

# Analysis of Haplotype Frequency of the Late Blight Resistance Gene, *RB*, in *Solanum bulbocastanum* using a Novel MAMA PCR-based Approach.

Ryan Syverson, Ben Millett, and Jim Bradeen.

Department of Plant Pathology. University of Minnesota.

495 Borlaug Hall/1991 Upper Buford Circle St. Paul, MN 55108.



## Introduction:

Potato, *Solanum tuberosum*, is the fourth most important world food crop and is the single most important dicot food crop (<http://apps.fao.org>). Belonging to *Solanaceae*, cultivated potato is related to a number of other well known crops and also to a group of wild potato species belonging to the *Superseries Stellata* which includes species such as *S. bulbocastanum*, *S. cardiophyllum*, *S. pinnatisectum*, *S. polyadenium*, *S. trifidum*, and *S. tamii* (Hawkes, 1990). More than 60 diseases of economic importance affect potato (Stevenson *et al.*, 2001) and the wild relatives display a wide range of resistance to many of potato's disease threats.

In particular, *S. bulbocastanum*, a diploid (2n), obligate outcrossing species that is distributed throughout southern Mexico and into Guatemala, is a potential source of genetic resistance to a variety of potato diseases, including late blight, caused by the oomycete *Phytophthora infestans* (Helgeson *et al.*, 1998 and Naess *et al.*, 2001) (Figure 1). Genes imparting resistance to *P. infestans* have been identified and cloned from *S. bulbocastanum*, including the *RB* gene, which imparts broad spectrum resistance to all known races of *P. infestans* (Song *et al.*, 2003; Vossen *et al.*, 2003). *S. bulbocastanum* also exhibits resistance to soft rot, potato cyst nematode and aphids (Hawkes, 1990). *S. bulbocastanum* is an attractive species for genetic research, not only because of its potential as a source of disease resistance, but also because of the availability of genetic resources for this species including nearly 60 accessions in the US Potato Genebank located in Sturgeon Bay, WI, a BAC library (Song *et al.*, 2000), and an F1 mapping population (*S. bulbocastanum* SB22 X *S. bulbocastanum* PT29) constructed by Dr. Chuck Brown (USDA-ARS) via a cross between *S. bulbocastanum* genotype SB22 and *S. bulbocastanum* genotype PT29.

Figure 1:



Fig. 1a: *S. bulbocastanum*, shown growing in Rosemount, MN, can be readily identified by its simple leaves and star-shaped flowers.

Fig 1b: A resistance gene, *RB*, derived from *S. bulbocastanum* imparts late blight resistance to cultivated potato; compare with susceptible cultivars (Hancock, WI; courtesy J. Helgeson)

The Potato Pathology and Genomics Laboratory at the University of Minnesota is focused on utilizing genetic resistance for a variety of economically important potato diseases. A major goal for our lab is to develop resources to facilitate the identification, location and isolation of potentially useful disease resistance genes. Specifically, we seek integrated genome-wide genetic maps and resistance (R) gene physical maps with associated markers which may be useful in mapping and map-based recovery of genes imparting agriculturally significant disease resistance. Towards this goal we are currently working on the construction of a whole genome linkage map for *S. bulbocastanum*. We are also developing methods for generating markers associated with R gene regions based on single nucleotide polymorphisms (SNPs), which is the focus of this poster.

## Objective:

**The goal of this research is to identify an efficient assay for SNP detection that allows marker association analysis of regions of interest**

Previously, we adopted a Mismatch amplification mutation assay (MAMA) approach to develop transgene-specific PCR and RT-PCR assays for the late blight resistance gene *RB* (B. Millett *et al.*, PAG Poster 439). Here, we adapt MAMA-PCR for analysis of SNP-based marker association near *RB*.

Address technical considerations of MAMA PCR

- Optimal annealing temperatures
- Nucleotide composition at the penultimate position (i.e. transition vs. transversion)

Demonstrate the utility of this approach

- Validate haplotype specificity through segregation analysis using a *S. bulbocastanum* F1 population
- Examine marker associations around *RB* using a survey panel of 60 *S. bulbocastanum* genotypes (two individuals from each of 30 US Potato Genebank accessions)

## MAMA PCR:

MAMA differentiates sequences based on SNPs (Cha *et al.* 1992)

The assay functions by design of a SNP-specific PCR primer incorporating the SNP at the ultimate (3') position and a mismatch at the penultimate position

Despite the mismatch at the penultimate position, the specificity at the 3' most nucleotide site allows the PCR primer to specifically anneal to a desired sequence, enabling amplification (Figure 5)

