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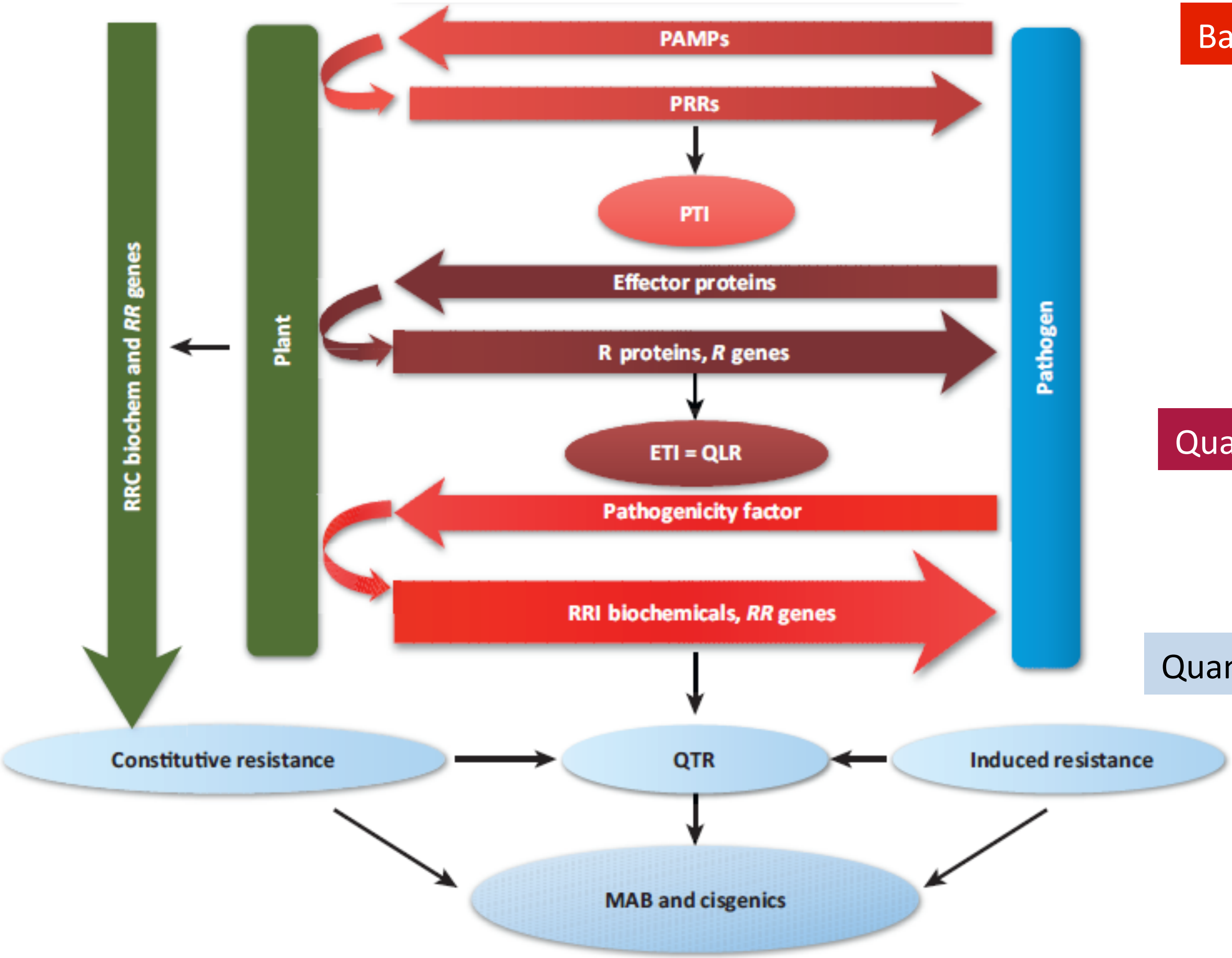
Novel candidate genes for quantitative resistance to late blight in potato

Teresa Mosquera-Vásquez, María Fernanda Álvarez, José M. Jiménez-Gómez,
Astrid Draffehn, Christiane Gebhardt

Types of genetic resistance identified



TRENDS in Plant Science

Basal resistance

Qualitative resistance

Quantitative resistance

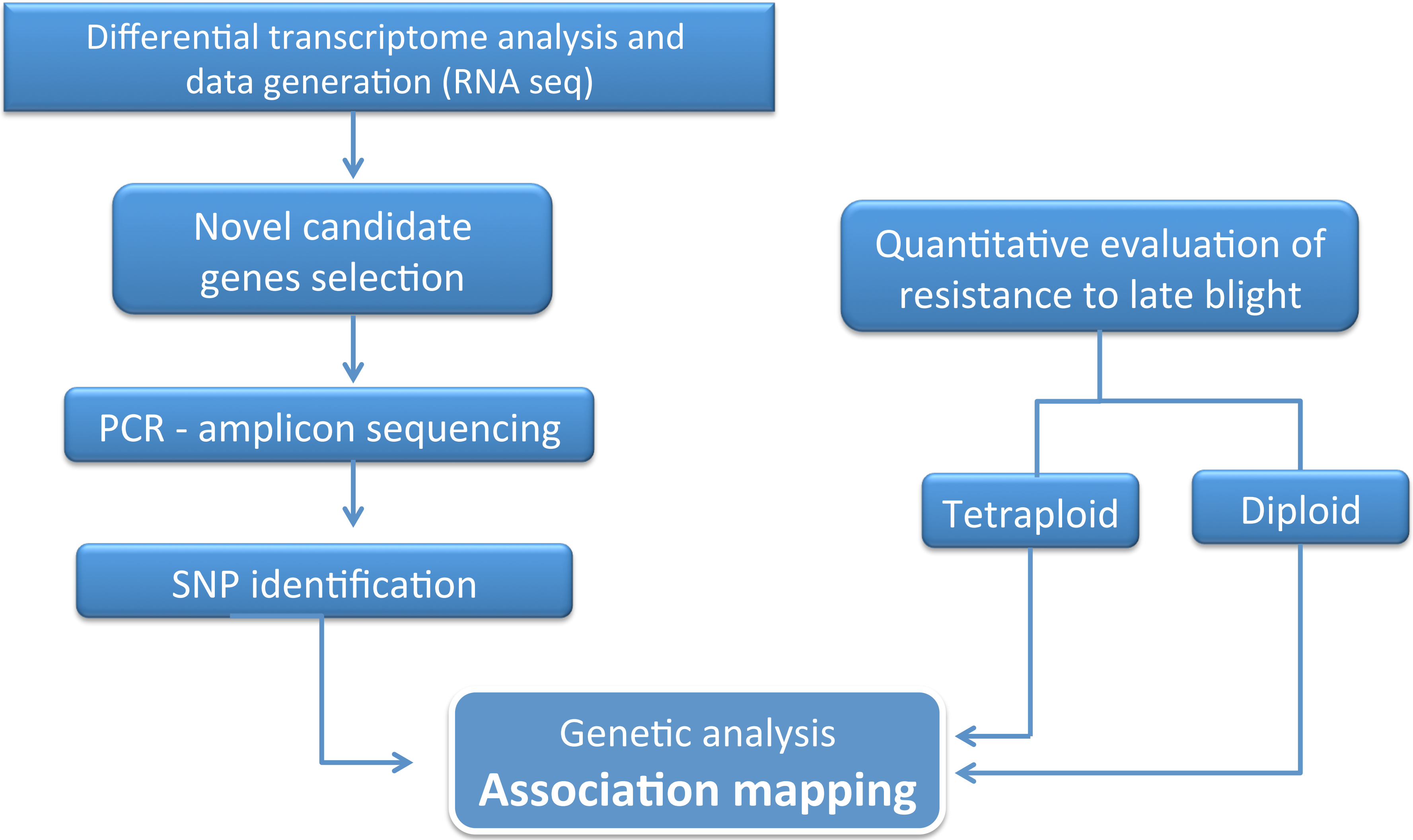
Ajjamada C. Kushalappa and Raghavendra Gunnaiah,
Trends in Plant Science XX (2013) 1-10

