Communicating Research to the General Public

The WISL Award for Communicating PhD Research to the Public launched in 2010, and since then over 100 Ph.D. degree recipients have successfully included a chapter in their Ph.D. thesis communicating their research to non-specialists. The goal is to explain the candidate's scholarly research and its significance—as well as their excitement for and journey through their area of study—to a wider audience that includes family members, friends, civic groups, newspaper reporters, program officers at appropriate funding agencies, state legislators, and members of the U.S. Congress.

WISL encourages the inclusion of such chapters in all Ph.D. theses everywhere, through the cooperation of PhD candidates, their mentors, and departments. WISL offers awards of $250 for UW-Madison Ph.D. candidates in science and engineering. Candidates from other institutions may participate, but are not eligible for the cash award. WISL strongly encourages other institutions to launch similar programs.

Wisconsin Initiative for Science Literacy

The dual mission of the Wisconsin Initiative for Science Literacy is to promote literacy in science, mathematics and technology among the general public and to attract future generations to careers in research, teaching and public service.

Contact: Prof. Bassam Z. Shakhashiri

UW-Madison Department of Chemistry

bassam@chem.wisc.edu

www.scifun.org

Genetic Dissection of Bacterial Leaf Spot Response in *Beta vulgaris* Through Genome-Wide Association and Linkage Mapping

By:

Audrey K. Morrison

A dissertation submitted in partial fulfillment of

the requirements for the degree of

Doctor of Philosophy

(Plant Breeding and Plant Genetics)

at the

UNIVERSITY OF WISCONSIN- MADISON

2026

Date of final oral examination: 06/19/2026

The dissertation is approved by the following members of the Final Oral Committee:

Irwin L. Goldman, Professor, Plant and Agroecosystem Sciences

Natalia de León Gatti, Professor, Plant and Agroecosystem Sciences

Andrew Bent, Professor, Plant Pathology

Sarah J. Pethybridge, Professor, Plant Pathology and Plant-Microbe Biology Section, Cornell University

Chapter 5: A Genomic Approaches to Understanding Bacterial Leaf Spot Response in Table Beet

As Part of Communicating Dissertation Research to the General Public
Wisconsin Initiative for Science Literacy

Wisconsin Initiative for Science Literacy

Thank you to the Wisconsin Initiative for Science Literacy (**WISL**) for the opportunity to dedicate a chapter of this dissertation to explaining my Ph.D. research to a general audience. The WISL, in collaboration with the UW-Madison Graduate school, provides an award for Ph.D. candidates who choose to write and include a chapter that communicates their scholarly research and its significance to a wider audience. In the writing process, I was assigned to work with Elizabeth Reynolds, an editor that worked with me to shape this chapter into its current form. WISL, directed by Professor Bassam Shakhashiri, was originally encouraged for all UW-Madison chemistry Ph.D. candidates, and has expanded across colleges and programs. I am excited to be able to take part in this initiative representing the Plant Breeding & Plant Genetics program.

As a graduate student, I attended several conferences where I presented a poster on my research. All of these presentations were to scientists, students, or industry personnel who had a science background, yet, each presentation was slightly altered depending on who was standing before me. In August of 2023, the Goldman Lab was invited by Pat Slattery, an organic vegetable farmer in the Driftless region, to present about beets, onions, and carrots to local farmers in that area. The Goldman Lab, led by Dr. Irwin Goldman, is a root vegetable breeding and genetics lab here at UW-Madison, and it is where I conducted my graduate research. In

existence for over 75 years, this program has developed numerous cultivars and breeding populations of table beets, onions, and carrots, and serves as the only public program that breeds table beets, and one of few that works with onions and carrots. Excited about the unexpected invitation, our lab traveled to Bangor, WI, to meet Pat and other farmers at a local church fellowship hall where they provided a potluck dinner for us.

For our presentations, we brought roots of our crops for tastings and were able to pass those around as we talked about our research, breeding efforts, and cultivation/management practices. Tailoring a research talk to an audience of farmers, most of whom were Amish, was a very different task for me compared to giving the same talk at academic conferences. I brought photos and *attempted* to present my work and objectives in ways that blended the importance with practicality for my audience. Frankly, this was a very difficult task compared to the academic conferences I had previously presented at. Those in attendance in the church basement asked wonderful questions and provided their own experiences growing these crops. I think I learned more from them than what they learned from me.