## Optimization:

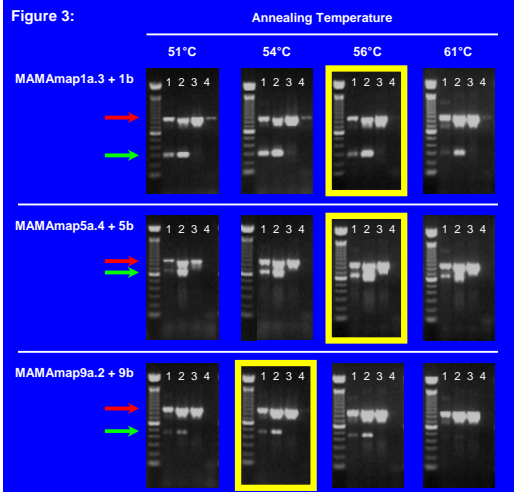
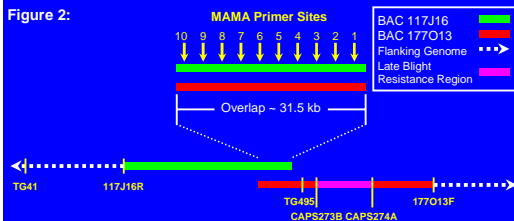
As a model system, we used two fully sequenced BAC clones associated with *RB* (117J16 and 177O13; GENBANK AY303171), originating from different haplotypes of *S. bulbocastanum* PT29 (Figure 2)

MAMA primers were designed at 10 SNPs, approximately 3kb apart, within the overlapping region. Other MAMA primers targeted SNPs within CAPS (273B & 274A) and RFLP (TG495) markers (Figure 2)

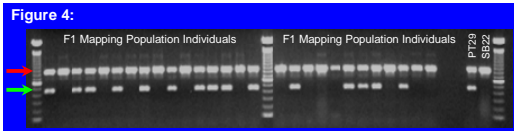
MAMA primers, paired with standard PCR primers, were singly tested over a range of annealing temperatures (51°C-61°C) on PT29 genomic DNA and BAC DNA from both haplotypes (Figure 3)

Positive control primers designed from the *RNA Polymerase II* (RP2) gene and the pBELOBAC11 vector (BACvector) were multiplexed with MAMA primer pairs

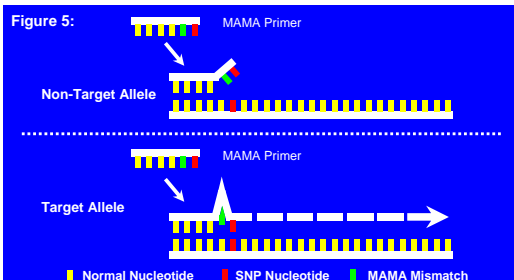
Optimized primer specificity was validated through screening of F1 population (Figure 4)



PCRs at varying annealing temperature (listed across the top) using 3 different MAMA primers from the BAC overlap region: MAMAmap1a.3 + 1b (amplicon=298), MAMAmap5a.4 + 5b (amplicon=858) and MAMAmap9a.2 (amplicon=471). Target Bands are marked with green arrows. These reactions were multiplexed with RP2 (amplicon=862) and BACvector (amplicon=849) control primers. Control bands are marked with red arrows. Reactions at optimal annealing temperature are highlighted with yellow boxes. Templates for these reactions are 1) *S. bulbocastanum* PT29 genomic DNA, 2) 117J16 BAC DNA (+ control), 3) 177O13 BAC DNA (- control) and 4) water.



PCRs at an annealing temperature of 54°C using MAMA primer pair MAMAmap9a.2 + 9b (target bands marked with green arrows) and RP2 control primers (control bands marked with red arrows). Templates for these reactions are genomic DNAs from F1 mapping population individuals and *S. bulbocastanum* PT29 & SB22 genomic DNA, where indicated.



## Results:

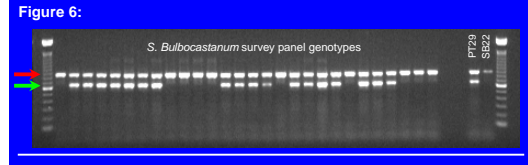
13 of 16 (81.25%) markers examined were successfully adapted for MAMA PCR (haplotype specificity could not be validated for markers MAMA 10, 177O13F and 117J16R) with all successful markers fitting the expected 1:1 segregation ratio ( $\chi^2$  ranged from 0.005 to 0.681; p-values ranged from 0.409 to 0.944) (Figure 4)

Optimal annealing temperatures varied. For most markers, amplification was best at 56°C, three markers were better at 54°C and one marker each was best at 51°C and 49°C (Figure 3)

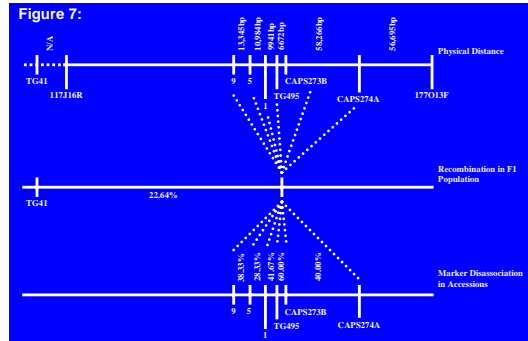
The effect of nucleotide composition on specificity also varied amongst MAMA primers tested. Of primers specific for the target, 46.7% were transitions and 53.3% were transversions