Objectives

1. To select novel candidate genes for quantitative resistance to late blight from genes showing high level of polymorphism in differential transcriptome analysis.

2. To validate the identified allelic variants for association with resistance to late blight in diploid and tetraploid germplasm.

Methodology



Differential transcriptome analysis and data generation (RNA seq)

Novel candidate genes selection

PCR - amplicon sequencing

SNP identification

Quantitative evaluation of resistance to late blight

Tetraploid

Diploid

Genetic analysis

Association mapping

Plant material

Diploid genotypes
(Colombian germplasm)

Colombian Core Collection: *Solanum phureja*

Tetraploid genotypes
(German germplasm)

Evaluation for resistance to late blight:

AUDPC and Maturity Corrected Resistance (MCR)

Late blight evaluation under field conditions

Methodology

Astrid Draffehn

José Jiménez-Gómez

Differential transcriptome analysis and data generation (RNA seq)

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Quantitative evaluation of resistance to late blight

Tetraploid

Diploid

Genetic analysis

Association mapping

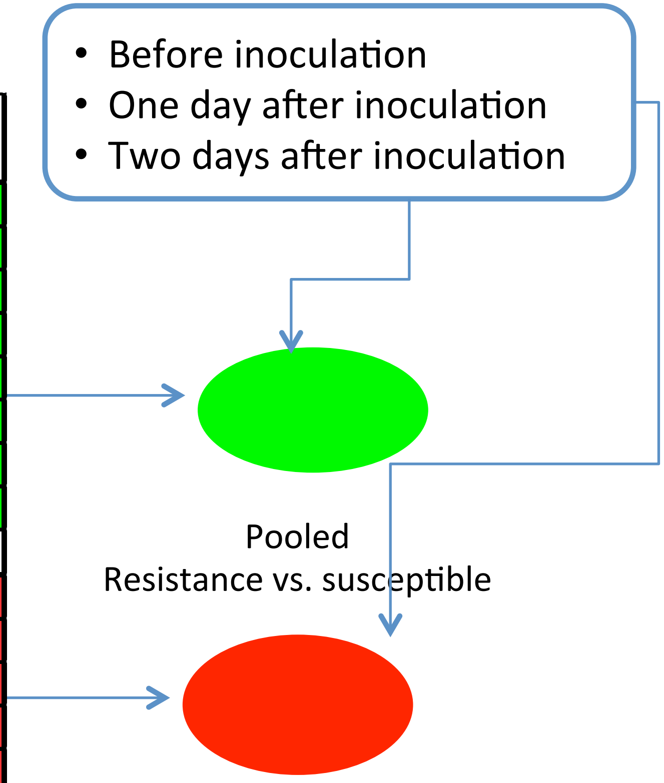
María Fernanda Álvarez

Differential transcriptome analysis (RNA seq experiment)

StAOS$_2$ *Allene Oxide Synthase 2*

Eight resistant and eight susceptible genotypes were selected

Genotype	Family	StAOS2 SNP691	StAOS2 SNP692	rAUDPC mean	Pool
BL114	BNA16 x BNA20	AAAA	CCCC	0.24909	R8
BL196	BNA16 x BNA20	AAAG	CCCG	0.36605	R8
BL201	BNA16 x BNA20	AAAG	CCCG	0.31999	R8
BL426	BNA40 X BNA22	AAAG	CCCG	0.24014	R8
BL499	BNA40 X BNA22	AAAG	CCCG	0.24384	R8
SL155	PapPhy20 x PapPhy14	AAAA	CCCC	0.33	R8
SL312	PapPhy20 x PapPhy16	AAAA	CCCC	0.23	R8
SL194	PapPhy20 x PapPhy14	AAAA	CCCC	0.465	R8
				0.30551	
BL013	BNA16 x BNA20	GGGG	GGGG	0.53493	S8
BL024	BNA16 x BNA20	AGGG	CGGG	0.5308	S8
BL141	BNA16 x BNA20	AGGG	CGGG	0.4886	S8
BL238	BNA16 x BNA20	AGGG	CGGG	0.554	S8
SL317	PapPhy20 x PapPhy16	AGGG	CGGG	0.53	S8
SL316	PapPhy20 x PapPhy16	AGGG	CGGG	0.615	S8
SL314	PapPhy20 x PapPhy16	AGGG	CGGG	0.62	S8
SL433	PapPhy20 x PapPhy16	AGGG	CGGG	0.595	S8
				0.55854	



Three time points:

- Before inoculation
- One day after inoculation
- Two days after inoculation

Pooled
Resistance vs. susceptible

454 Sequencing

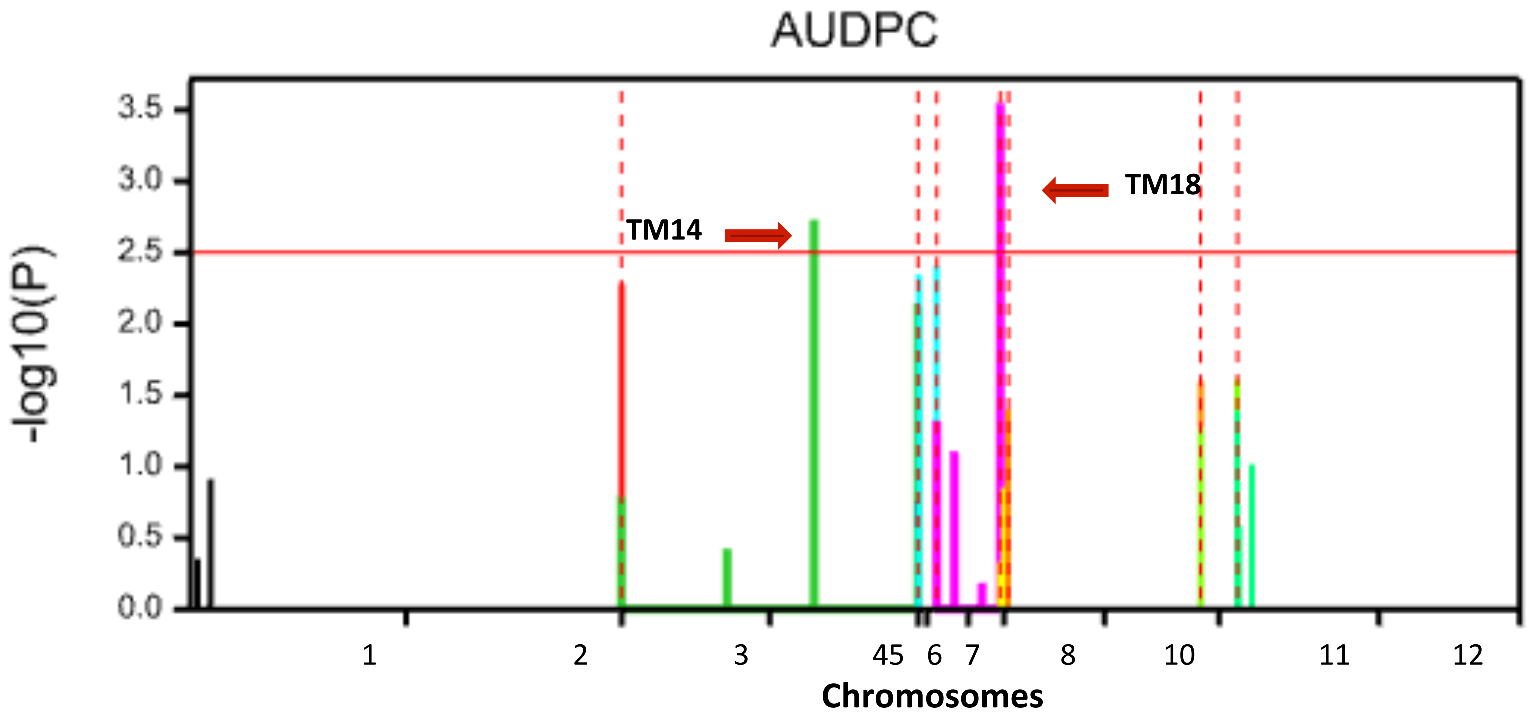
Normalized cDNA library

GenXPro

Criteria for candidate genes selection for quantitative resistance to late blight

- Genes that co-localize in the genetic map with QTL for resistance to late blight.
- Genes that are involved in fundamental pathways and participate in different regulations that might influence the defense response.
- Genes showing at least two SNPs (haplotype) that differentiate (significantly) resistant from susceptible genotypes.
- Genes across the 12 potato chromosomes.

SNPs associated to quantitative resistance to late blight in diploid potatoes



Chr.	SNP Identification	-log10(P)	Effect
3	TM14 SNP61	2.6	10.5%
3	TM14 SNP199	2.7	10.5%
3	TM14 SNP211	2.6	10.5%
6	TM18 SNP88	3.5	13%
6	TM18 SNP108	3.5	13%

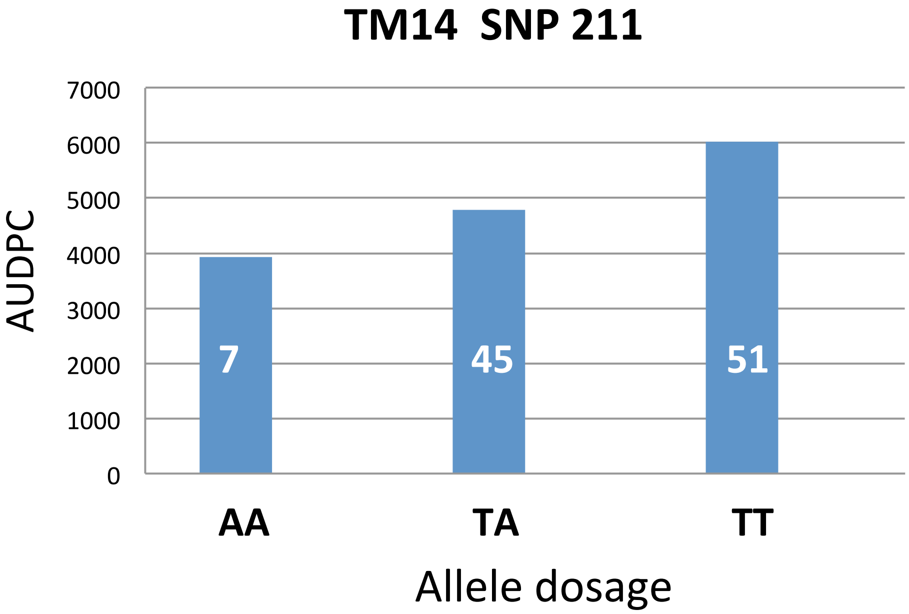
●	●	●	●	●	●	●	●
1	**2**	**3**	**4**	**6**	**7**	**8**	**10**

