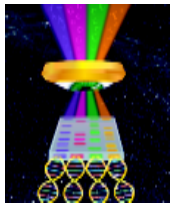

Focal Points

UVP BioImaging Systems

Application Note FP-120

Genetic Mapping of Newly Identified Mutations Using the Arabidopsis Genome and CAPS Markers

Marcela Rojas-Pierce and Patricia S. Springer
Department of Botany and Plant Sciences
University of California, Riverside, CA

INTRODUCTION

Arabidopsis thaliana is an important model system in plant biology because of its small genome size, short life cycle and relatively high gene density. It is also the first plant to have its genome completely sequenced (The Arabidopsis Genome Initiative, 2000). A rich assortment of tools such as morphological mutations and molecular markers are readily available, making it an excellent teaching tool for molecular genetics. Here we describe a laboratory exercise in which a new morphological mutation is placed on the Arabidopsis genetic map using PCR-based molecular markers.

Genetic mapping of a mutation is the first step towards the identification of the corresponding gene. Two elements are needed to map a mutation: 1) a population in which the mutation is segregating, 2) genetic markers that can be tested for linkage to mutation. Either morphological or molecular markers can be used as long as they are segregating in the mapping population. Crossing mutant individuals to individuals that carry different alleles of the markers in question generates a mapping population. In Arabidopsis, this is accomplished by crossing plants of a different ecotype. In the F_2 generation, both the mutation and the markers will segregate. Individual plants are scored for the presence of the mutation and for the different alleles of each individual marker. In this way, linkage between the mutant phenotype and each marker can be tested.

Cleaved Amplified Polymorphic Sequence (CAPS) markers are PCR-based markers that can detect single nucleotide polymorphisms (SNPs) between two DNA samples (Konieczny and Ausubel, 1993). These markers are co-dominant and easy to use. Each marker is based on the PCR amplification of a fragment of genomic DNA that contains one or more SNPs. The PCR products are digested with restriction enzymes that differentiate between the two polymorphic alleles and the digestion products are resolved by agarose gel electrophoresis. If the markers chosen are well dispersed in the genome, then linkage to marker can be established using a small number of markers and a small number of individuals (Konieczny and Ausubel, 1993).

We have developed a laboratory exercise that exposes students to all aspects of the process of placing a mutation on a genetic map using molecular markers. The goal of this laboratory exercise was to map a newly identified mutation to a chromosome arm in the Arabidopsis genome using CAPS markers.

MATERIALS AND METHODS

Plant Material

Wild type Arabidopsis seeds from *Landsberg erecta* (*Ler*) and *Columbia* (*Col*) ecotypes were originally obtained from the Arabidopsis Biological Resource Center (ABRC). The *senescence* (*sen1*) mutation arose in an *Ac*/*Ds* transposon tagging population (Sundaresan *et al*., 1995). Co-segregation analyses had previously shown that the *sen1* mutation was not caused by a transposon insertion. The cause of the mutation remains unknown. Inter-crossing homozygous *sen1* plants in the *Ler* ecotype to wild type *sen1* plants in the *Col* ecotype generated a mapping population. The F_1 progeny of this cross were allowed to self-pollinate and F_2 seed were collected. Approximately 200 F_2 seeds were sown in soil and scored for the *sen1* phenotype at 3 weeks of age. Plants were grown as previously described (Springer *et al*., 2000).

