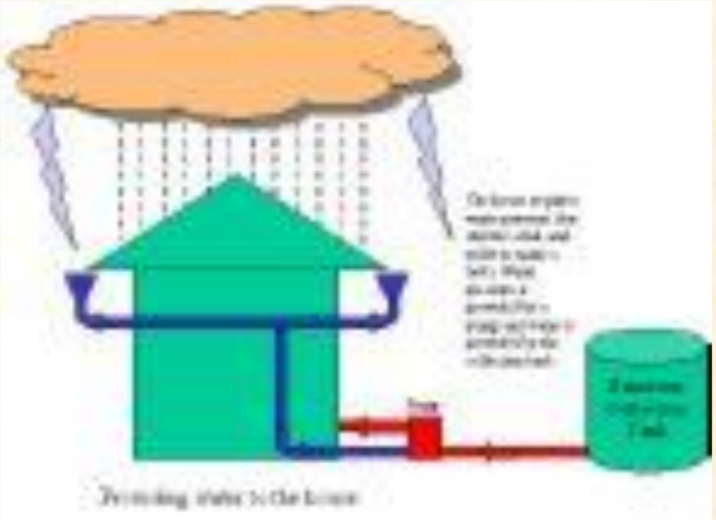
- Leaves & Stems
  - 72 h post inoculation
  - Ground in liquid nitrogen



Extraction - Metabolites

Problems and progress

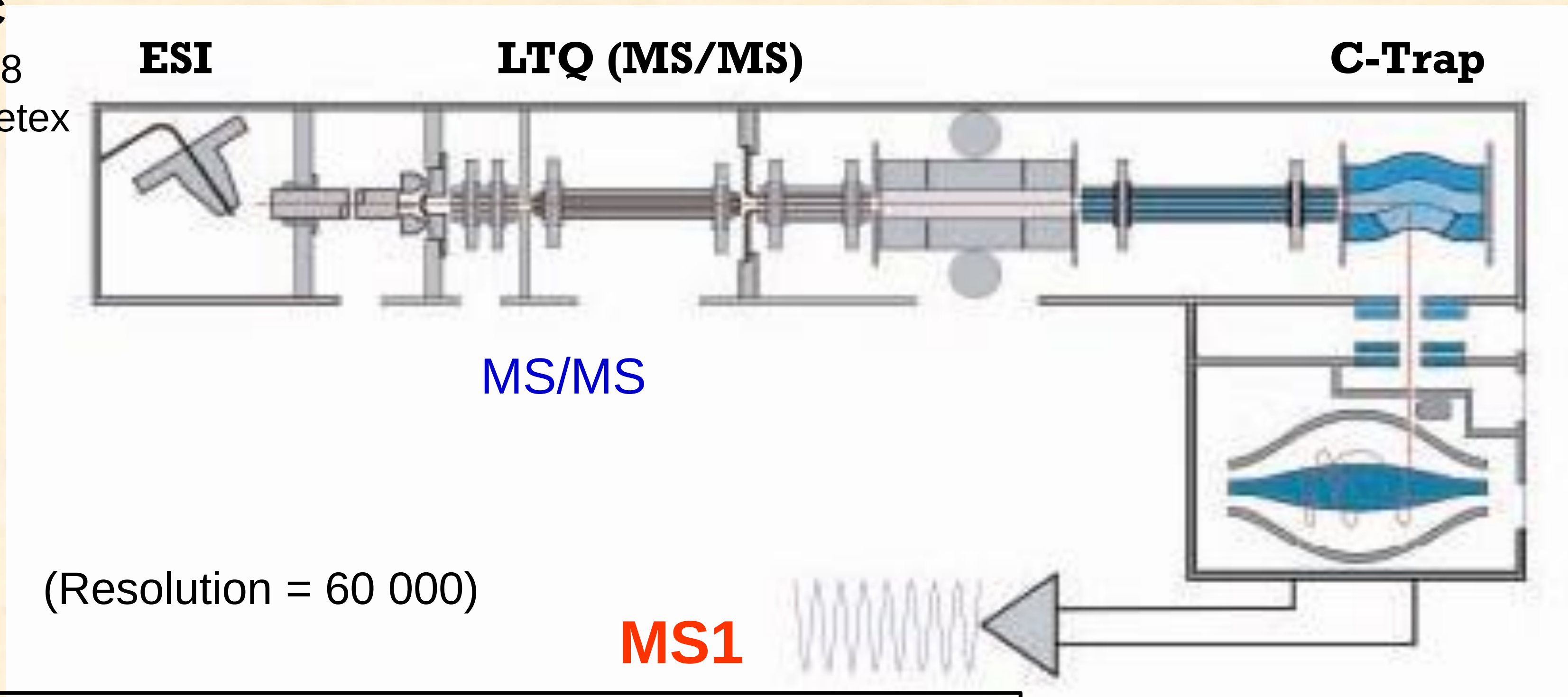
- No single solvent can extract all the metabolites
- Methanol + water
  - Most of the metabolites are extracted (semipolar)