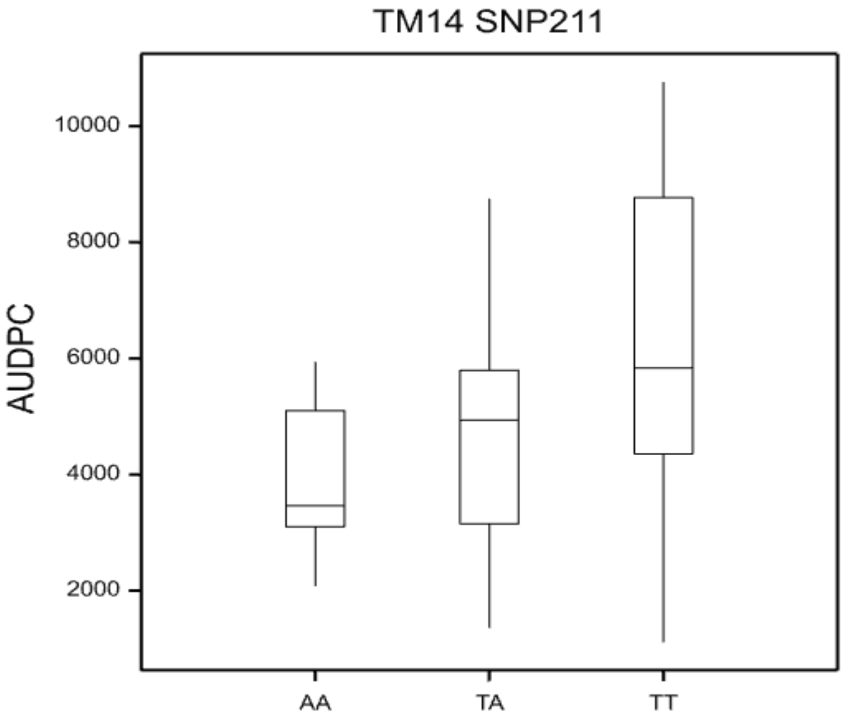
chromosomes

Quantitative resistance and allele dosage

TMV - induced protein I

Chromosome 3, SNP 211: **CATC(T/A)GAAG**

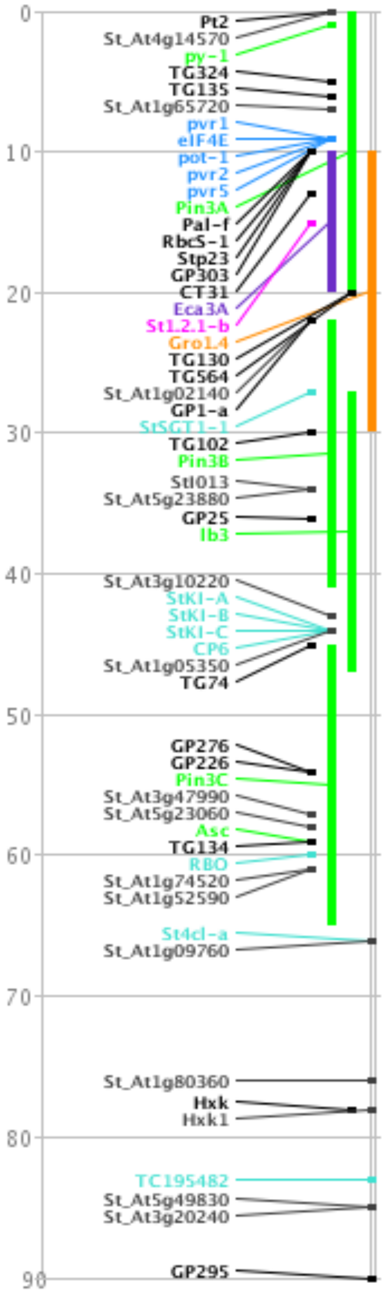




Allele dosage

p-value 0.009

Novel candidate gene validated for quantitative resistance to late blight in diploid potatoes



17.220.914 bp

TMV- induced protein 1

29.900.189 bp

53.228.703 bp

Chromosome III

🟩 QTL for late blight resistance

SNP 211 TMV – induced protein I:

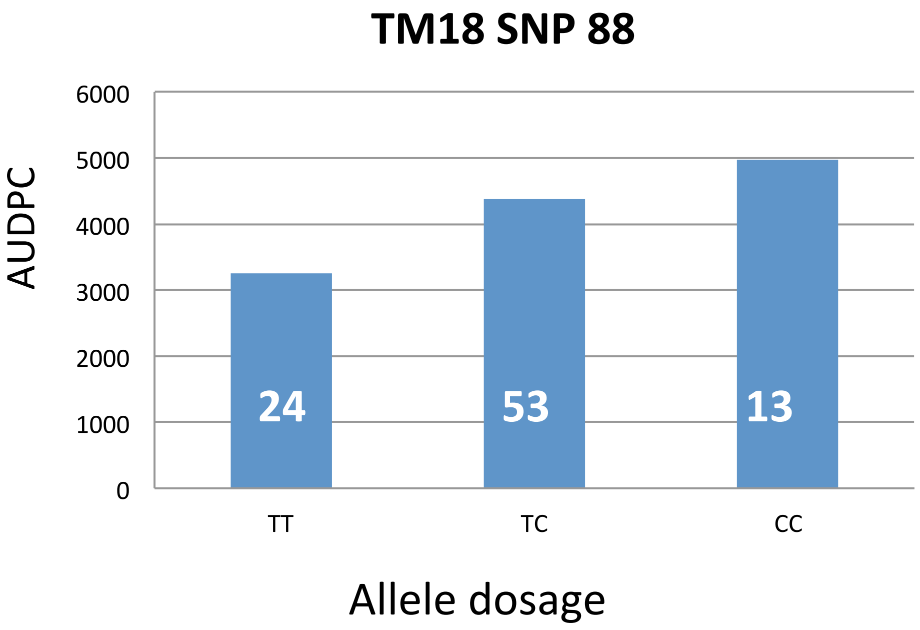
- has a lypoxigenase domain.
- is found in a variety of membrane or lipid associated proteins.
- is a protein binding domain.

PoMaMo database
http://www.gabipd.org/projects/Pomamo/

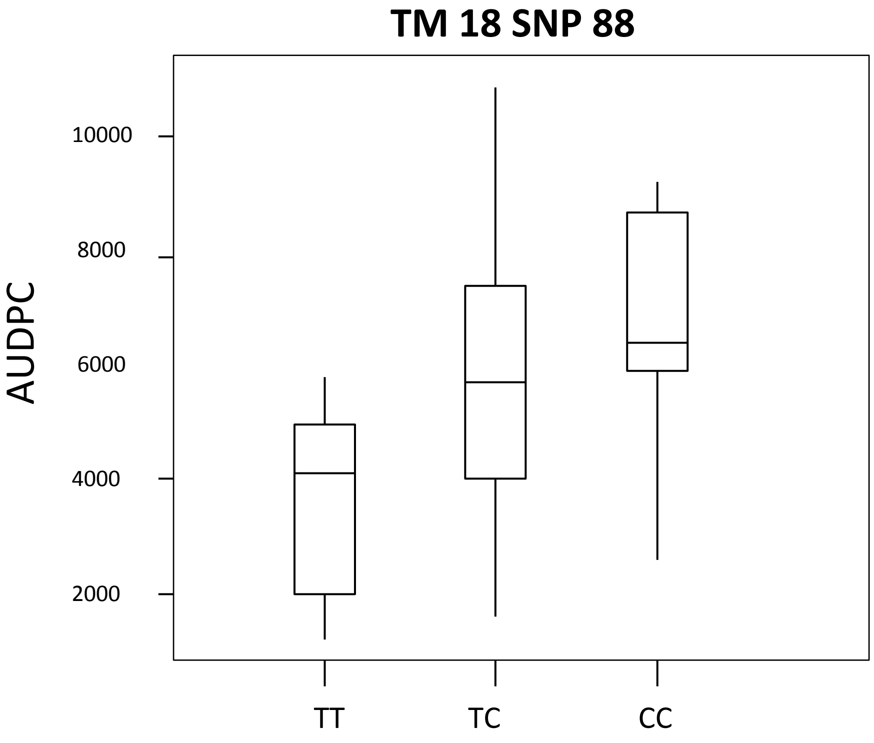
Quantitative resistance and allele dosage