In all, this was a unique event that not many graduate students experience in contrast with academic research talks, seminars, or conferences. It really tested me to be able to communicate my work to the individuals whom it impacts the most. When I heard about the WISL opportunity to write a dissertation chapter to the general public, I was instantly reminded of this 'farmer extension day' and wanted to take part in this opportunity to continue to work on my science communication skillset. Communication skills are necessary for every position and relationship you have in life, and communicating my dissertation research is one step in continuing to improve on this skill. Since most of this work was funded by a United States

Department of Agriculture Specialty Crops Research Initiative grant, I do feel a sense of responsibility to communicate these results not only to the science community, but to the general public. The Wisconsin Idea states, “The boundaries of the University are the boundaries of the state.” I believe WISL encourages students to live up to this proclamation; rather than confining research findings to the ivory tower of academic research journals or university institutions, devoting a chapter instigates us to disseminate that information.

Overview

In 2019, the Goldman Lab began participating in a multi-disciplinary team spanning many universities that set out to study bacterial leaf spot (**BLS**). Many different crops are affected by bacterial leaf spot, which is a general type of a foliar disease identified by lesions (dead leaf tissue). While the grant covers the study of bacterial pathogens affecting cucurbits (pumpkin, squash, watermelon, etc.) and Amaranthaceae crops (beet, Swiss chard, etc.), our lab is focused on BLS caused by the bacterium *Pseudomonas syringae* pathovar *aptata* (***Psa***), which causes BLS in table beet and Swiss chard.

Psa enters the plant through natural openings or through wounds, causing BLS. BLS is a foliar disease emerging in production areas for the affected crops. Lesions caused by *Psa* appear tan, brown, or black, and the healthy tissue surrounding the lesion often puckers, is distorted, and/or produces other stress responses like pigmentation (Figure 5.1). These symptoms are favored by cool, wet conditions, which coincide with conditions experienced in the early growing season. The baby leaf salad industry, for which beet and chard leaves are grown, has a strict threshold: if greater than 5% of the crop shows leaf infection, it can be rejected from the processing plants due to difficulty of sorting.

In a market where the beet crop is grown for root production, an extreme foliar disease causing complete defoliation would be devastating. Root crops are typically harvested by machine top-pullers, which require intact foliage to function. BLS has been diagnosed in both Swiss chard and table beet crops across many states: New York, Arizona, California, Georgia, Oregon, Ohio, and others in which a formal report has not been made.

Figure 5.1. Bacterial leaf spot lesions, caused by bacterium *Pseudomonas syringae* pathovar *aptata* observed on a) Swiss Chard and b) table beet foliage following inoculations. Swiss chard photo was taken at a 2015 field Washington State University field trial and provided by Dr. Lindsey du Toit. Table beet photo taken in 2025 by Audrey Morrison following inoculations at West Madison Agricultural Research Station (Verona, WI).

Some genotypes of *Psa* can infect beet and chard, while some only affect beet. BLS is a **seedborne disease**, allowing it to spread rapidly as seed is produced, transported, and planted. For the disease to be seedborne, it means the pathogen resides in certain parts of the seed (specifically, *Psa* resides in the corky outer layer of the seed called the pericarp) and can cause foliar damage to the seedling and crop as it grows. In some cases, removal of the pericarp in seed processing can limit the spread of BLS. In order to control, prevent, and/or limit BLS

prevalence, crop rotation, seed testing, drip irrigation, and tilling of crop residue after harvest may help. Copper based bactericides (like a disinfectant spray) have been shown to slow disease progression, but there is nothing on the market that will eradicate BLS.

Prior to my start as a graduate student in January of 2022, there was a previous student in the Goldman Lab, Emilee Gaulke, that started the work on this grant, screening both table beet and Swiss chard cultivars ('cultivated varieties'). Emilee inoculated plants in both vegetative growth stages (production of swollen roots, leafy greens) and in reproductive growth stages (production of seed). She observed differences in cultivars and found that there was no strong correlation between BLS response in vegetative versus reproductive life cycle. I was hired as a graduate student to follow up on this work. Because UW-Madison houses the only table beet breeding program in a public institution, the only other research being done is either by industry (private) or international institutions. I was excited to be able to contribute to the table beet community through this research.

