Functional genomic research on the potato quantitative resistance to late blight

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Disease resistance - Late blight
(*Phytophthora infestans*)

- $1$ billion loss each year.
- Pathogen race changes frequently and fungicide resistance builds up quickly.
Breeding strategies

Strategies for utilizing R genes

1. *R* gene polyculture strategy:

To create a resistance polymorphism in the field utilizing several *R* genes simultaneously.
2. Using new ‘broad-spectrum’ resistance genes

Gene RB cloned from *Solanum bulbocastanum* confers broad spectrum resistance to potato late blight

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An ancient *R* gene from the wild potato species *Solanum bulbocastanum* confers broad-spectrum resistance to *Phytophthora infestans* in cultivated potato and tomato

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Quantitative or horizontal resistance is controlled by a number of genes that do not prevent infection but slow down the development of the pathogen. This resistance seems more durable than that mediated by R genes.

Strategies for utilizing horizontal resistance

| Susceptible | Horizontal resistance |
Durable resistance is a promising but challenge way.
Up to now, a number of quantitative trait loci (QTLs) for late blight have been mapped in many experimental populations of potato, but the molecular basis of this phenomena is poorly understood.
How many genes involved in the durable resistance & what are they?
How are they organized in plant defense system?

For look into the molecular factors involved in the horizontal resistance …..
Enrich horizontal resistance-related genes

SSH library construction
suppression subtractive hybridization

**Plant material**: B population breeding line
B3C0 386209.10 from CIP
Horizontal resistance

The synthesized dcDNA from leaves challenged by *P. infestans* was used as tester, while cDNA from leaves sprayed with water as driver.

*P. infestans* strains (1, 3, 4 and 1.3)
**Expression profile in response to *P. infestans***

ESTs from SSH library were used to prepare cDNA microarrays.

Leaves were inoculated with mixture of *P. infestans* strains (1、3、4 and 1.3) and sampled at nine time points ranging from 2, 4, 6, 8, 12, 24, 36, 48 and 72 h after inoculation.
Based on the criteria of a normalized expression ratio over 2.2-fold between the inoculated and control samples, a total of 669 significantly differentially expressed ESTs were identified.

After sequencing and merging the repeated ones, 348 singleton ESTs were identified.

234 had significant matches with the functional genes or ESTs, whereas the rest (32.8%) had low sequence similarity.
The ESTs with known function were classified into 13 categories.

These functional genes are mostly related to metabolism, plant defense, signaling and transcription regulation, involving the whole process of plant defense response to pathogens.
Clustergram shows the different classes of gene expression profiles.

Variation in the distribution percentage of 348 genes induced by *P. infestans*.
Overview of the metabolic pathways activated by P. infestans

98 genes related to metabolism which belongs to 11 pathways
The dynamic expression patterns of 348 genes under the treatments of salicylic acid (SA), methyl jasmonate (MJ), and ethephon (ETH).

**Material:** 386209.10
**SA:** 10 mmol/L
**MJ:** 50 μ mol/L
**ETH:** 200 μ l/L
**Time point:** 2, 8, 12, 24, 36 h
The majority of *P. infestans*-responsive genes could be regulated by SA and MeJA, suggesting that the SA-dependent systemic acquired resistance (SAR) pathway and the JA/ET-dependent induced systemic resistance (ISR) pathway exist and participate in the defense response of potato to *P. infestans*.
The different expression profiles of 348 genes associated with quantitative resistance under the treatments of SA, MJ and ETH.
The different expression profiles of WRKY, ERF and MYC under the treatments of SA, MJ and ETH implied that the three signaling pathways finally converged on these transcription factors (TFs) and integrated into the consistent defense process of potato against P. infestans.

A hypothesized model for the generation and integration of SA, JA and ET signaling pathways of potato in response to P. infestans infection.
Comparison of the expression profiles of 348 genes among different resistance level potato cultivars or line.

QR  Horizontal resistance line
E3  Vertical resistance cultivar
JX  Susceptible cultivar
Time point: 8, 24, 72 hip
The results indicated that the most of these genes could be induced in E3, JX and QR. Only few of the identified ESTs were specific to either the E3 and QR.

But obvious differences in expression patterns and transcriptional abundance of many genes were observed among the three cultivars (clone).
Different expression profiles of 348 potato genes in three potato cultivars or clone.

Few differences in the category and number of genes participating in the defense response to *P. infestans* exist in the three potato materials.

But the differences of their resistance might depend on the special expression of the “genes induced by *P. infestans* specially”, or the time of initial expression of certain genes related to resistance response and/or their induced expression level.
Summary

- These resistance related genes are mostly related to metabolism, plant defense, signaling and transcription regulation.
- The majority of *P. infestans*-responsive genes could be regulated by SA and MeJA and ETH.
- Genes involved in basal defense in different resistance level materials overlap to a great extent. The expression differences of these genes involved in basal defense are mainly in timing and intensive.
Some works on the way

Mapping of some candidate genes
21 full-length cDNAs were cloned

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Functional investigation

Stable transformation

Over expression
Transient expression

Gene silence (VIGS system)

PVX201 vector        TRV vector
Resistance-related gene function investigation.

◆ POTH-1 expression and functional research.

Induction expression profiles.

Gene transformation.
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