DNA Fingerprinting of *Brassica Rapa* Using SRAP—Sequence-Related Amplified Polymorphism DNA Fingerprinting: An Experiment for use in a College Biology Course

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Abstract

We used a novel approach that combined direct PCR of plant tissue and SRAP DNA fingerprinting to seek to identify the crosses made in *Brassica rapa* used in the Wisconsin Fast Plants. Our goal was to develop a rapid method for students to use in a biology, botany, or genetics class to identify the parental types (purple stem, non-purple stem) and the F1 generation for *Brassica rapa*. Our data indicates that there is detectable polymorphism in the *Brassica Rapa* plants with which to identify the three types of plants.

Introduction

We continually look for new experiments that offers our students a "taste" of exploration of the real world, allow them to become familiar with biotechnology tools, and capture their attention, setting them on a path of discovery in our amazing world. The basis for this experiment is to have students identify the first filial generation (F1) plants from the parental types by screening for genetic variations between the three.

SRAP uses primer pairs in PCR. By using a forward and reverse primer pairs that are 17 or 18 nucleotides long, SRAP can amplify open reading frames and test for the presence of polymorphism, or variations in the nucleotide sequences. This method is simpler and overcomes several problems with other PCR marker systems used to create genetic maps of plant species. Primers were originally created by Li and Quiros using the strategy of filler sequence, an open reading frame (ORF) region, and a selective, sequence. Starting at the 5' end of the forward primer bases 1-10 are filler and nonspecific; bases 11-14 are specific and use CCGG which targets exons in the plant chromosome; and bases 15-17 are selective. For the reverse primer bases 1-10 are filler (different than the forward primer filler); bases 11-14 are specific and use AATT which targets introns and promotor in the plant chromosome; and bases 15-17 are selective (Li and Quiros). These primers enable the system to detect polymorphisms.

This method can be applied to a rapid cycling version of *Brassica rapa*. Carolina Biological Supply company and University of Wisconsin-Madison teamed up to market a fast growing plant for use in the educational classroom. They call their system Wisconsin Fast Plants. These rapid-cycling brassicas were developed over a 15 year period by Professor Paul H. Williams. *B. rapa* plants are related to mustards and cabbages. The rapid-cycling brassicas are an excellent tool for classrooms because they take up little space, 2500 plants per square meter, grow from seed to flower in 18 days, and produce seed by day 28. All of this is accomplished using fluorescent lighting (Williams, P.H.).

With the addition of rapid-cycling brassicas to our lab we felt we could develop a simple system whereby students could identify *B. rapa* plants with different traits. In fact, the goal was to find polymorphisms that would distinguish the two true-breeding *B. rapa* parents from their subsequent F1 offspring. To test this idea we recruited three of our biology students to test this project.

Results

Using primer pair combinations (Me1/Em1, Me4/Em1, Me4/Em3, Me4/Em6, Me5/Em6) the parental purple stem, parental non-purple stem and F1 purple stem plants were tested for polymorphisms. With the Wisconsin Fast plants we were able to get numerous bands, representing polymorphisms, from each of the three plants tested. Figures 1, 2, and 3 are DNA gels that show the banding patterns for each plant. Because we did not target one specific gene the F1 purple plant is not exactly the same banding pattern as the purple parent. This is reasonable because other genes were inherited from both parents.

Primer pairs that worked well and gain different banding patterns for all three samples were Me1/Em1, Me4/Em1, Me5/Em6, and Me4/Em6. Me4/Em3 was a smear on the two parents (probably degraded DNA) and good bands on the F1 plant. In figures 1, 2, & 3 the banding patterns in the red box indicate polymorphisms for all three plant sample and are the best to use in a classroom setting.

Figure 1

Parental type (Purple Stem) SRAP PCR products using different primer pairs for the Purple Stem parent plant