Biochemical analysis: LC-ESI-LTQ-Orbitrap (High resolution mass spectrometer)

LC

C-18
Kinetex



Detects more than 10 000 peaks in one run

Orbitrap (AME < 5 ppm)

Peak deconvolution: dataset: XCMS-Output

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
1	name	fold	mzmed	mzmin	mzmax	rtmed	rtmin	rtmax	npeak	k	v	metlin	LO_20080318	LO_20080:	LO_2008031	LO_20080318_C
2	M89T31	Inf	88.96807	88.96807	88.96807	30.8442	30.8442	30.8442	2	0	2	http://metlin.scripps.edu/metabo_list.php?mass_mi	0	0	5835170.5	5835170.487
3	M98T31	Inf	97.9685	97.9685	97.9685	30.8442	30.8442	30.8442	2	0	2	http://metlin.scripps.edu/metabo_list.php?mass_mi	0	0	1308435	1308435.046
4	M123T11	19.41283	122.9288	122.9288	122.9288	117.6339	117.6339	117.6339	2	2	0	http://metlin.scripps.edu/metabo_list.php?mass_mi	1643182.391	1643182	84644.156	84644.15621
5	M126T15	10.11179	125.986	125.986	125.986	158.6538	158.6538	158.6538	2	2	0	http://metlin.scripps.edu/metabo_list.php?mass_mi	16735064.65	16735065	1655005.7	1655005.671
6	M128T32	46.8373	127.9802	127.9802	127.9802	31.7739	31.7739	31.7739	2	0	2	http://metlin.scripps.edu/metabo_list.php?mass_mi	35814.62955	35814.63	1677460.7	1677460.711
7	M128T11	8.106151	127.9835	127.9835	127.9835	116.7139	116.7139	116.7139	2	2	0	http://metlin.scripps.edu/metabo_list.php?mass_mi	31971952.09	31971952	3944159.4	3944159.366
8	M129T11	14.15418	129.0266	129.0266	129.0266	115.7345	115.7345	115.7345	2	2	0	http://metlin.scripps.edu/metabo_list.php?mass_mi	7678711.564	7678712	542504.91	542504.9092
9	M131T99	5.035987	130.9876	130.9876	130.9876	98.6841	98.6841	98.6841	2	2	0	http://metlin.scripps.edu/metabo_list.php?mass_mi	51958780.93	51958781	10317496	10317496.07
10	M132T11	7.352355	131.9522	131.9522	131.9522	118.5643	118.5643	118.5643	2	2	0	http://metlin.scripps.edu/metabo_list.php?mass_mi	13020646.31	13020646	1770949	1770949.021
11	M132T18	14.70526	132.1017	132.1017	132.1017	184.724	184.724	184.724	2	2	0	http://metlin.scripps.edu/metabo_list.php?mass_mi	119058333.1	1.19E+08	8096311.6	8096311.614
12	M133T11	12.21818	132.9582	132.9582	132.9582	119.4845	119.4845	119.4845	2	2	0	http://metlin.scripps.edu/metabo_list.php?mass_mi	405048230.5	4.05E+08	33151278	33151278.49
13	M134T11	1.744102	133.9597	133.9597	133.9597	118.5643	118.5643	118.5643	2	2	0	http://metlin.scripps.edu/metabo_list.php?mass_mi	7408023.263	7408023	4247471.2	4247471.19
14	M135T12	13.58004	134.9587	134.9587	134.9587	120.3543	120.3543	120.3543	2	2	0	http://metlin.scripps.edu/metabo_list.php?mass_mi	45417675.17	45417675	3344444.2	3344444.161