Thylakoid lumenal 15 kDa protein 1, chloroplastic

Chromosome 6, SNP 88: **CCTT(T/C)CCT**



TM18 SNP 88

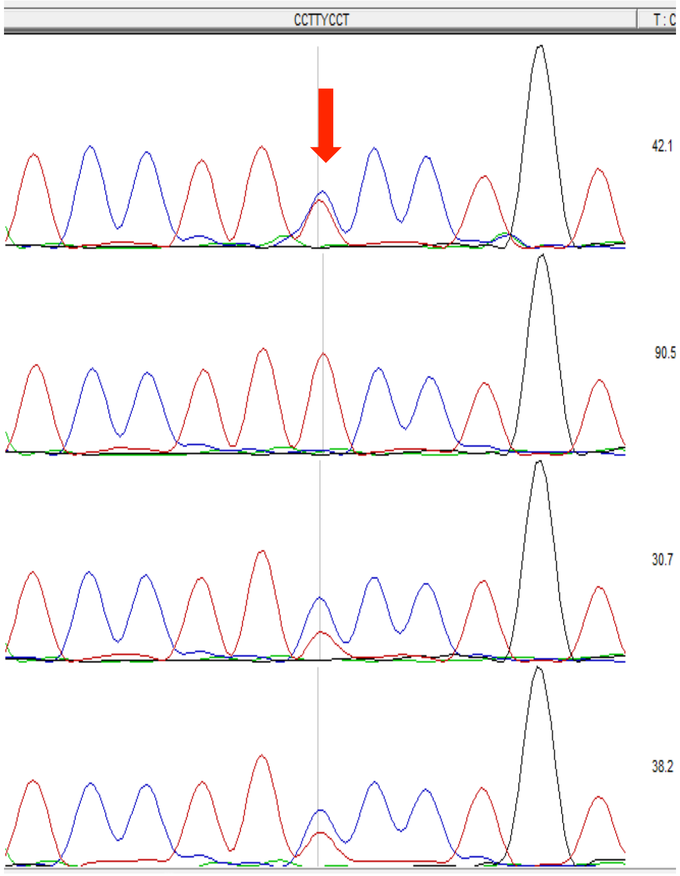


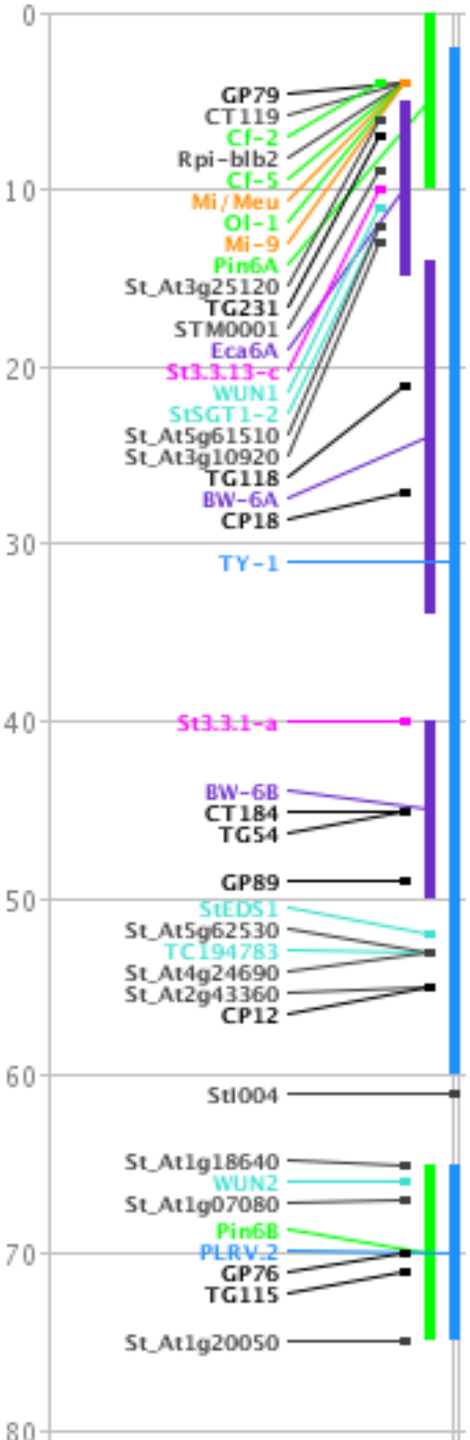
TM 18 SNP 88

p-value <.001

Novel candidate gene validated for quantitative resistance to late blight in diploid potatoes

Phu TM18 CCTT(TC)CCTG p.88





Chromosome VI

🟩 QTL for late blight resistance

50.394.411 bp

Thylakoid lumenal 15 kDa
52.355.675 bp

58.913.746 bp

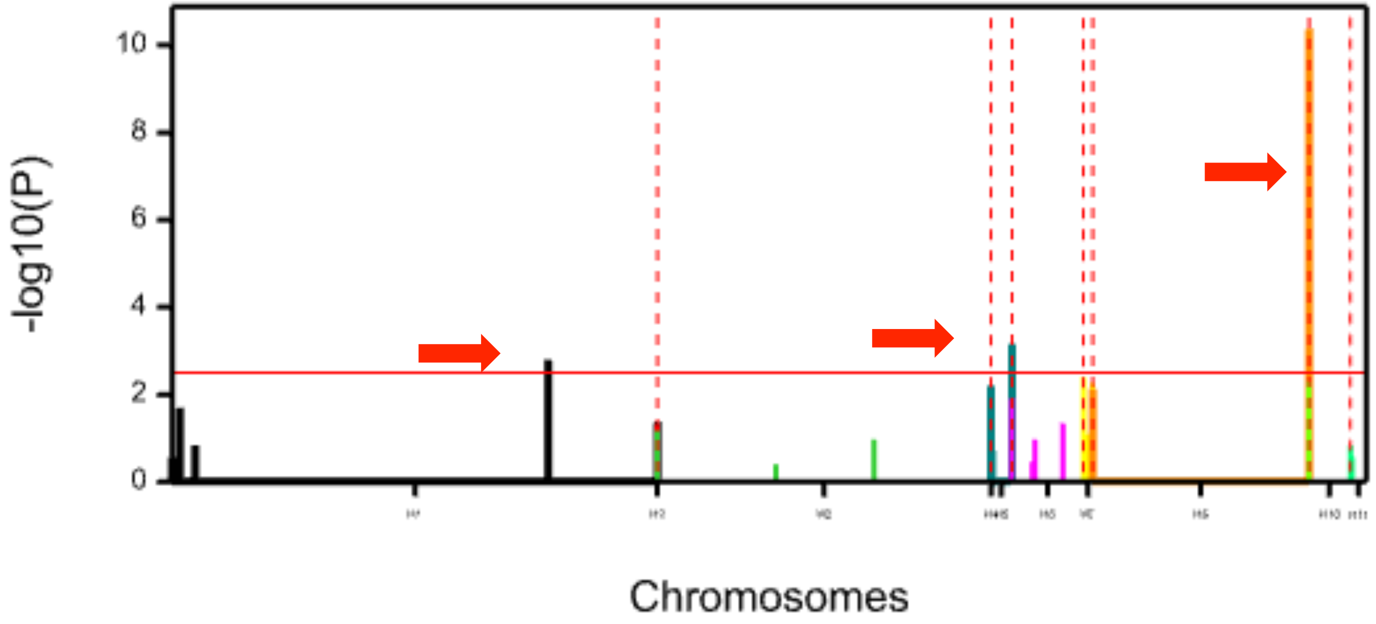
PoMaMo database
http://www.gabipd.org/projects/Pomamo/

