Exposing Students and Teachers to Plant Molecular Genetics with Short-Term Barley Gene Mapping Projects

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ABSTRACT

Many universities sponsor science research programs during the summer to provide hands-on laboratory experience to high school students and teachers. Our objective was to design a project that exposes the students to the full range of research, from developing and testing a hypothesis through presenting research results. The project goal is to demonstrate mapping of morphological traits using simple sequence repeat (SSR) markers. Students learn how to evaluate plants for morphological marker traits, extract DNA, conduct PCR (polymerase chain reaction) and gel electrophoresis, evaluate results, and conduct linkage analysis between traits and markers. Mapping data are presented for five of the barley (*Hordeum vulgare* L.) traits analyzed since 1997. The genes bracteatum (*bra-a.001*) and short awn (*lks.o*) were located on the short arm of chromosome 7H, the glossy phenotype conferred by *cer-zv.268* was located near the centromere of chromosome 4H, fragile stem (*fst2.b*) was located near the centromere of chromosome 1H, and intermedium spike (*int-h.42*) was located on the short arm of chromosome 5H. The high school teachers have used their experiences to help teach genetics and molecular biology, and several have included the projects in their portfolio for an advanced degree. Many students have used their projects for science fairs and their experience to help obtain part-time jobs during their undergraduate studies. These experiments provide the necessary components of a successful research project for short-term programs and provide meaningful data to advance our ongoing projects, benefitting both the students and our laboratory.

MANY universities host summer science programs to involve high school students and teachers in hands-on research projects. North Dakota State University (NDSU) has hosted the North Dakota Governor's School for high school juniors and seniors since 1990 and hosted the North Dakota Science Teacher Enhancement Project from 1997 to 2000. The goal of these programs was to provide a hands-on, 6-wk research experience mentored by a faculty member. The participants conducted research, wrote a research paper, and gave an oral presentation of their research project at the end of the 6-wk session.

A successful research project for these short programs must provide:

1. A testable hypothesis
2. Exposure of the participants to published research articles
3. Training in new techniques, including dealing with technical problems
4. Generation and analysis of data
5. Meaningful data for the host laboratory's research program
6. Close interaction with the project leader and support staff

During the last 6 yr, we have developed and refined a summer research program in barley genetics that meets these requirements, which is described in this article. The success of this program has come from the availability of numerous morphological marker lines in barley and PCR (polymerase chain reaction)–based simple sequence repeat (SSR) markers that provide nearly complete coverage of the barley genome (Ramsay et al., 2000).

More than 1000 morphological markers have been identified in barley (Franckowiak and Lundqvist, 2002). These phenotypic traits were observed as spontaneous or artificially generated mutants in a wide range of cultivars over decades of research. Traits identified with alternate alleles include plant height, spike morphology, and seed size among others. Approximately 275 of these markers have been placed on the morphological marker linkage map of the seven barley chromosomes (Franckowiak, unpublished, 2002) and/or on the consensus molecular marker linkage map (Kleinhofs, 2002); approximately 150 to 200 additional markers have been placed on other maps but the rest have not been mapped.

Simple sequence repeat markers are PCR-amplified regions of two or three base DNA repeats. Primers were designed to anneal to DNA on either side of each repeated segment, so size differences in amplification products are caused by different numbers of repeats in different genotypes. The PCR reactions are easy to set up, do not use any hazardous chemicals, and only take a few hours for amplification. Products can be separated on agarose or acrylamide gels and results can be obtained in 1 d, which made these molecular markers ideal for short-term projects.

MATERIALS

Near-isogenic lines, each containing a single morphological marker, were created by backcrossing the cultivar or genetic stock expressing the trait four to nine times to 'Bowman', a two-rowed barley cultivar developed at NDSU (Franckowiak et al.,1985). Each backcross (BC) involved selection of F_2 plants that expressed a morphological trait and that most resembled Bowman for other traits. By the BC_3, most (>90%) genetic differences should be linked to the trait.