Ph.D. Research Project One: GWAS

Introduction and experimental methods

A Genome Wide Association Study (**GWAS**) is a statistical study that attempts to find a statistical association between a **genotype** (an organism's genetic information) and **phenotype** (its observable physical traits), utilizing a large, diverse collection of individuals (in my case, plants). It asks the question: "**Is there a position in the genome** (genetic instructions, DNA) **responsible for trait X?**". That question is asked at every genetic maker position available. If the

answer to that question is “yes”, then those genetic variant positions are said to be **associated** with the trait of interest.

This type of study relies on using what is called a diversity panel: diverse collections of germplasm (broad pool of genetic plant material) that differ in the trait you are studying. To conduct the GWAS, the Wisconsin Beta Diversity Panel was assembled by researchers here at UW-Madison in the Goldman Lab. This diversity panel included germplasm within the *Beta vulgaris* crop complex: table beets, Swiss chard, sugar beets, fodder beets, and *Beta vulgaris* subsp. *maritima* (the wild ancestor to beets/chard) (Figure 5.2). This panel comprised over two hundred distinct germplasms within this crop family, with a wide variety of phenotypic expression for all traits. Some of the accessions (plant material specimens: cultivars, populations, etc.) in the diversity panel had been screened by prior researchers, so I had some indication on how they would perform with additional BLS screenings. For most, however, I did not have prior knowledge of their BLS resistance/susceptibility.

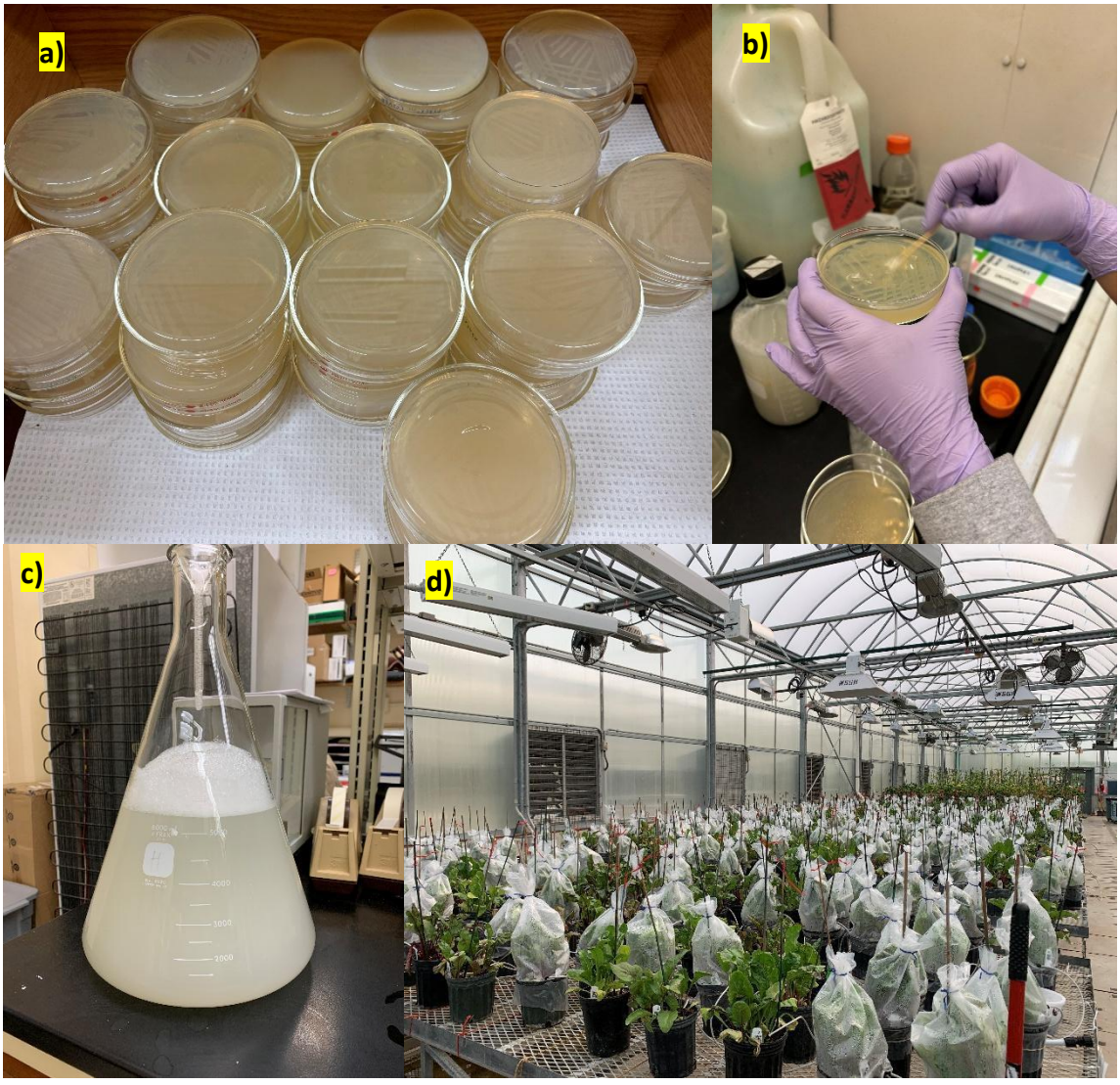
Figure 5.2. The *Beta vulgaris* crop complex and crop types used in the Wisconsin Beta Diversity Panel. Swiss chard (top left), table beet (left center), sugar beet (right center), Beta maritima (top right), and fodder beet (bottom).