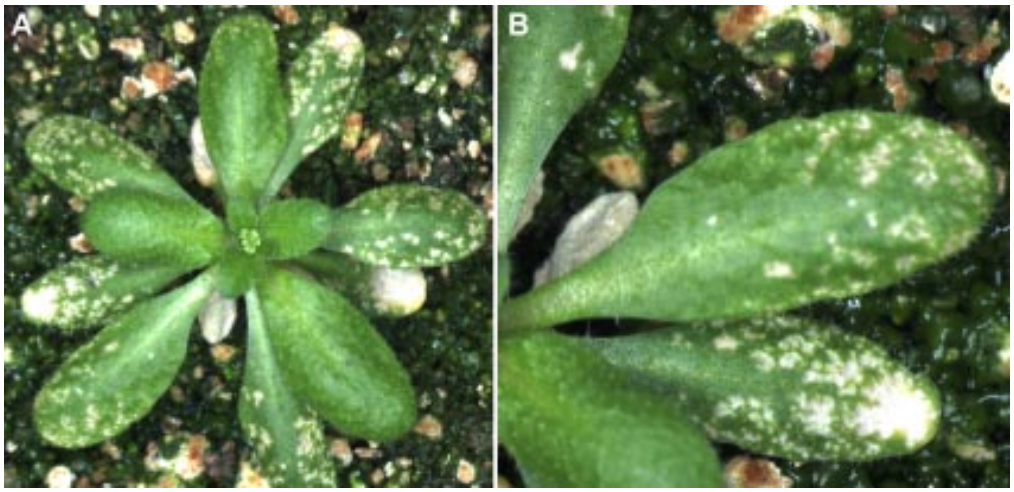

Fig. 1. *sen1* homozygotes show premature senescence. This is manifest as chlorotic mottling on the leaves. The mottling follows a developmental progression, appearing first at the leaf tip.

Genetic analysis

Genomic DNA was isolated from *sen1* homozygotes as well as wild type *Ler* and *Col*

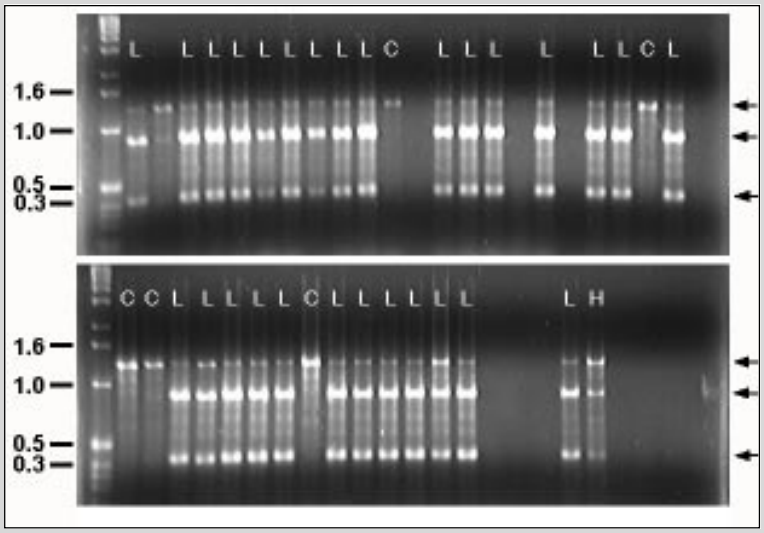

Fig. 2. Segregation of CAPS marker BGL1 among *sen1* homozygotes. Ethidum bromide stained gel of BGL1 PCR products following digestion with *Rsa*I. Arrows show the bands produced by the *Col* (C) and *Ler* (L) alleles. Genotypes are shown above each lane (C = *Columbia* homozygote; L = *Landsberg erecta* homozygote; H = heterozygote).

parental plants as described (Campisi *et al.*, 1999). Two CAPS markers from each Arabidopsis chromosome were chosen for use (Table 1). A description of all available CAPS markers can be found at http://www.arabidopsis.org/search/marker_search.html. MAPPAIRS PCR primers were purchased from Research Genetics (Huntsville, AL). PCR reactions and restriction digests were performed as described by Konieczny et al. (1993). Digested products were visualized in 1.5 % agarose gels. Following electrophoresis, gels were stained in ethidium bromide and visualized using the BioDoc-It System (UVP Inc., Upland, CA). Recombination frequencies between each marker and *sen1* were calculated as the number of chromosomes containing the *Col* allele divided by the total number of chromosomes scored (number of heterozygous individuals plus 2x the number of *Col* individuals divided by 2x the number of plants scored).