METHODS

Several steps were conducted before the students arrived. First, a set of backcross-derived morphological marker parent

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Abbreviations: BC, backcross; NDSU, North Dakota State University; PCR, polymerase chain reaction; RCF, relative centrifugal force; SSR, simple sequence repeat.

lines and the recurrent parent Bowman were grown in 15-cm clay pots in a potting mix (Sunshine mix no. 1, SunGro Horticulture Canada Ltd., Bellevue, WA) supplemented with a slow-release fertilizer (Osmacote 14–14–14, Scotts-Sierra Horticultural Products Co., Marysville, OH) in the greenhouse, maintained at 20 to 25°C, with natural lighting supplemented with sodium vapor bulbs to provide 16 h of light per day. DNA was extracted from leaf tissue of each parent line and screened for polymorphism using SSR markers. As many as five morphological marker lines that showed several SSR polymorphisms compared to Bowman were selected for each summer session. A population of 50 BC_xF_2 plants per line was grown so the trait was visible during the 6-wk program.

When students arrived, they received a handout (available on request) describing some basic genetic concepts and terminology, such as DNA, alleles, types of gene action, phenotype, genotype, gene segregation, and recombination. The handout also described molecular and morphological markers, chi-square goodness-of-fit tests, PCR, SSRs, DNA extraction, gel electrophoresis, mapping, and specifics on barley and the morphological marker lines selected for study. After the students had a chance to read through the handout, we discussed the project and answered any initial questions. We then initiated their project by collecting leaf tissue and extracting DNA, emphasizing the importance of accurate labeling of the tubes used in each step.

The DNA extraction procedure was modified to exclude highly hazardous chemicals. Approximately 100 mg of leaf tissue (a 5- to 7-cm piece) from each BC_xF_2 plant and the parents was collected in a 1.5-mL microcentrifuge tube and frozen in liquid N. The tissue was ground in 500 μL extraction buffer (0.1 *M* Tris-HCl, pH 8.0; 0.05 *M* EDTA, pH 8.0; 0.5 *M* NaCl; 0.32% 2-mercaptoethanol), vortexed, and 33.3 μL 20% sodium dodecyl sulfate added. Samples were incubated at 65°C for 10 min. Potassium acetate (166 μL, 5 *M*) was added, and samples were mixed gently and incubated on ice for 20 min. Samples were centrifuged at 15 800 relative centrifugal force (RCF) in a microcentrifuge at 4°C for 10 min; then the supernatant was transferred to a fresh tube and centrifuged again. After the supernatant was transferred to another tube, 550 μL isopropanol was added, and the solution gently mixed until the DNA precipitated. The tube was centrifuged at 4000 RCF for 1 min to pellet the DNA, and the supernatant was removed. The pellet was washed with 500 μL 70% ethanol, centrifuged at 15 800 RCF for 2 min and the ethanol was removed. The pellets were air-dried for at least 10 min, 200 μL TE (10 m*M* Tris-Cl, pH 7.4 and 1 m*M* EDTA, pH 8.0) were added, and the samples were refrigerated overnight to allow the pellet to dissolve.

Simple sequence repeat reactions were conducted in 96-well plates in a Tetrad thermocycler (MJ Research, Waltham, MA). Each 20-μL reaction contained 1 unit Taq DNA polymerase (Promega, Madison, WI), 1x PCR buffer (supplied by the manufacturer), 2 m*M* $MgCl_2$, 800 μ*M* dNTPs, 0.3 μ*M* forward and reverse primers (Ramsay et al., 2000), and approximately 50 ng template. The temperatures for PCR cycles depended on the marker being analyzed (Ramsay et al., 2000). After PCR was completed, 4 μL of gel loading buffer (46% glycerol, 3.8X TBE [5X = 450 m*M* Tris-borate, 10 m*M* EDTA], bromophenol blue to color) was added to each sample and SSR products were separated on 20 by 25 cm 4% Agarose SFR (Amresco, Solon, OH) gels run at 130 V for 6 h. Gels were stained in ethidium bromide (10 mg mL^{-1}) for 30 min and photographed under UV light using a UV transilluminator (Fotodyne, Hartland, WI) and a Polaroid MP-4 camera system (Cambridge, MA). As an alternative, gels were stained in SYBR-Green I nucleic acid gel stain (Molecular Probes, Eugene, OR), which can be used for science fair projects and has a lower health risk than the mutagen ethidium bromide. The students then determined whether each BC_xF_2 plant had the SSR marker from Bowman, the morphological marker parent, or was heterozygous.