15	M136T18	14.11849	136.1106	136.1106	136.1106	185.7043	185.7043	185.7043	2	2	0	http://metlin.scripps.edu/metabo_list.php?mass_mi	4511548.371	4511548	319548.96	319548.9644
16	M137T11	38.45625	136.9568	136.9568	136.9568	119.4845	119.4845	119.4845	2	2	0	http://metlin.scripps.edu/metabo_list.php?mass_mi	16241208.13	16241208	422329.48	422329.4752
17	M137T31	1.386795	136.9608	136.9608	136.9608	30.8442	30.8442	30.8442	2	0	2	http://metlin.scripps.edu/metabo_list.php?mass_mi	2215903.198	2215903	3073004.2	3073004.225
18	M138T18	28.88862	138.0548	138.0548	138.0548	185.7043	185.7043	185.7043	2	2	0	http://metlin.scripps.edu/metabo_list.php?mass_mi	9373485.271	9373485	324469.83	324469.8267
19	M138T82	1.294953	138.066	138.066	138.066	81.584	81.584	81.584	2	2	0	http://metlin.scripps.edu/metabo_list.php?mass_mi	4066823.899	4066824	3140517.8	3140517.793
20	M140T11	58.03697	139.9791	139.9791	139.9791	112.9439	112.9439	112.9439	2	2	0	http://metlin.scripps.edu/metabo_list.php?mass_mi	14162833.82	14162834	244031.24	244031.2434
21	M140T18	7.409712	140.0681	140.0681	140.0681	183.8639	183.8639	183.8639	2	2	0	http://metlin.scripps.edu/metabo_list.php?mass_mi	34348852.04	34348852	4635652.7	4635652.656
22	M142T30	128.3109	141.9584	141.9584	141.9584	29.904	29.904	29.904	2	0	2	http://metlin.scripps.edu/metabo_list.php?mass_mi	102354.5808	102354.6	13133205	13133204.9
23	M142T11	86.46465	141.9586	141.9586	141.9586	115.7345	115.7345	115.7345	2	2	0	http://metlin.scripps.edu/metabo_list.php?mass_mi	165717866.2	1.66E+08	1916596.7	1916596.709
24	M144T11	47.51712	143.959	143.959	143.959	115.7345	115.7345	115.7345	2	2	0	http://metlin.scripps.edu/metabo_list.php?mass_mi	22262860.73	22262861	468522.93	468522.9298
25	M144T32	7.702688	143.9734	143.9734	143.9734	31.7739	31.7739	31.7739	2	0	2	http://metlin.scripps.edu/metabo_list.php?mass_mi	364312.2249	364312.2	2806183.4	2806183.388
26	M146T11	68.84263	145.9654	145.9654	145.9654	113.9339	113.9339	113.9339	2	2	0	http://metlin.scripps.edu/metabo_list.php?mass_mi	13152168.12	13152168	191046.85	191046.8519
27	M147T11	11.82794	146.9747	146.9747	146.9747	116.7139	116.7139	116.7139	2	2	0	http://metlin.scripps.edu/metabo_list.php?mass_mi	101670753.2	1.02E+08	8595809.3	8595809.319
28	M148T11	12.98288	147.9811	147.9811	147.9811	116.7139	116.7139	116.7139	2	2	0	http://metlin.scripps.edu/metabo_list.php?mass_mi	3244844.729	3244845	249932.64	249932.6428
29	M150T11	38.00306	149.9973	149.9973	149.9973	118.5643	118.5643	118.5643	2	2	0	http://metlin.scripps.edu/metabo_list.php?mass_mi	37074571.15	37074571	975568.01	975568.01
30	M151T11	13.65674	150.9699	150.9699	150.9699	116.7139	116.7139	116.7139	2	2	0	http://metlin.scripps.edu/metabo_list.php?mass_mi	2914071.412	2914071	213379.8	213379.7974