Thylakoid lumenal 15 kDa protein 1, chloroplastic:

- is involved in light dependent reactions.
- has a tetratricopeptide repeat (TPR)-like.
- presents protein – protein interaction.

SNPs associated to quantitative resistance to late blight in tetraploid potatoes

MCR : maturity corrected resistance



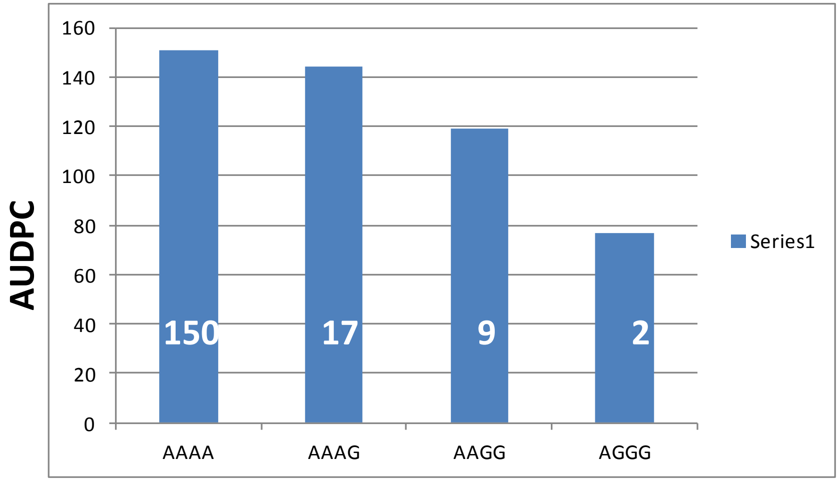
● ● ● ● ● ● ● ●
1 2 3 5 6 7 8 10
chromosomes

Chr.	SNP Identification	-log10(P)	Effect
1	MF22 SNP 8	2.7	15%
5	TM35 SNP 14	3.1	11.5%
8	TM27 SNP 505	5.3	12.5%
8	TM27 SNP 548	10.3	14.5%

Quantitative resistance and allele dosage

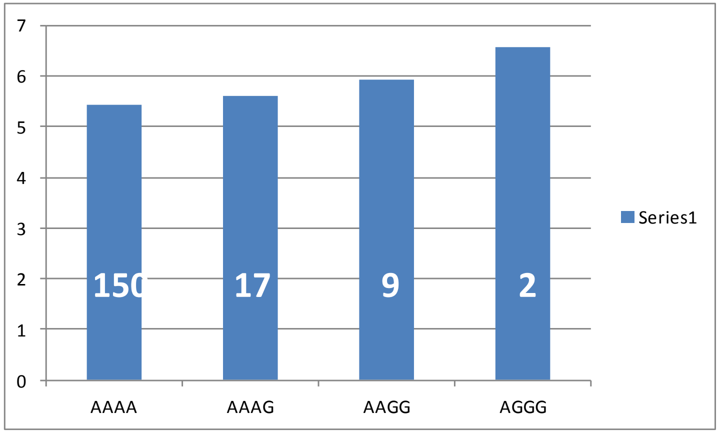
Cytochrome P450 71D11

Chromosome 8, SNP 505: **CTGA(A/G)GTAT**



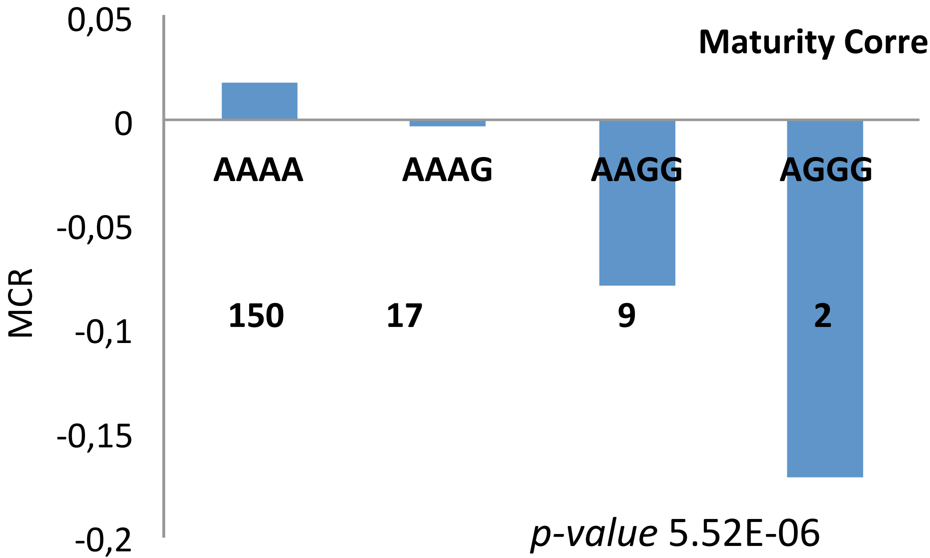
P-value **0.000346780**

Maturity



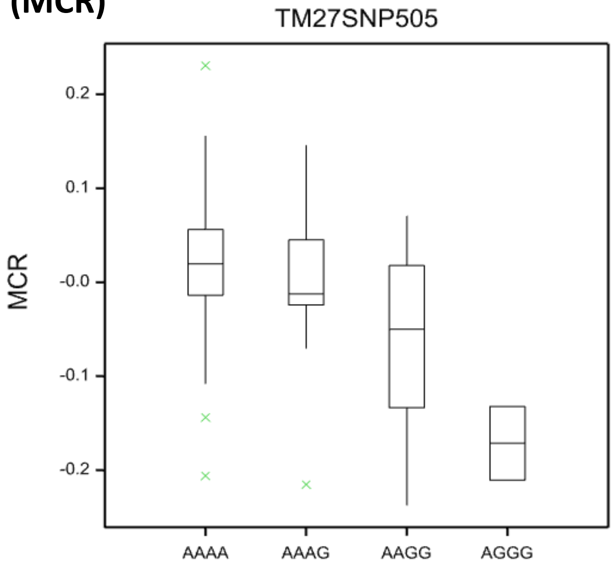
P-value **0.44971309**

Maturity Corrected Resistance (MCR)