At the appropriate time, the students were taught how to score the BC_xF_2 population for the phenotypic trait. Most of the morphological traits were recessive, so the populations were tested for a 3:1 normal/mutant single gene segregation ration using the chi-squared test (Steel and Torrie, 1980). The students were then taught how to put their data into the proper format for linkage analysis, and how to run the MAPMAKER program (Lander et al., 1987; Lincoln et al., 1992). MAPMAKER uses multipoint linkage analysis to construct maximum likelihood maps for any set of markers segregating in an experimental cross. The program determines which marker order is most likely and calculates the centiMorgan distances between markers for the entered data. The BC_xF_2 plants were grown to maturity and harvested. Nine BC_xF_3 plants from each dominant BC_xF_2 were grown to separate heterozygotes from homozygotes. Knowing the trait's allelic genotype on all 100 chromosomes that carry the gene in the plants instead of only the phenotype of 50 chromosome pairs increased the accuracy of the linkage analysis. In many years, the high school students returned during the school year to fill in missing SSR data, test BC_xF_3 plants, and map additional marker traits. They then used the completed experiments for science fair projects.

RESULTS AND DISCUSSION

The project handout used each summer has been modified over the years to expand discussion of more difficult concepts, especially chi-square analysis. Literature provided to all students included Ramsay et al. (2000), the MAPMAKER tutorial and reference manual (Lincoln et al., 1992), and *The Cartoon Guide to Genetics* (Gonick and Wheelis, 1991). Other literature provided specific information on questions often asked by the students.

Populations of at least three different morphological markers were used each year since 1997. Because of the limited number of SSR markers available (40, Liu et al., 1996), the first 2 yr met with limited success. Linked markers to only 2 of the 11 morphological traits tested in 1997 and 1998 were discovered. Other markers tested were not linked or not polymorphic. In 1999, the Ramsay et al. (2000) SSR primer sequences became available. They represented a much better coverage of the barley genome and we have used 200 of the higher quality SSRs to identify polymorphisms. We could then select populations that had at least two SSR polymorphisms on the same chromosome between the morphological marker parents and Bowman. This increased the success of linkage discovery. We also selected traits that were easy to evaluate visually, and timed the planting so all traits were expressed during the 6-wk program. A single student usually could determine linkage in two to three populations, while groups of

two teachers and a student could map three to five populations. Teams of teachers and students were particularly successful, increasing project discussions and allowing them to divide the work between them and accomplish more in a shorter time.

The first week, the students were trained in all the techniques, including collecting leaf tissue, extracting DNA, conducting PCR and gel electrophoresis, evaluating marker data, and, if possible, scoring morphological marker data. At the end of the week, we reviewed the handout with the students, discussed any questions, and provided additional literature. By this time, the students could estimate the length of time needed for each part of the project. We then discussed the objectives of the full project and helped them determine a time-line to finish the experiments and prepare their talks and papers. Participants in both programs were required to write a short research paper (5–10 pages) following the standard format of abstract, introduction with hypothesis and objectives, materials and methods, results and discussion, and references. Oral presentations (30–45 min for North Dakota Science Teachers Enhancement Program; 20 min for North Dakota Governor's School) also were required. These were prepared using PowerPoint software (Microsoft, Seattle WA), after training as part of the 6-wk program.

We found it useful to have at least one morphological marker that could be evaluated early in the 6-wk program. This allowed the students to conduct linkage analysis as soon as they had SSR marker data. MAPMAKER is a MS DOS-based program, and we used the DOS editor to create the data files. Few if any students had experience with DOS, so running these programs often was a challenge for them. Also, when linkage was identified between a SSR marker and the morphological trait, students became more excited about the project and were eager to generate more data for linkage analyses.

Five of the mapped morphological marker traits and their pedigrees are listed in Table 1. Lines from this subset of those evaluated were backcrossed four to seven times to generate segregating BC_xF_2 populations. When possible, BC_xF_3 trait data were used for the final linkage analysis, providing more information for these recessive traits. All five traits showed 3:1 segregation, indicating they were controlled by single genes.

The first population segregated for the bracteatum (*bra-a.001*) trait (Lundqvist and Franckowiak, 2002). Neither the parent nor BC_4F_3 plants expressed bracteatum in the summer greenhouse, indicating that expression of this trait was environmentally sensitive. Linkage analysis was based on BC_4F_2 evaluation. The *bra-a.001* gene was linked to three SSR markers on the short arm, proximal to the centromere of chromosome 7H (Fig. 1a). The BC_5F_3 data were unavailable for the

Table 1. Morphological marker lines, pedigrees, and number of backcrosses to 'Bowman' barley (*Hordeum vulgare* L.).