31	M153T11	33.62651	152.9491	152.9491	152.9491	113.9339	113.9339	113.9339	2	2	0	http://metlin.scripps.edu/metabo_list.php?mass_mi	1789179.352	1789179	53207.413	53207.41333
32	M154T11	8.172404	153.9818	153.9818	153.9818	112.0641	112.0641	112.0641	2	2	0	http://metlin.scripps.edu/metabo_list.php?mass_mi	3208394.283	3208394	392588.79	392588.7876
33	M155T83	2.061696	154.9778	154.9778	154.9778	82.554	82.554	82.554	2	2	0	http://metlin.scripps.edu/metabo_list.php?mass_mi	6678562.075	6678562	3239353.7	3239353.685
34	M156T18	412.3029	156.0421	156.0421	156.0421	185.7043	185.7043	185.7043	2	2	0	http://metlin.scripps.edu/metabo_list.php?mass_mi	210461120.8	2.1E+08	510452.73	510452.7345
35	M156T45	134.0888	156.0507	156.0507	156.0507	44.714	44.714	44.714	2	0	2	http://metlin.scripps.edu/metabo_list.php?mass_mi	720346.456	720346.5	96590375	96590374.92
36	M157T18	8.528035	157.0453	157.0453	157.0453	185.7043	185.7043	185.7043	2	2	0	http://metlin.scripps.edu/metabo_list.php?mass_mi	7868813.169	7868813	922699.46	922699.4613
37	M158T18	147.2994	158.0401	158.0401	158.0401	186.5639	186.5639	186.5639	2	2	0	http://metlin.scripps.edu/metabo_list.php?mass_mi	12119022.47	12119022	82274.736	82274.73625
38	M158T45	16.71503	158.0513	158.0513	158.0513	44.714	44.714	44.714	2	0	2	http://metlin.scripps.edu/metabo_list.php?mass_mi	288874.2012	288874.2	4828542.3	4828542.323
39	M159T97	13.075	158.9849	158.9849	158.9849	96.8038	96.8038	96.8038	2	2	0	http://metlin.scripps.edu/metabo_list.php?mass_mi	25318502.71	25318503	1936405.7	1936405.718
40	M160T95	20.62193	159.973	159.973	159.973	94.8837	94.8837	94.8837	2	2	0	http://metlin.scripps.edu/metabo_list.php?mass_mi	11516549.62	11516550	558461.37	558461.3741
41	M161T10	9.546419	161.0121	161.0121	161.0121	99.6238	99.6238	99.6238	2	2	0	http://metlin.scripps.edu/metabo_list.php?mass_mi	16258445.31	16258445	1703093.6	1703093.619
42	M163T27	5.834003	162.9687	162.9687	162.9687	27.0839	27.0839	27.0839	2	0	2	http://metlin.scripps.edu/metabo_list.php?mass_mi	1340470.424	1340470	7820308.5	7820308.536
43	M163T11	36.1406	162.9691	162.9691	162.9691	118.5643	118.5643	118.5643	2	2	0	http://metlin.scripps.edu/metabo_list.php?mass_mi	43373521.05	43373521	1200132.8	1200132.785
44	M164T11	32.13913	163.9766	163.9766	163.9766	118.5643	118.5643	118.5643	2	2	0	http://metlin.scripps.edu/metabo_list.php?mass_mi	36166087.22	36166087	1125297.5	1125297.48
45	M164T18	78.44214	164.0739	164.0739	164.0739	188.4542	188.4542	188.4542	2	2	0	http://metlin.scripps.edu/metabo_list.php?mass_mi	7097484.758	7097485	90480.507	90480.50667