I utilized 219 accessions as part of this diversity panel, and 152 of them were table beets. None of the accessions I used were bred, developed, or created for this project: they were already on the market, collected, or part of existing populations. I ran BLS screening experiments at the West Madison Agricultural Research Station (Verona, WI) greenhouses. Because BLS has proven to be devastating for the baby leaf industry, I focused on growing conditions that might emulate those of the baby leaf industry. Therefore, when I chose to inoculate the accessions in the experiment, I chose to do so at the two true leaf stage, which is about when the baby leaf growers begin to harvest. **Inoculation** is the process of introducing a pathogen into a plant using **inoculum**, or in my case, a bacterial solution with my pathogen of interest (*Psa*).

To generate inoculum, I utilized storage reserves (frozen) of a *Psa* strain, or sample, that was used in previous BLS screenings and was found to be **pathogenic** (disease-causing) on both beet and chard. This was advantageous since I had so many different crop types in the diversity panel. I made nutrient agar plates and swabbed the *Psa* on the plates which allowed bacterial colonies to grow and populate (Figure 5.3 a)). I used sterilized water to wash the colonies off the plate (Figure 5.3 b)), forming a very concentrated bacterial solution (Figure 5.3 c)). I adjusted this concentration to 1×10^8 colony-forming units per milliliter using a spectrophotometer, to ensure I had a standard inoculum solution for each experimental screening. A spectrophotometer is an instrument that measures how much light a solution (in my case, bacterial solution) absorbs or transmits by passing a beam of light through it across specific wavelengths. Using this information, researchers can determine the concentration of

the solution tested. In bacterial terms, colony forming units, or CFUs, per milliliter refers to an estimate of the number of viable bacterial cells per mL.

Figure 5.3. GWAS inoculation materials. Panel **a)** shows agar plates with *Pseudomonas syringae* pathovar *aptata* colonies growing. Panel **b)** depicts making a bacterial solution, before concentration adjusting, by washing the colonies off of the agar plates. Panel **c)** shows an adjusted bacterial solution ready for inoculation. Panel **d)** shows experimental design at West Madison Agricultural Research Station in Verona, WI. Due to the *Beta vulgaris* diversity panel having different germination and maturity rates, "batches" of inoculations were conducted, each batch including the pots in which accessions were at the two true leaf stage. Bagged humidity chambers were placed over each pot 24 hours before and after inoculation. Photos taken by Audrey Morrison.



I put a “bagged humidity chamber” over each pot 24 hours before inoculation, removed as I inoculated, and then put back in place for 24 hours post inoculation before permanent removal (Figure 5.3 d)). These humidity chambers increased leaf wetness, and the humidity in the bag helped to open stomata (pores on plant leaf and stem surface) to assist bacterial delivery into the plant. It is common for baby leaf growers/producers to utilize low tunnels over beds of the crop for protection (Figure 5.4). This is the inspiration for creating a humidity chamber on each pot, and foreshadowing for the next project (I refined the design and made it a bit closer to Figure 5.4 in the second part of my Ph.D. research).