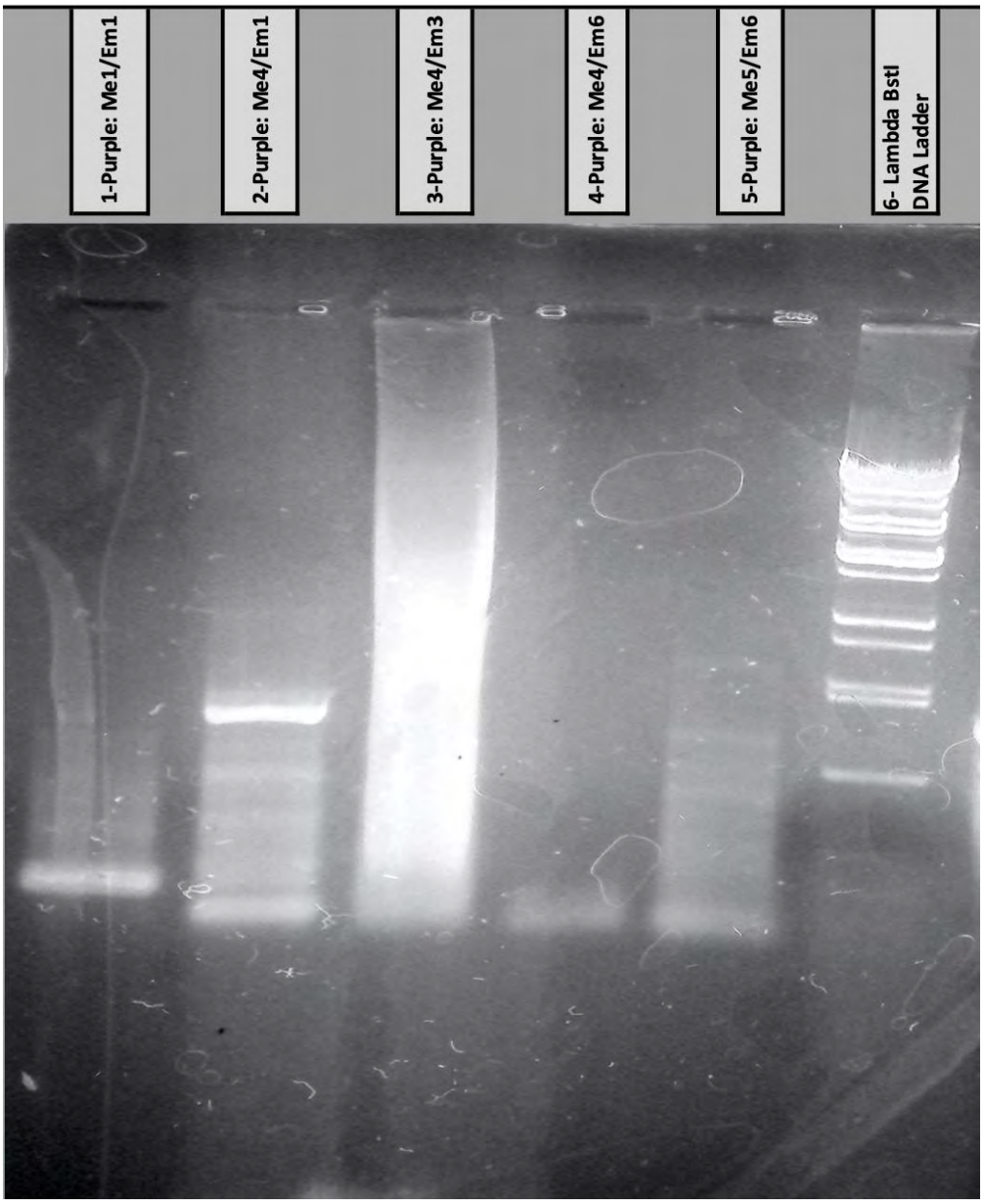


Figure 2

Parental type (Non-Purple Stem) SRAP PCR products using different primer pairs for the Non-Purple Stem parent plant