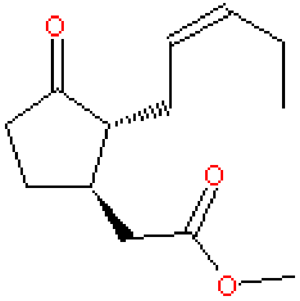
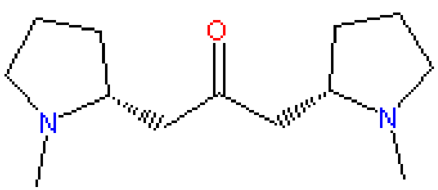
example

Information extraction: Metabolite ID (Based on MS1)

METLIN

Metabolites

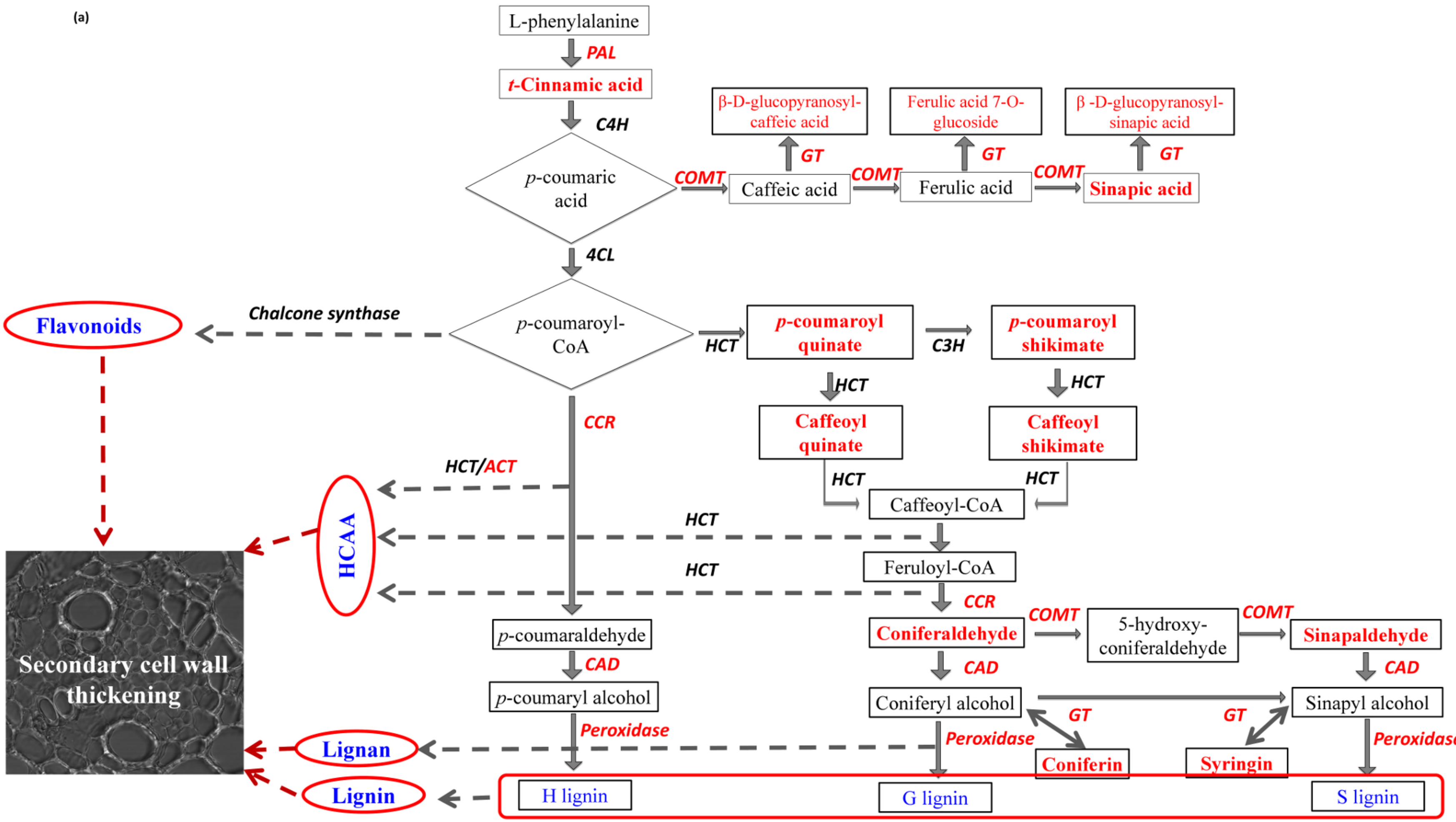
(Metabolites 1-24 of 24) Change Query

MID	Mass	Name	Formula	CAS	KEGG	Structure
3361	224.1412	Methyl jasmonate	$C_{13}H_{20}O_3$	1211-29-6	C11512	
3362	224.1889	Cuscohygrine	$C_{13}H_{24}N_2O$	454-14-8	C06521	

Resistance related constitutive and induced metabolites

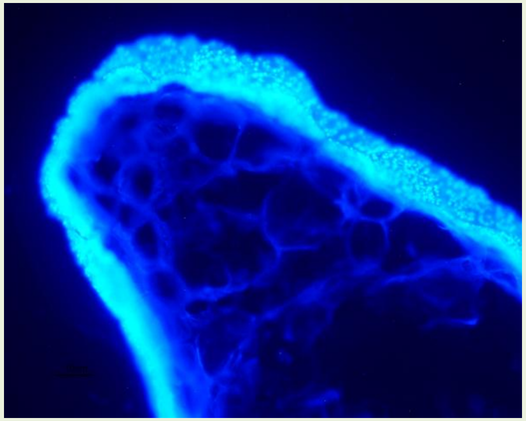
- **Statistical analysis: *t*-test**
- **Fold change in resistant genotype relative to susceptible genotype**
- **RRC = RM/SM**
- **RRI = (RP/RM)/(SP/SM)**