Figure 5.4. Low tunnels using agricultural plastic are commonly used by baby leaf salad green producers to protect beds from cold temperatures or weather elements. When the sun is out and temperatures are warmer, these low tunnels are often vented (right most photo). Figure courtesy of the Washington State University Extension publication ‘Baby-leaf salad green production guide for Western Washington’ (1).

I sprayed inoculum onto the leaves of the diversity panel accessions in each pot in the greenhouse, and seven days later I went through and visually gave each pot a BLS response score based on the percentage of diseased leaf area. I used a scoring rubric with intervals from

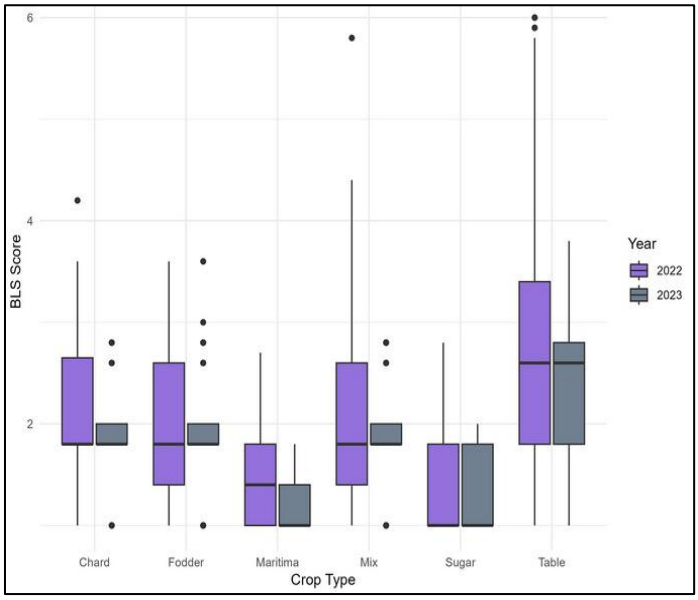
0-7, with each number equating to a percentage of diseased leaf area (Table 5.1). It was critical to conduct this rating with as much accuracy and consistency as possible.

Table 5.1. Scoring rubric used for GWAS assessments following inoculation with *Psa*. Visual ratings were based on percentage of diseased leaf area.

Percentage of diseased leaf area (%)	Score
0	0
1–15	1
16–30	2
31–45	3
46–60	4
61–75	5
76–90	6
91–100	7

Looking at these BLS response scores, it was obvious for some accessions if they fell into the susceptible disease category, resistant (or tolerant) disease category, or somewhere in the middle (Figure 5.5). Overall, sugar beet and *Beta vulgaris* subsp. *maritima* accessions had low disease scores, and the table beet crop type was quite variable.

Figure 5.5. Phenotypic distributions for the GWAS Bacterial Leaf Spot screenings. BLS score shown on the Y axis, the crop type on the X axis, and boxplots are colored by year.



With phenotypes (BLS response score) in hand, the only other piece needed for a GWAS is the genotype of each accession. I took leaf samples of accessions grown (non-inoculated leaf samples!) and submitted those tissues to the UW Biotechnology Center for genotyping. In return, I received one file with genetic marker (sequence) data belonging to every individual that was in the diversity panel.

GWAS results

The goal of a GWAS is to survey the genome and identify if there is a gene region (referred to as 'quantitative trait locus' or 'QTL') that is statistically associated with the trait of interest. A **QTL** is a central theme in this research and in many other genetic studies. A QTL can be thought of as a chromosomal segment, identified primarily through **molecular markers**, that contributes to the expression of a trait.

Finding a QTL is similar to looking at a roadmap: the roadmap is the plant's genome, each chromosome a different highway, and mile markers or exit numbers are like molecular markers. Molecular markers (locations on the chromosomes) can help give us directions of locations near genes of interest (our destination!). If we know the destination, in our case, BLS resistance, then identifying exit numbers/mile markers can be useful in communicating information about said destination and making it reproducible for other scientists. So here, a QTL would be a region on the highway, perhaps identified by a mile marker or exit number.