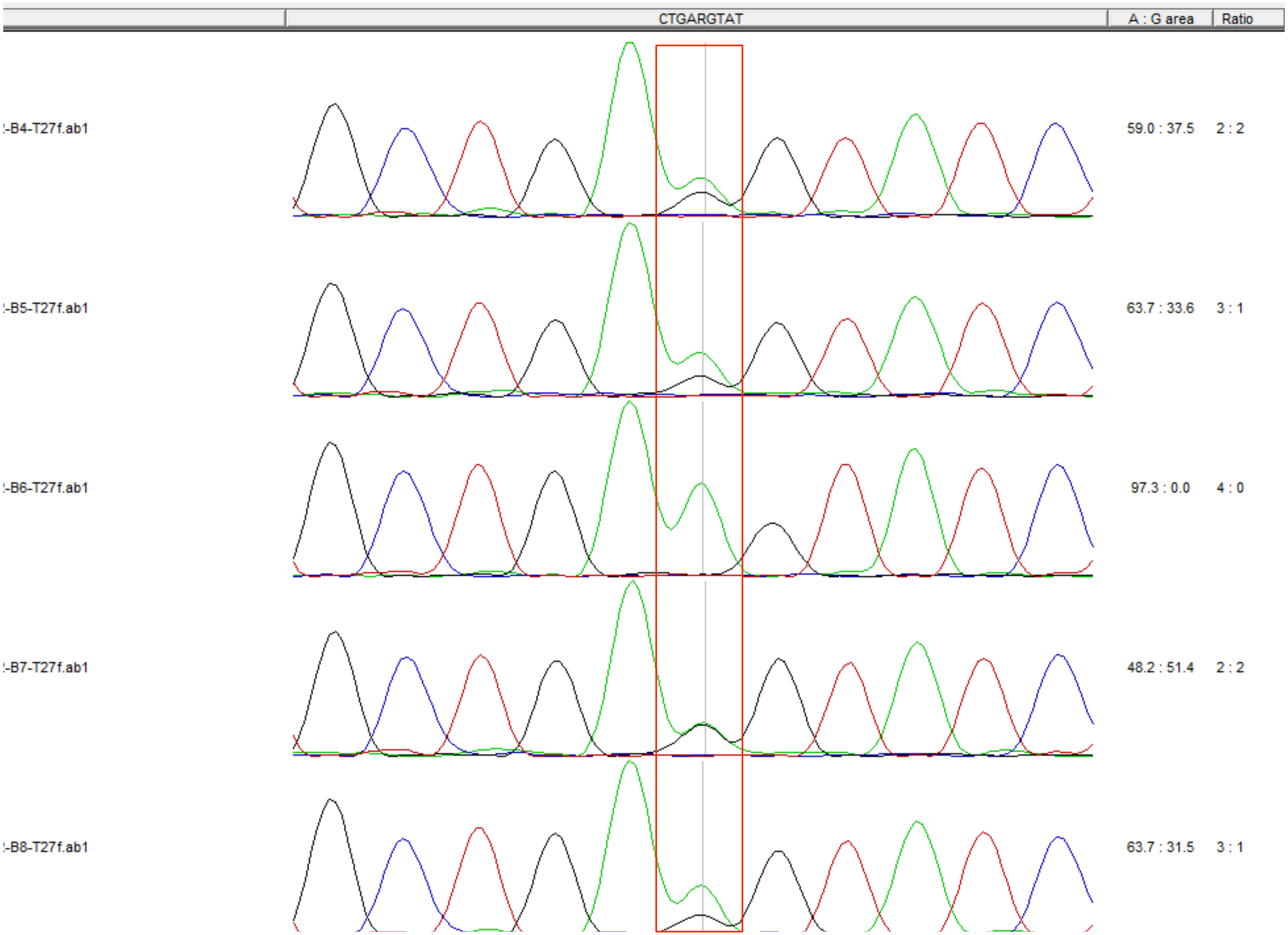


p-value 5.52E-06

12.5% of phenotypic variance

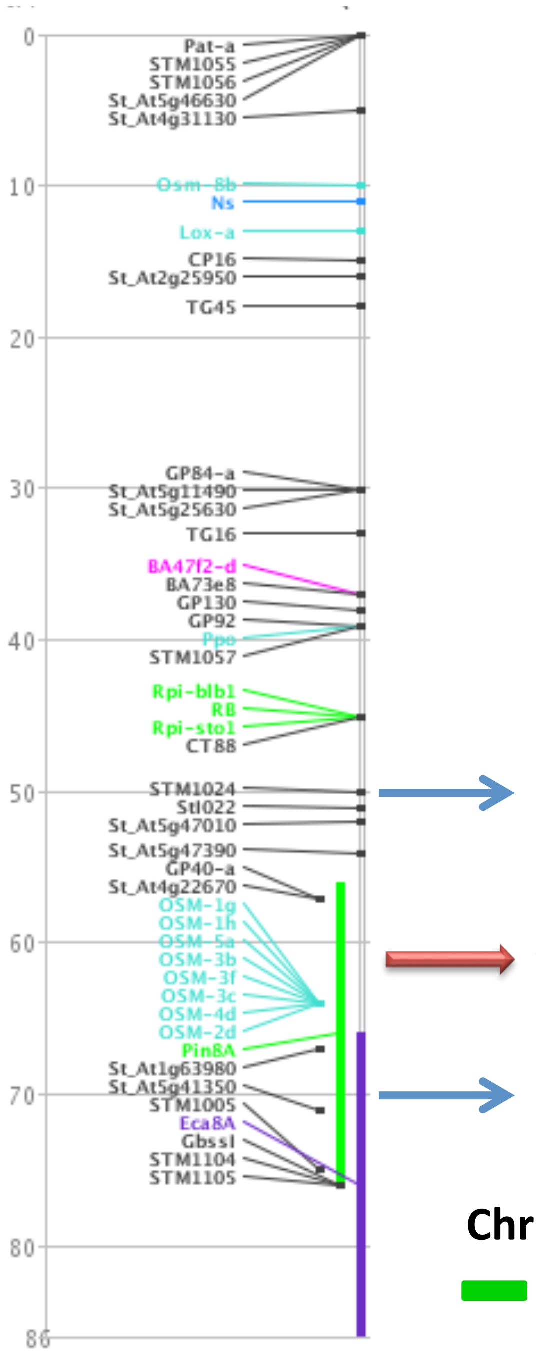


Chromosome 8: cytochrome P450 71D11 SNP CTGA(A/G)GTAT



	CTGARGTAT	A : G area	Ratio
-B4-T27f.ab1		59.0 : 37.5	2 : 2
-B5-T27f.ab1		63.7 : 33.6	3 : 1
-B6-T27f.ab1		97.3 : 0.0	4 : 0
-B7-T27f.ab1		48.2 : 51.4	2 : 2
-B8-T27f.ab1		63.7 : 31.5	3 : 1