Cell wall thickness – prevent pathogen spread

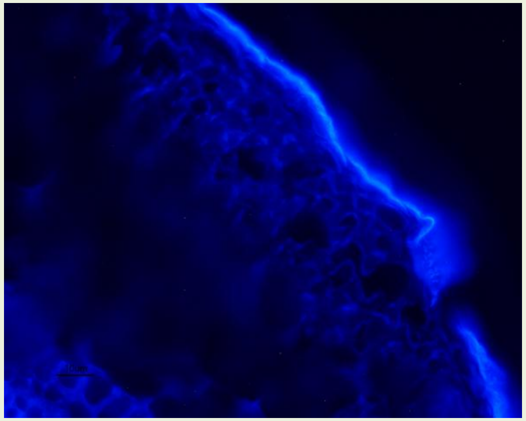


RR metabolite
Cell wall thickening

Hydroxycinnamic acid amides (HCAAs) = high fold change

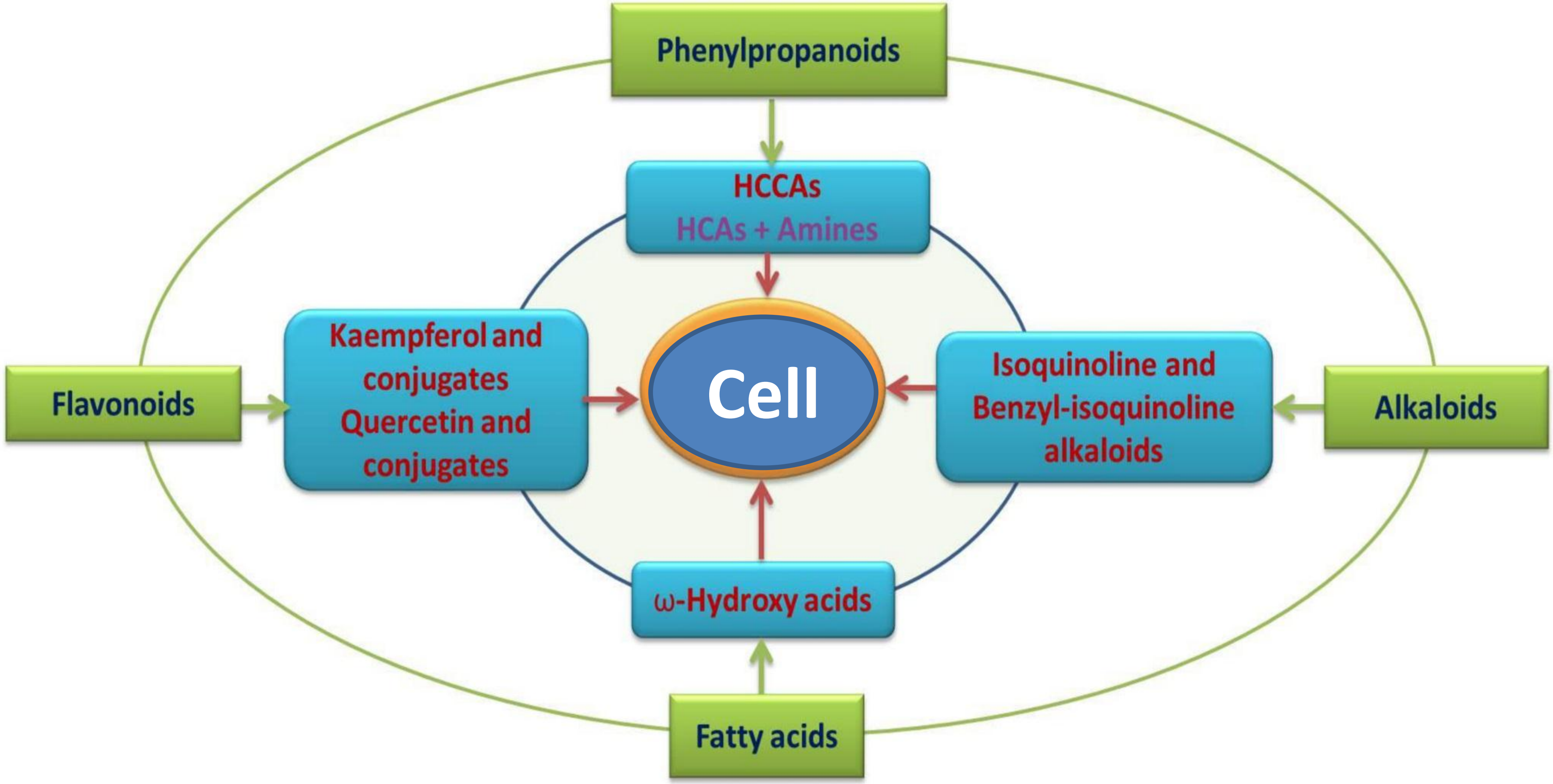


Resistant cultivar
Pathogen inoculated



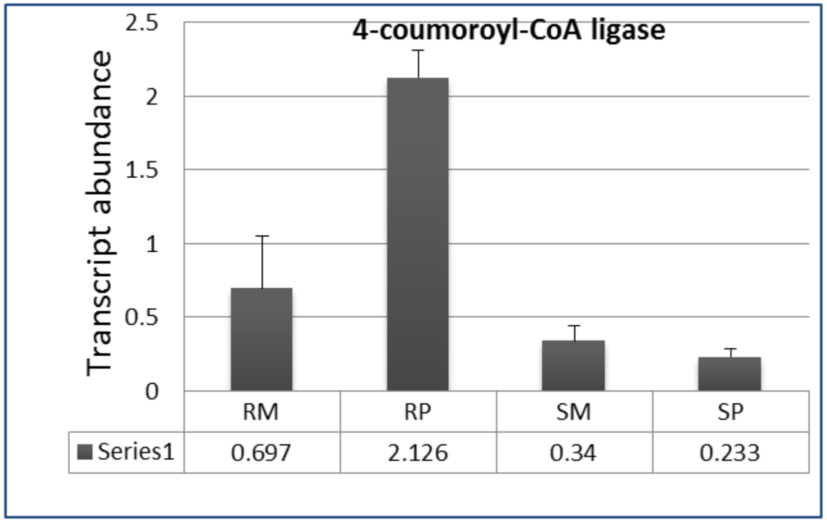
Susceptible cultivar
Pathogen inoculated