In the studies conducted for this dissertation research, molecular markers were used to identify QTLs. These markers "define" the location of the QTL, but it is important to note that with a QTL in these studies, we have only defined a **gene region**. I cannot conclude that the molecular markers within the QTL region are causal, meaning I cannot make the statement that "Marker X directly contributes to BLS response in *Beta vulgaris*". The markers can be a proxy for nearby causal variants. However, identifying a QTL is a step in the right direction towards better understanding the possible genetics responsible for BLS response in beet and beet relatives. There are many future studies and experiments that could be done with the QTLs identified in this dissertation work that could pinpoint causation, but that is beyond the scope of these initial studies.

It was clear to me a few days after inoculations that the *Beta vulgaris* germplasm that contained red pigmentation, **betacyanin**, appeared to be more susceptible than the green germplasm. I was observing betacyanin "bursts" on the red leaf material; red/purple splotches all over the leaves, in addition to lesions (Figure 5.6). On the green leaf material, I was seeing lesions that appeared more "water soaked" than the lesions on the red material which looked

more tan and crispy. Upon statistical analysis of this observation, I found that there was a correlation between leaf color and disease response; the relationship being that red leaf material appeared to have higher disease response scores (greater % of diseased leaf area) than green leaf types. Our lab previously identified differential responses in red and green leaf types, and industry beet breeders have commented that green leaf types appear to be more resistant.

Figure 5.6. Table beet leaf reaction following *Psa* inoculation. Foliage is showing few lesions, with mostly betacyanin pigment accumulation in the leaf as a response to the inoculation. Photo taken by Audrey Morrison.

Betacyanin pigments have a variety of functions in different plant species. In table beet, betacyanin is indirectly involved in plant defense response. Betacyanin is produced by plants not only in reaction to pathogens, but also to other stressors such as U.V. radiation.

Knowing this information, I considered that there was bias in my disease scoring; because I saw pigmentation and stress, I considered it part of the percentage of diseased leaf area even though the pigmentation may not have been lesions or disease, but rather a different type of defense response. However, I considered the practical application of this work. As

mentioned, any baby leaf crop load with greater than 5% of cosmetic flaws would be rejected. These facilities would include the betacyanin accumulations, produced in response to *Psa* presence, as a cosmetic flaw. Therefore, I felt as if my scoring rubric was more encompassing towards "**BLS response**" as a whole, rather than just lesion production. Cosmetic flaws are not as concerning for crops destined for root production, but the baby leaf, Swiss chard, and bunching beet markets would consider the betacyanin bursts unacceptable for marketing standards.

Because there was a weak but significant correlation of leaf color with BLS response, meaning that red leaf types tended to have higher disease ratings whereas green leaf types tended to have lower disease ratings, I factored this into my analysis. Adjusting for leaf color upfront, I can be more confident that my genetic signals were truly tied to disease response, rather than being related to leaf color. Although leaf color is not something that I am studying directly, if I ignore it in analysis, it can skew my results. There are several software programs publicly available for GWAS use in crops.

My GWAS analysis pinpointed a **QTL on chromosome one**, meaning that there is a gene region on chromosome one of *Beta vulgaris* that is associated with BLS response. Beets and other *Beta vulgaris* crops have nine total chromosomes. I found that this QTL explains upwards of **21% of variation** in BLS response. Identification of a QTL that explains a large portion of trait variation is significant information to know, as it holds more importance and hopefully would be valuable to the beet/chard research community.

Different models and parameters used within the GWAS software identified different markers, but there was consistency across them being on chromosome one. Because a large

portion of this diversity panel was table beets, I also conducted a GWAS using a **table beet subset**, removing the other crop types from analysis. This also resulted in a QTL on **chromosome one**.

Identifying a statistical association between a region on chromosome one and BLS response is promising for researchers and *Beta vulgaris* breeders. We hoped that this project would provide valuable insight for the industry and research community. However, future work needs to be done to narrow in on this region on chromosome one and conduct experiments to validate its true function and what genes are present in that region.

We can hypothesize what genes might be there based on what has been identified and annotated in the past, but at this stage we cannot conclude that the chromosome one QTLs are causal, and more work would need to be done to further explore this region. In addition to the genomic insight provided, the inoculation and screening work provides researchers, farmers, and growers with the results of cultivar performance. Beyond identifying genetic associations with disease response (like our mile marker or exit number on a highway analogy), this project also identifies which beet/chard varieties are most productive when BLS was present.