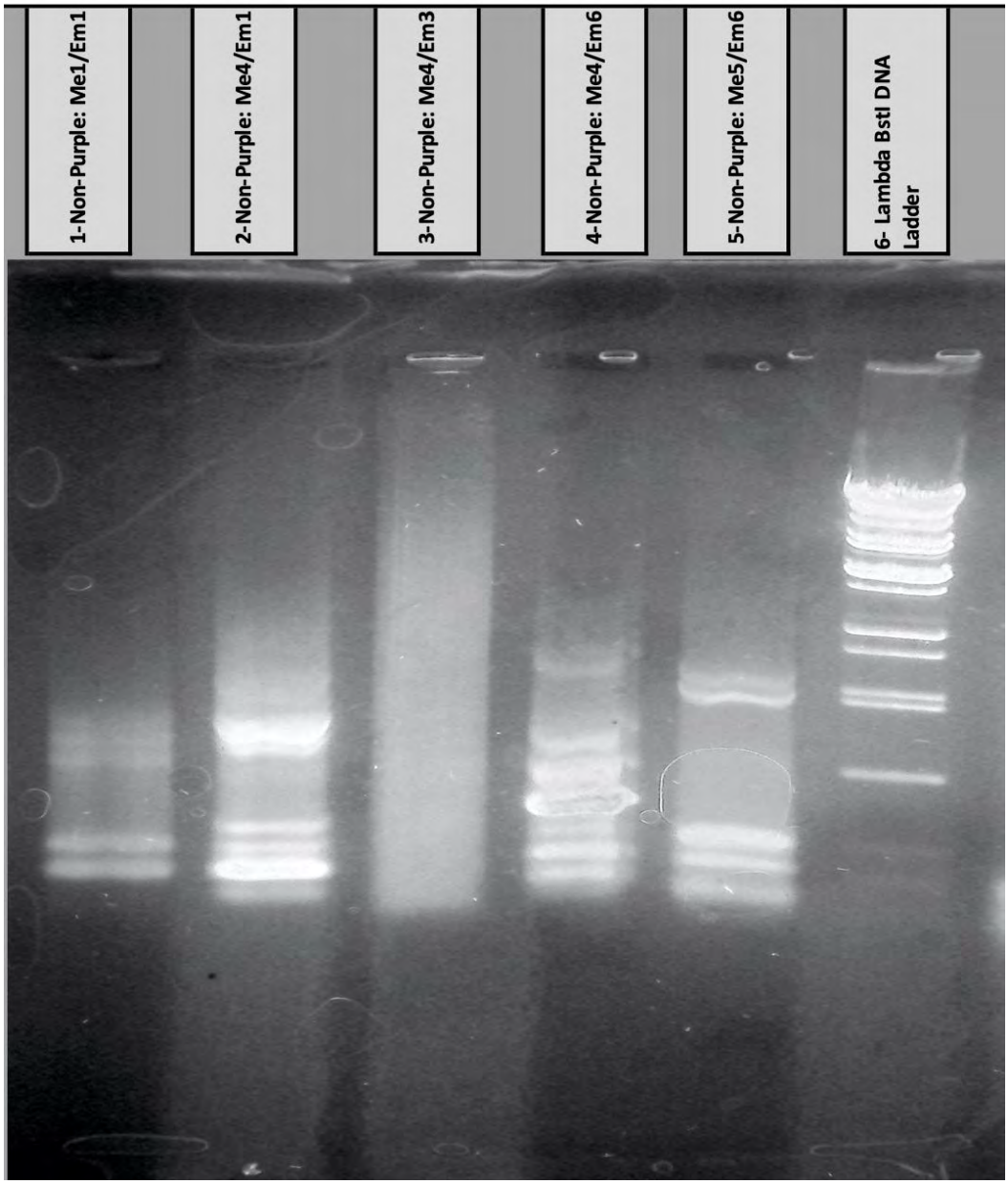
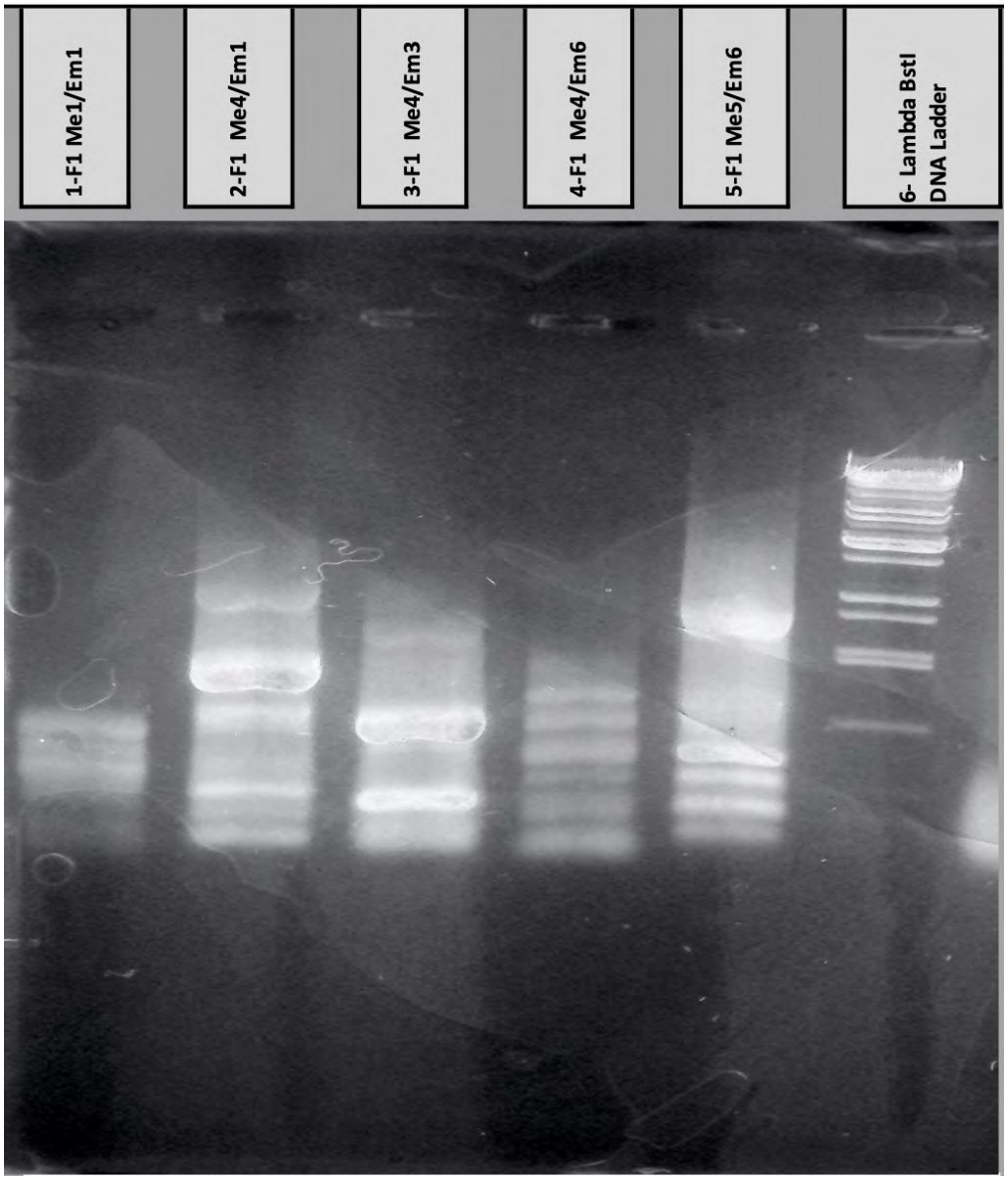