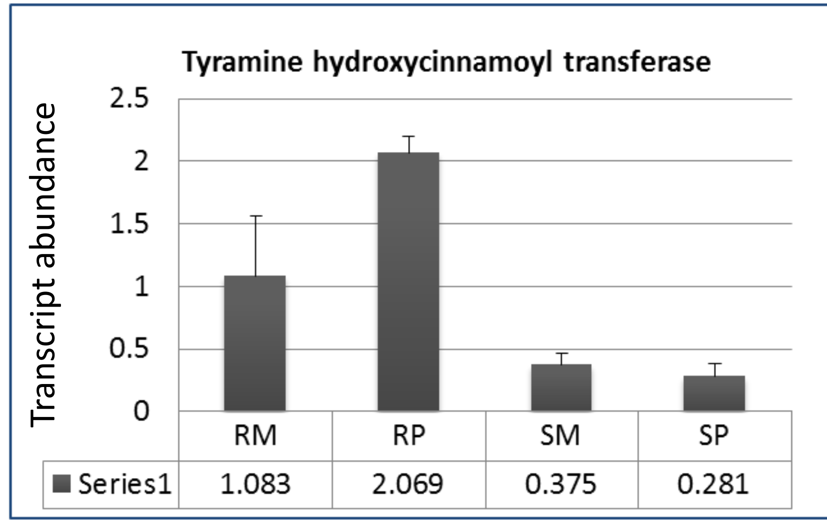
Cell wall thickening

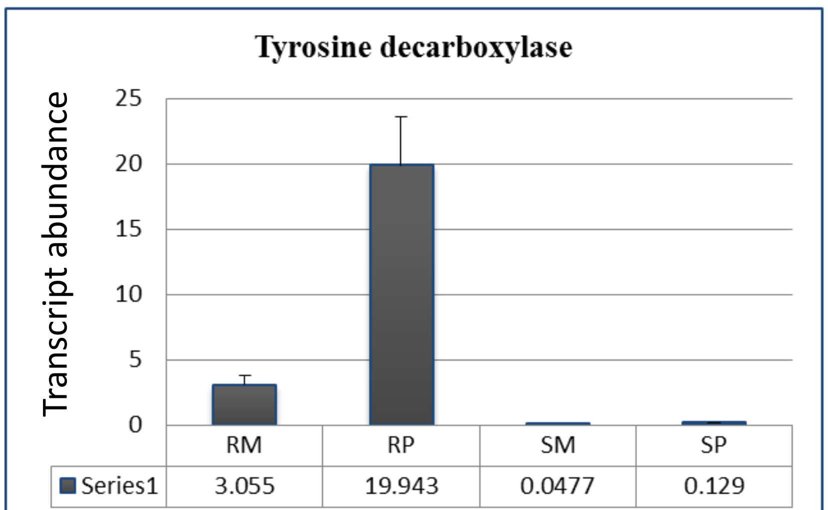


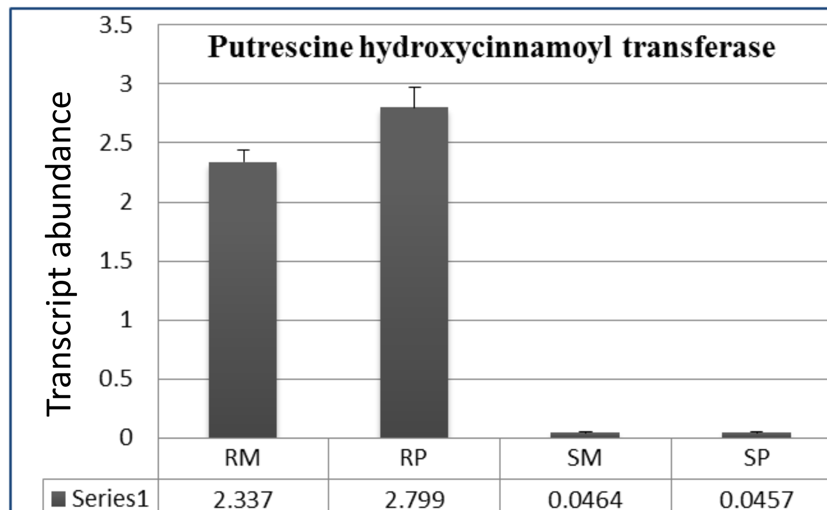
Multiple disease/pest resistance?

Candidate genes involved in Cell wall thickening

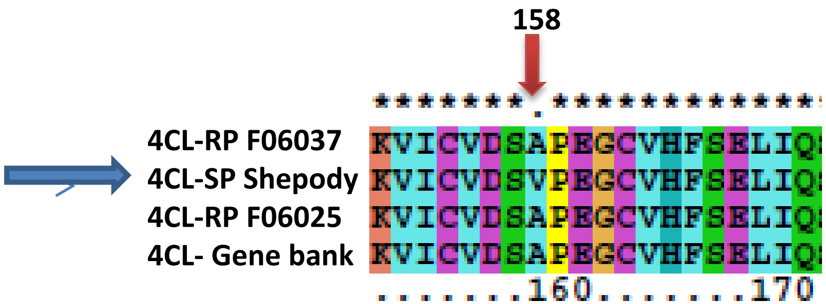


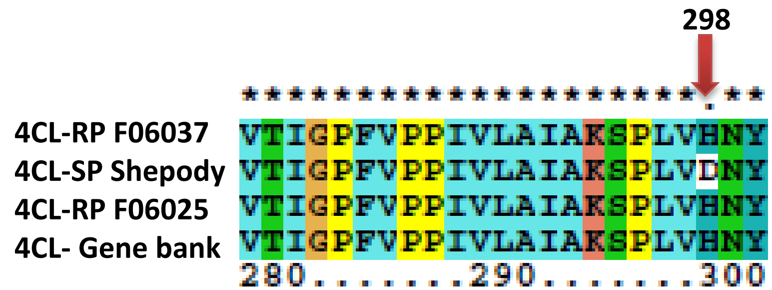






Amino acid substitution leads to instability of 4-coumaryl ligase protein





Substitution of Alanine (A) with Valine (V) at position 158 leads to decrease in protein stability by ΔΔG= -0.80 (negative value)

Substitution of Histidine (H) with Aspartic acid (D) at position 298 leads to decrease in protein stability by ΔΔG= -0.70

Where as,

ΔΔG- Free energy

ΔΔG: ΔG(New Protein)-ΔG(Wild Type) in Kcal/mol

ΔΔG<0: Decrease Stability

ΔΔG>0: Increase Stability

(Capriotti et al. 2006)

Gene resistance function validation based on VIGS

RR Gene not silenced