RESULTS AND DISCUSSION

The *sen1* mutation is characterized by the presence of necrotic lesions on the rosette leaves of young plants and premature

Table 1. CAPs markers used to map *sen1*

Marker	Chromosome	Map position (cM)	Restriction Enzyme	# Col homozygotes	# Ler homozygotes	# Hets	FR (%)
m235	1	34.01	*Hin*dIII	8	17	17	39.2
m305	1	91.89	*Hae*III	3	3	21	50.0
PhyB/hy3	2	34.45	*Xho*I	8	7	22	51.3
m429	2	73.19	*Scr*FI	10	5	19	57.3
g4711	3	38.06	*Hin*dIII	10	6	9	58.0
BGL1	3	75.25	*Rsa*I	6	28	1	18.5
GA1	4	17.72	*Bsa*BI	13	10	18	53.6
AG	4	63.16	*Xba*I	4	9	12	40.0
r89998	5	38.65	*Rsa*I	9	11	23	47.6
DFR	5	89.51	*Bsa*AI	13	5	24	59.5

senescence (Figure 1). *sen1* is recessive and behaves as a single genetic locus (data not shown). Homozygous *sen1* plants (in the *Ler* ecotype) were crossed to wild type *sen1* plants in the *Col* ecotype. The F_1 generation was allowed to self-fertilize and approximately 200 F_2 plants were scored for the presence or absence of the mutant phenotype at 3 weeks of age. Students performed phenotypic scoring during the laboratory period. Students, working in pairs, collected plant tissue from *sen1* homozygotes and isolated genomic DNA. A total of 48 *sen1* plants were used in the subsequent analysis.

Ten CAPS markers, two per chromosome, were tested for linkage to the *sen1* mutation (Table 1). DNAs from all 48 homozygous *sen1* plants as well as both *Ler* and *Col* parental types were used as templates for the each CAPS reaction. Digested PCR products were separated by gel electrophoresis and visualized after ethidium bromide staining. Each agarose gel was photographed using the **UVP BioDoc-It gel imaging system.** Each group of students performed the PCR, digestion and electrophoresis for two markers. Due to variable success of the PCR reactions, all 48 *sen1* plants could not be genotyped for each marker. Nonetheless, recombination frequencies between *SEN1* and each marker could be calculated. Individual lab groups performed the scoring and pooled the data for analysis by all students. Each marker was tested for linkage to *sen1* (see Materials and Methods). Markers that were not linked to *sen1* were expected to show a recombination frequency of ~50%, while linked markers would show a recombination frequency of <50%. One marker, BGL1, showed a recombination frequency substantially less than 50% (Table 1). Fig. 2 shows the frequency of recombination between BGL1 and the *SEN1* locus was 18.5 % (χ^2 = 58.92, P < 0.005). This places the *SEN1* locus somewhere on the lower arm of chromosome III. Additional CAPS markers are available in this region (http://www.arabidopsis.org/search/marker_search.html) and can be used to more precisely map the *SEN1* gene.

CONCLUSIONS

We have developed a laboratory exercise in which students determine the chromosomal map location of a previously unknown morphological mutation in Arabidopsis. This is the first step toward cloning the corresponding gene. This exercise exposes students to a range of different molecular genetic techniques and could be performed using any morphological mutation. CAPS markers are especially convenient molecular tools that can be easily scored. CAPS markers are readily available in Arabidopsis, but may not be applicable to species where significant genomic sequence has not been determined. In this case, other easily scorable molecular markers could be used.

This experiment was performed as part of the laboratory course “Methods in Arabidopsis Research,” taught in Fall, 2000 in the Department of Botany and Plant Sciences, University of California, Riverside.

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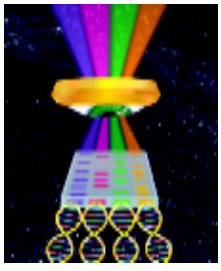

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