Figure 3

First filial generation (F1) SRAP PCR products (Parents-Purple vs Non-Purple) using different primer



Discussion

Three students tested the system, and the results indicate this system works for differentiating the parental and F1 Wisconsin Fast Plants. The experiment is very easy to do. Students could be given a tube of the PCR master mix to which they will add a leaf sample. From there it will go into a thermocycler so that it will be ready to run on gel electrophoresis the next day or next class. Gels can be premade for the students or students can make their own if the instructor is comfortable with the addition of ethidium bromide (a mutagen). Subsequently, our lab has switched from ethidium bromide to a non-toxic stain called Gel Green Nucleic Acid Gel Stain from Minipcr.com. We run mini-gels which takes about an hour after which it is ready to view on the UV transilluminator. All in all this experiment can be completed over two lab days once the Wisconsin Fast plants have germinated for a minimum of three days.

Methods

Plant Material

From each plant a 1mm piece of leaf was excised just prior to the experiment. Fast-cycling brassicas were planted from seed using Parental types of Purple Stem (dominant) and a mutant non-purple stem (recessive). The F1 plant was a cross between two parental types and yielded the dominant phenotype of purple stem. The Fast-cycling brassica seeds were purchased from Carolina Biological Supply, Burlington, North Carolina.

Protocol for the SRAP Marker System

Primer pairs were selected from the methods of Li and Quiros. The following primers were ordered from Operon Technologic Inc., Alameda, CA:

Sequence (5'—3')

- Me1 (forward)—TGAGTCCAAA CCGG ATA
- Me4 (forward)—TGAGTCCAAA CCGG TAG
- Me5 (forward)—TGAGTCCAAA CCGG TGT
- Em1 (reverse)—GACTGCGTACG AATT TGC
- Em3 (reverse)---GACTGCGTACG AATT AGC
- Em6 (reverse)---GACTGCGTACG AATT AAT

DNA Amplification

DNA amplification was completed using Terra PCR Direct Polymerase Mix from Clonetech, Mountain View, CA

A 1mm piece of fresh plant leaf was added directly to the Terra PCR Direct Polymerase Mix per Table 1 (3). We followed the thermocycler settings used by Li, Y, Fan, X., Shi, T., Zhang, Q., & Zhang, Z as follows: One cycle at 94 C for five minutes, five cycles at 94 C for one minute, 35 C for one minute, and 72 C for one minute, then thirty-five to forty cycles at 94 C for one minute, 50 C for one minute, and 72 C for 1 minute. PCR ended with one cycle at 72 C for five minute and then held at 4 C.

Table 1.

Recipe list to make the master mix for Terra PCR Direct (4*)*

Table I: Recommended Master Mix		
Reagent	**Volume**	**Final Conc.**
2X Terra PCR Direct Buffer (with Mg^{2+}, dNTP)	25 μl	1X[a]
Primer 1	15 pmol	0.3 μM
Primer 2	15 pmol	0.3 μM
Tissue Sample/Extract	≤ 5 μl[b]	
Terra PCR Direct Polymerase Mix	1 μl	1.25 U
Sterile Water	up to 50 ul	
Total Volume per reaction	50.0 μl[c]	

Gel Electrophoresis

DNA from PCR was separated by size on a 1% agarose gel and visualized using 1ul of 10mg/ml stock of ethidium bromide added to the gel while still molten and allowed to solidify.

Acknowledgments

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References

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