

# Comparative Structural Genomics Of The Potato Tertiary Genepool: Improving Access To Disease Resistance Genes



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## Premise

The genomes of potato and tomato were shown 20 years ago to be syntenic, with clear conservation of overall marker order and chromosome structure [Genetics (1988) 120: 1095-1103]. More recently, it has been demonstrated that disease resistance (R) genes occupy orthologous regions of the genomes of potato, tomato, and pepper [Genetics (2000) 155: 873-887]. There are approximately 200 potato species, each a potential source of genes for improvement of the cultivated potato. The tertiary genepool of potato includes 20 wild species rich in R genes (Figure 1). In this project, we build upon observations of genome-wide synteny and conservation of R gene locations in the genus *Solanum* to improve access to unique R genes found in wild potato species.



**Figure 1: The potato tertiary genepool is a rich source of disease resistance.** (A) Wild potato *Solanum bulbocastanum* growing in a late blight nursery in Minnesota. Compare with diseased and dead cultivated potato. (B) Wild potato *S. polyadenium* (right) growing in *Verticillium* infested soil in Minnesota. Compare with diseased and dead wild potato (left).

## Strategy

- 1 We are developing a series of linkage maps for key wild potato species. Markers previously mapped in cultivated potato and tomato and sequencing of markers mapped in wild potato will facilitate extension of observations of genome-wide synteny to the potato tertiary genepool.
- 2 BAC clones harboring candidate R genes will be isolated and mapped in wild potato. Comparison of genome-wide R gene organization in the genus *Solanum* will ensue.
- 3 The resulting integrated linkage maps, R gene physical maps, and DNA markers will be employed to map one R gene in a disease resistant wild potato species.

## Constructing linkage maps for wild potato species

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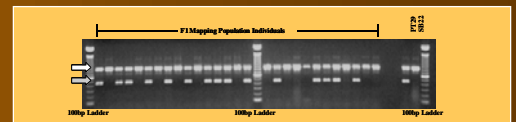
**Objectives and methods:** Linkage maps for three wild potato species will be constructed: one each for *Solanum bulbocastanum*, *S. pinnatisectum*, and *S. commersonii*. *Solanum bulbocastanum* and *S. pinnatisectum* are noted sources of late blight resistance. *Solanum commersonii* is a source of frost tolerance. These three species represent phylogenetically distinct series within the potato tertiary genepool. Maps will be constructed using a variety of markers, including AFLP markers, Conserved Orthologous Sequence (COS) markers, PCR-RFLP markers, and DArT (Diversity Arrays Technology) markers. Markers previously mapped in tomato and cultivated potato (e.g. COS and RFLP) will be used for gross comparison of genome structures within the genus *Solanum*. Finer scale comparisons will be accomplished by sequencing DArT markers mapped in wild potato followed by *in silico* analysis against burgeoning potato and tomato genome sequences.

### Expected outcomes:

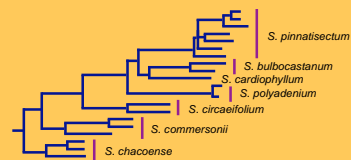
- Medium density linkage maps for three important wild potato species
- Extension of observations of genome-wide synteny to the potato tertiary genepool
- DNA sequence of anchor markers suitable for marker development in related species and populations
- A DArT microarray platform for the potato tertiary genepool, a community resource that will facilitate phylogenetic analyses, mapping, and cross-laboratory comparisons

### Progress to date:

- 58 AFLP markers have been mapped in *S. bulbocastanum*
- 10 COS markers on chromosome 5 are being adapted for PCR mapping in *S. bulbocastanum*; this serves as a case study for the mapping utility of this marker class
- Protocols for PCR-RFLP have been tested and optimized (Figure 2). RFLP markers near the *S. bulbocastanum* late blight resistance gene *RB* have been mapped
- A preliminary DArT array has been constructed. Phylogenetic examination of species relationships demonstrate the utility of these markers for the study of the potato tertiary genepool (Figure 3). An expanded, diversity enriched array is currently being constructed in collaboration with Andrzej Kilian, DArT, Pty. Ltd.
- Mapping populations have been constructed for *S. bulbocastanum*, *S. commersonii*, and *S. pinnatisectum*



**Figure 2: PCR-RFLP.** We have tested and optimized a PCR method for mapping RFLPs and BAC ends in wild potato (Syverson and Bradeen, submitted). Results from mapping in *S. bulbocastanum*. Upper fragment is an internal control (positive in all reactions); lower fragment is target specific, is polymorphic between mapping parents PT29 and SB22, and segregates at a 1:1 ratio in F1 progeny.



**Figure 3: Phylogenetic analyses of wild potato species using DArT.** Preliminary results from a potato DArT array confirm species affinities and assist in selection of polymorphic mapping parents. Analysis conducted by Andrzej Kilian, DArT Pty. Ltd.