RR Gene silenced

RR Metabolomics

Disease severity

Pathogen biomass

Resistant

Susceptible

Knowledge application: Plant resistance improvement

- Transfer of RR genes to elite cultivars
  - Marker Assisted Breeding
  - Cisgenics (gene transfer between sexually compatible genotypes)

Second phase?

Cisgenic and transgenic to improve potato resistance to late blight

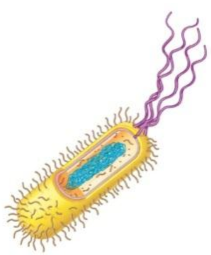
Wild potato

Gene replacement

Elite potato cultivar

Tissue culture Potato plant

Cisgenic potato plants



Bacteria or Other organisms

(http://www.glogster.com)

Late blight resistance gene

Elite potato cultivar

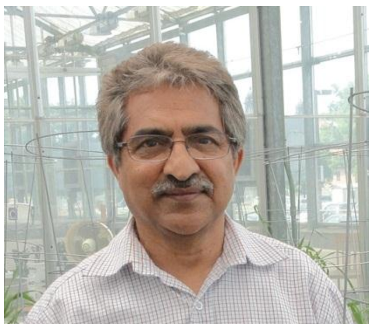
Tissue culture Potato plant

Transgenic potato plants

Thank you all

FACE BOOK

McGill University



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Pushpa

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