Analysis of marker data from the 60 *S. bulbocastanum* genotypes revealed no statistically significant correlation between physical distance and observed marker disassociation ( $r = 0.167$ ,  $p > 0.05$ ) (Figure 7)

Further pairwise analysis exhibited an overall trend of marker association decay with increasing physical distance, although deviations from this trend were observed (data not shown)



PCRs at an annealing temperature of 56°C using MAMA primer pair MAMAmap5a.4 + 5b (target bands marked with green arrows) and RP2 control primers (control bands marked with red arrows). Templates for these reactions are genomic DNA from *S. bulbocastanum* survey panel genotypes and *S. bulbocastanum* PT29 & SB22 genomic DNA, where indicated.



Shown are the physical distances, observed recombination, and observed marker disassociation between *RB*-associated MAMA PCR markers. The topmost horizontal line indicates the physical distances between MAMA PCR markers (determined from SNP to SNP). The dashed horizontal line connecting markers 117J16R and TG41 represents a region of unknown sequence and undetermined physical distance. The middle horizontal line indicates the observed recombination percentage between MAMA PCR markers in an F1 *S. bulbocastanum* mapping population (109 individuals). The bottom horizontal line indicates the observed marker disassociation between adjacent MAMA PCR markers in a collection of 60 *S. bulbocastanum* genotypes from 30 distinct populations. All marker positions are based on known physical distances from *S. bulbocastanum* genotype PT29, except as noted. Drawing is to scale.

## Conclusions:

- MAMA PCR is an efficient and sensitive assay for SNP detection
- MAMA markers were developed from BAC sequence and CAPS and RFLP markers and are suitable for linkage mapping and haplotype characterization
- Because of variation in optimal conditions such as temperature and nucleotide composition, technical issues of this assay need to be optimized for each primer pair

## Future Research:

Generation of new markers in closer proximity to *RB* itself for further association mapping applications in *S. bulbocastanum*. We have developed and optimized a LR-PCR approach for multi-genotype allelic mining at the *RB* locus (Sanchez and Bradeen 2006). MAMA PCR combined with these *RB* allele characterization efforts can be used to test for correlation between specific MAMA markers and specific *RB* alleles.

Target/develop new markers for association mapping of additional R genes in *S. bulbocastanum*

**Acknowledgements:** This research was partially funded by the National Research Foundation (NSF) Potato Genome Project (DBI0218166)

**References:** Cha RS, Zabel H, Keohavong P, Thilly WG (1992) Mismatch Amplification Mutation Assay (MAMA): Application to the *c-H-ras* Gene. *Genomic Research* 2:14-20  
 Hawkes JG (1990) The Potato: Evolution, Biodiversity and Genetic Resources. Smithsonian Institution Press, Washington, D. C.  
 Helgeson JP, Palmann JD, Austin S, Haberlach GT, Wielgus SM, Ronis D, Zambolin L, Tooley P, McGrath JM, James RV (1998) Somatic hybridization: *Solanum bulbocastanum* and potato: a new source of resistance to late blight. *Theor Appl Genet* 90:738-742  
 Naess SK, Bradeen JM, Wielgus SM, Haberlach GT, McGrath JM, Helgeson JP (2001) Analysis of the Introgression of *Solanum bulbocastanum* into potato breeding lines. *Mol Genet Genomics* 265:694-704  
 Sanchez MJ, JM Bradeen (2006) Towards efficient isolation of R gene orthologs from multiple genotypes: optimization of Long Range-PCR. *Molecular Breeding* 17:133-148  
 Song J, Bradeen JM, Naess SK, Raasch JA, Wielgus SM, Haberlach GT, Liu J, Kuang H, Austin-Phillips S, Buell CR, Helgeson JP, Jiang J (2003) Gene *RB* cloned from *Solanum bulbocastanum* confers broad spectrum resistance to potato late blight. *PNAS* 100:9128-9133  
 Song J, Dong F, Jiang J (2003) Construction of a bacterial artificial chromosome (BAC) library for potato molecular cytogenetics research. *Genome* 43:199-204  
 Stevenson WR, Loda R, Franz GD, Weingartner DP (2001) Compendium of Potato Diseases, 2nd edn. APS Press, St. Paul  
 Vossen E.v.d., Sillekens A., Hecker B.T.L., Gros J., Stevens P., Muskens M., Wouters D., Pereira A., Sillekens W., Allers S. (2003) An ancient R gene from the wild potato species *Solanum bulbocastanum* confers broad spectrum resistance to *Phytophthora infestans* in cultivated potato and tomato. *Plant J* 37:867-882