## Mapping candidate R genes

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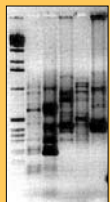
**Objectives and methods:** More than a dozen R genes have been cloned from Solanaceous species. Most cloned R genes belong to the NBS-LRR class, sharing structural motifs that can be targeted for amplification via degenerate primer PCR [Nature Genetics (1996) 14: 421-429]. The resulting resistance gene analogs (RGAs) represent intact R genes. Candidate R gene fragments and RGAs will be generated from *S. bulbocastanum* and used to probe an existing *S. bulbocastanum* BAC library [Genome (2000) 43: 199-204]. BAC clones will be end sequenced and integrated into the *S. bulbocastanum* linkage map, enabling comparison of genome-wide R gene locations between wild potato, cultivated potato, and tomato.

### Expected outcomes:

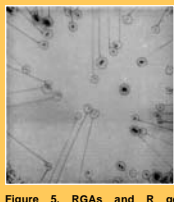
- Enriched RGA resources for the genus *Solanum*
- Observations of genome-wide conservation of R gene locations in the genus *Solanum*, including the potato tertiary genepool
- A collection of 100 or more BAC clones harboring candidate R genes
- DNA sequence associated with candidate R genes suitable for marker construction

### Progress to date:

- A library of more than 100 *S. bulbocastanum* RGA clones has been generated (Figure 4)
- Probes have been developed from 13 previously cloned R genes (Table 1); three probes have used to identify corresponding *S. bulbocastanum* BAC clones (Figure 5)
- Protocols for BAC end integration into the *S. bulbocastanum* linkage map have been tested and optimized (Figure 2)



**Figure 4. RGA resources for *S. bulbocastanum*.** We have generated an RGA library of more than 100 clones for this disease resistant species.



**Figure 5. RGAs and R gene fragments used to probe a BAC library.** Fragments generated from the tomato *Mi* gene identified multiple *S. bulbocastanum* BAC clones. BAC clones harboring R genes will be integrated into the *S. bulbocastanum* linkage map.

## Correlating disease resistance phenotypes with candidate R genes

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**Objectives and methods:** The *S. bulbocastanum* integrated linkage map, R gene physical maps, and associated markers that will result from our efforts will be useful for mapping resistance phenotypes in multiple *S. bulbocastanum* populations and in related species. To demonstrate this, one R gene will be mapped in a potato tertiary genepool species. This proof-of-concept will demonstrate one strategy to map disease resistance by targeting genome locations known to harbor R genes. Our ongoing efforts to characterize late blight resistance in wild potato species will provide targets for this effort; leading contenders including the wild potato *S. polyadenium* (Figure 6).

### Expected outcomes:

- Proof-of concept and illustrated strategy for utilization of the integrated mapping resource to map resistance phenotypes
- Known map location and associated markers for a previously undescribed late blight resistance gene

### Progress to date:

- Potato tertiary genepool species are annually evaluated for late blight resistance in University of Minnesota field trials
- Crosses amongst resistant and susceptible materials are ongoing

R Gene	Donor Species	Pathogen (Disease)	Structural Class
CS9	<i>S. peruvianum</i>	<i>Gliocladium fulvum</i>	LRR-Transmembrane
Gpa2	<i>S. tuberosum</i> sp. <i>andigena</i>	<i>Gliocladium pallida</i> (potato cyst nematode)	NBS-LRR
Gpa2.2	Cultivated potato	<i>Gliocladium roscocheni</i> (potato cyst nematode)	NBS-LRR
Hena	Tomato	<i>Potato cyst nematode</i>	NBS-LRR
I2	Tomato	<i>Fusarium oxysporum</i> fsp. <i>Eschscheriae</i>	NBS-LRR
Mi-1	Tomato	<i>Rice late necrotic, aphids</i>	NBS-LRR
Ppf	Tomato	<i>Pseudomonas syringae</i> pv. <i>Tomato</i>	NBS-LRR
Pur	Tomato	<i>Pseudomonas syringae</i> pv. <i>Tomato</i>	Protein Kinase
R1	<i>S. demissum</i>	<i>Phytophthora infestans</i> (late blight)	NBS-LRR
RB	<i>S. bulbocastanum</i>	<i>Phytophthora infestans</i> (late blight)	NBS-LRR
R2.2	<i>S. acule</i>	Potato virus X	NBS-LRR
SvS-d	Tomato	Tomato spotted wilt virus	NBS-LRR
Ve	<i>S. aethiopicum</i>	<i>Verticillium</i> spp.	Surface Receptor

Table 1: R genes cloned from Solanaceous species



**Figure 6. *Solanum polyadenium*: a potential target for mapping late blight resistance.** The wild potato *S. polyadenium* growing in a late blight nursery in Minnesota. Resistance from this species has been transferred to cultivated potato via somatic hybridization (J.P. Helgeson, personal communication). Crosses with this and other species are ongoing with the goal of generating segregating populations.



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This poster will be given as an oral presentation as part of the PAG 2008 Solanaceae Workshop (Tues 15 Jan at 5:20 pm)

A PDF reprint of this poster is available at our lab website: [ppg.cfans.umn.edu](http://ppg.cfans.umn.edu)