Gene	Trait	Pedigree†	No. backcrosses
bra-a.001	Bracteatum	Bowman*5/bra-a.001	4
cer-zv.268	Glossy	Bowman*6/cer-zv.268	5
fst2.b	Fragile stem	Bowman*8/BGS208 DWSI171	7
int-h.42	Intermedium spike	Bowman*6/int-h.42	5
lks.o	Short awn	DWS 1233 10015/7*Bowman	6

† Franckowiak and Lundqvist (2002).

population segregating for the glossy plant trait provided by *cer-zv.268* (Lundqvist and Franckowiak, 1997a). This glossy gene was located near the centromere of chromosome 4H, linked to two SSR markers (Fig 1b).

The BC_xF_3 populations were available for the remaining three morphological markers, which allowed identification of the heterozygotes, providing more accurate mapping. Analysis of *fst2.b*, which confers a fragile stem and leaf phenotype (Franckowiak and Konishi, 1997), showed that this gene was linked to two SSR loci near the centromere of chromosome 1H (Fig. 1c). The intermedium spike locus (*int-h.42*) (Lundqvist and Franckowiak, 1997b) was located on the short arm of chromosome 5H, linked to three SSR markers (Fig. 1d). The short awn locus (*lks.o*) was linked to two SSR markers on the short arm near the centromere of chromosome 7H (Fig 1e). The *fst2.b* gene was previously mapped on the long arm of chromosome 1H near the centromere (Tsuchiya, 1972). The *lks.o* locus had been previously assigned to chromosome 7H, based on linkage drag with the red stem locus (*Rst1*) (Franckowiak, unpublished data, 2002). We have located this locus more precisely using the SSR markers. The other three loci (*bra-a.001*, *cer-zv.268*, and *int-h.42*) had not been mapped previously, so these summer projects provided new information for barley geneticists by using near-isogenic lines, SSR markers, and linkage mapping to locate gene positions on chromosomes.

Since 1997, eight high school teachers and six high school students have participated in this research. The projects successfully exposed students to the concepts of genetics and to hands-on research, including developing a hypothesis, conducting all aspects of a research project including troubleshooting technical problems, analyzing data, and presenting research results orally and in short papers. Several of the teachers have used the research papers in their portfolios to support advanced degrees, and used the knowledge gained when discussing genetics and molecular biology in their classes. One teacher in a local high school has brought her Advanced Placement Biology class to visit our laboratory each

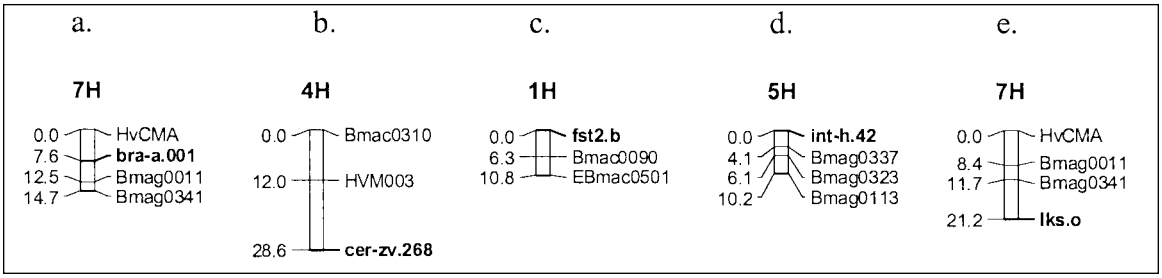

Fig. 1. Linkage groups showing the distances between morphological marker traits (bold) and simple sequence repeat (SSR) markers; (*a*) bracteatum (*bra-a.001*); (*b*) glossy (*cer-zv.268*); (*c*) fragile stem (*fst2.b*); (*d*) intermedium spike (*int-h.42*); and (*e*) short awn (*lks.o*). CentiMorgan distances using the Kosambi mapping function are shown on the left side of each map.

year to learn about mapping genes, and she describes her project to her students when they have a laboratory class on gel electrophoresis. Another teacher from a more distant high school has brought students to our laboratory every few years for a tour and discussion. Governor's School students have competed in regional, national, and international science fairs using their summer project as a base for their presentations. Most of the students chose a science major as they entered college, and their hands-on experience has helped them obtain laboratory jobs as undergraduate students. Similar projects could be done with any plant species that has morphological marker lines available and a reasonable number of PCR-based molecular markers.

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