Novel candidate gene validated for quantitative resistance to late blight in tetraploid potatoes



51.488.760 bp

Cytochrome P450 71D11

29.253.8 82 bp

Chromosome VIII

🟩 QTL for late blight resistance

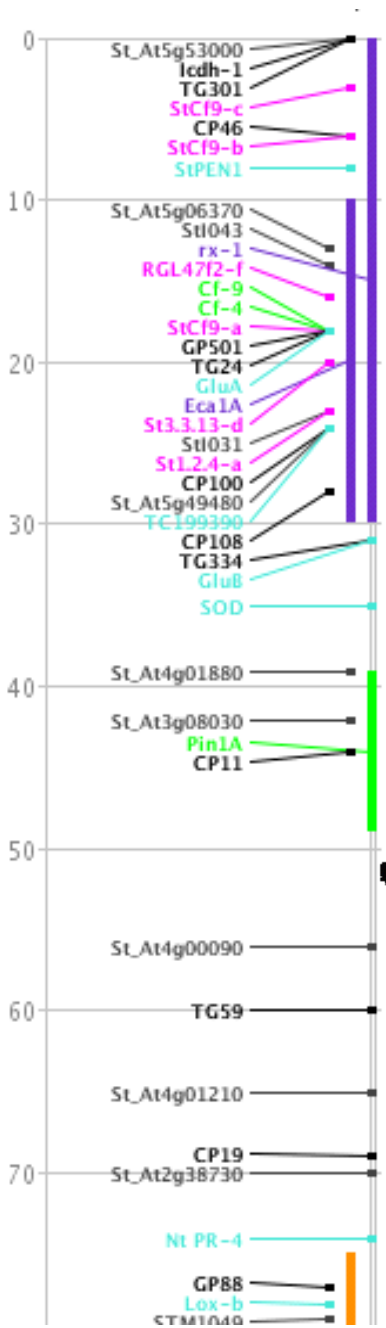
Cytochrome P450 71D11:

- ✓ has oxidoreductase activity, acting on paired donors.
- ✓ incorporates or reduces molecular oxygen and is oxygen binding.
- ✓ participates in regulation of defense response, response to bacterium, systemic acquired resistance with incorporation or reduction of molecular oxygen.

PoMaMo database

http://www.gabipd.org/projects/Pomamo/

Novel candidate genes validated for quantitative resistance to late blight in tetraploid potatoes

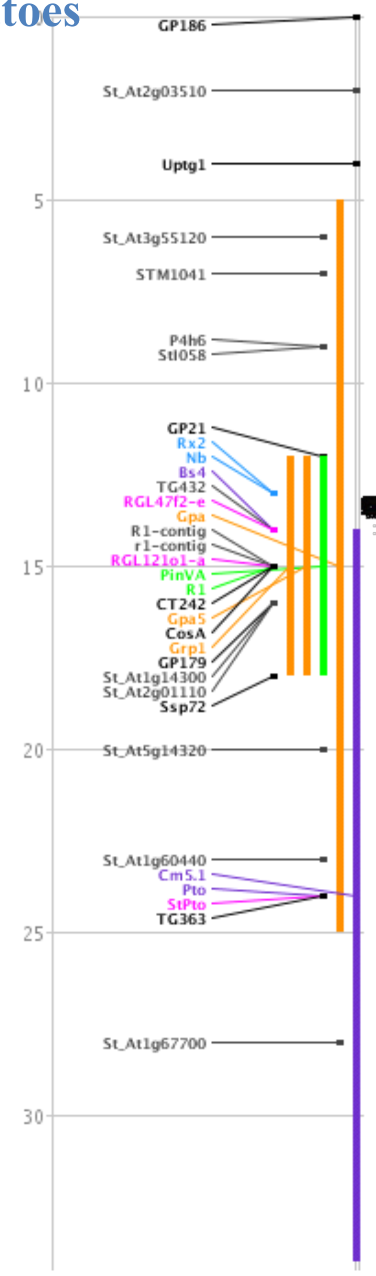


31.442.042 bp

Defective in induced resistance protein 1

76.955.498 bp

Chromosome I



4.037.739 bp

AAA ATPase domain

5.575.541 bp

Chromosome V

🟩 QTL for late blight resistance

PoMaMo database
http://www.gabipd.org/projects/Pomamo/

Summary	
Candidate genes selected	57
Candidate genes analyzed	30
Populations studied	3
Number of individuals analyzed	327
Total of SNPs scored	564
SNPs scored in tuberosum populations	322
SNPs scored in *S. phureja*	242
SNPs are showing association with late blight resistance	9
Candidate genes associated to late blight resistance	5
Chromosomes covered with 11 significant SNPs	1, 3, 5, 6, 8

Genes associated with resistance to late blight In diploid and tetraploid germplasm

Chr.	Gene (ID)	Function described	Population	SNP (ID)	-log10(P)	Effect %	Primer Name
1	PGSC0003DMG400011323	Defective in induced resistance 1 protein	Tub (4x)	8	2.7	15	MF22
3	PGSC0003DMG400016749	TMV - induced protein I	Phu (2X)	61	2.6	10.5	TM14
				199	2.7	10.5	
				211	2.6	10.5	
5	PGSC0003DMB000000609	AAA ATPase domain	Tub (4X)	14	3.1	11.5	TM35
6	PGSC0003DMG400034939	Thylakoid lumenal 15 kDa protein 1, chloroplastic	Phu (2X)	88	3.5	13	TM18
				108	3.5	13	
8	PGSC0003DMG400020809	Cytochrome P450 71D11	Tub (4x)	505	5.3	12.5	TM27
				548	10.3	14.5	

Conclusions and perspectives

- Differential transcriptome analysis has shown to be useful to identify novel candidate genes for quantitative resistance to late blight.

- The results regarding independence between traits: maturity and resistance to late blight open new opportunities for potato breeding.

- Our results show novel genes involved with basic process like light dependent reactions, growth and development, pest resistance, and senescence or responses to wounding, effects that are of potential relevance for potato resistance.

- The next step is to develop diagnostic molecular markers for resistance to late blight and to validate them in breeding programs for tetraploid and diploid germplasm.

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Plant Breeding Research

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Acknowledges

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DAAD
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German Academic Exchange Service

MinAmbiente
Ministerio de Ambiente
y Desarrollo Sostenible

PROSPERIDAD
PARA TODOS

Contract to access genetic resources RGE0069

Foreign Affairs, Trade and Development Canada
Affaires étrangères, Commerce et Développement Canada

IDRC | CRDI
International Development Research Centre
Centre de recherches pour le développement international

Thanks for your attention