Ph.D. Research Project Two: Linkage Mapping

Comparison between GWAS and Linkage Mapping

In genetic research, there are two common ways to connect DNA to traits like disease resistance: studying natural populations (GWAS) or studying families created and bred by researchers (linkage mapping). In GWAS, scientists look at a large, diverse group of plants that have been breeding and reproducing naturally over many generations. Over time, their DNA

has been mixed and reshuffled many times. Researchers scan across the genome and look for places where certain DNA patterns tend to be present more often in plants with a particular trait. Because these plants come from many different backgrounds, this approach can be very powerful—but it can also be "noisy," since individuals may be related in unexpected ways or differ in many traits at once. This GWAS approach was just demonstrated above in my first Ph.D. project.

In linkage mapping, researchers take a more controlled breeding approach. They start with parents that differ in a trait (for example, resistant vs. susceptible), cross them, and study their offspring. These offspring carry different combinations of DNA from the parents. By tracking the pieces of DNA that are passed down and reshuffled, researchers can identify regions of the genome that are linked to the trait. GWAS is quicker than linkage mapping, and although both studies yield QTL, there are slight differences in interpretation.

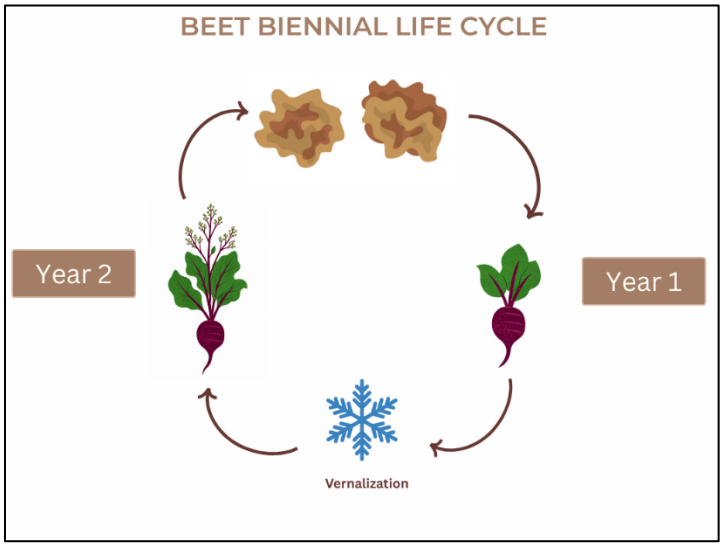
Instead of focusing on specific genomic variants (markers), linkage mapping asks a slightly different question: "**which pieces of chromosome inherited from each parent are associated with influencing the trait phenotype?"**. Scientists use genetic markers as reference points (like a coordinate, mile marker, or exit number) along the genome to test this, but the goal is to identify broader regions rather than single changes in DNA. Very few linkage mapping studies have been conducted in table beet, and even fewer GWASs have been produced. Doing both types of studies gives great insight not only to BLS resistance and inheritance, but also to the genomic architecture of table beet.

Linkage Mapping Research Project

In the first year of GWAS screens, I noted accessions that had a range of BLS response scores. Specifically, I was interested in accessions that scored high (susceptible) or low (resistant) for their use as parents, or founders, in linkage mapping populations. I identified 'White Detroit' and 'Touchstone Gold' to be BLS tolerant/resistant table beet cultivars, and cultivars 'Pablo', 'Forono', and 'Akela' were used as susceptible cultivars. When making these pairings, I aimed to cross resistant (R) and susceptible (S) individuals together. Three populations were generated for linkage mapping analysis: **Touchstone Gold x Pablo**, **White Detroit x Akela**, and **Touchstone Gold x Forono**. With these pairings, I went through the breeding cycle: crossing parents, producing F_1 (first generation after a cross) seed, growing the F_1 seed into roots, and vernalizing the roots to be able to **self-pollinate** (cross the plant to itself- not using pollen from another plant!) the offspring. **Vernalization** is the process in which some plants require a prolonged exposure to cold temperatures in order to induce them into the flowering and reproductive life cycle.

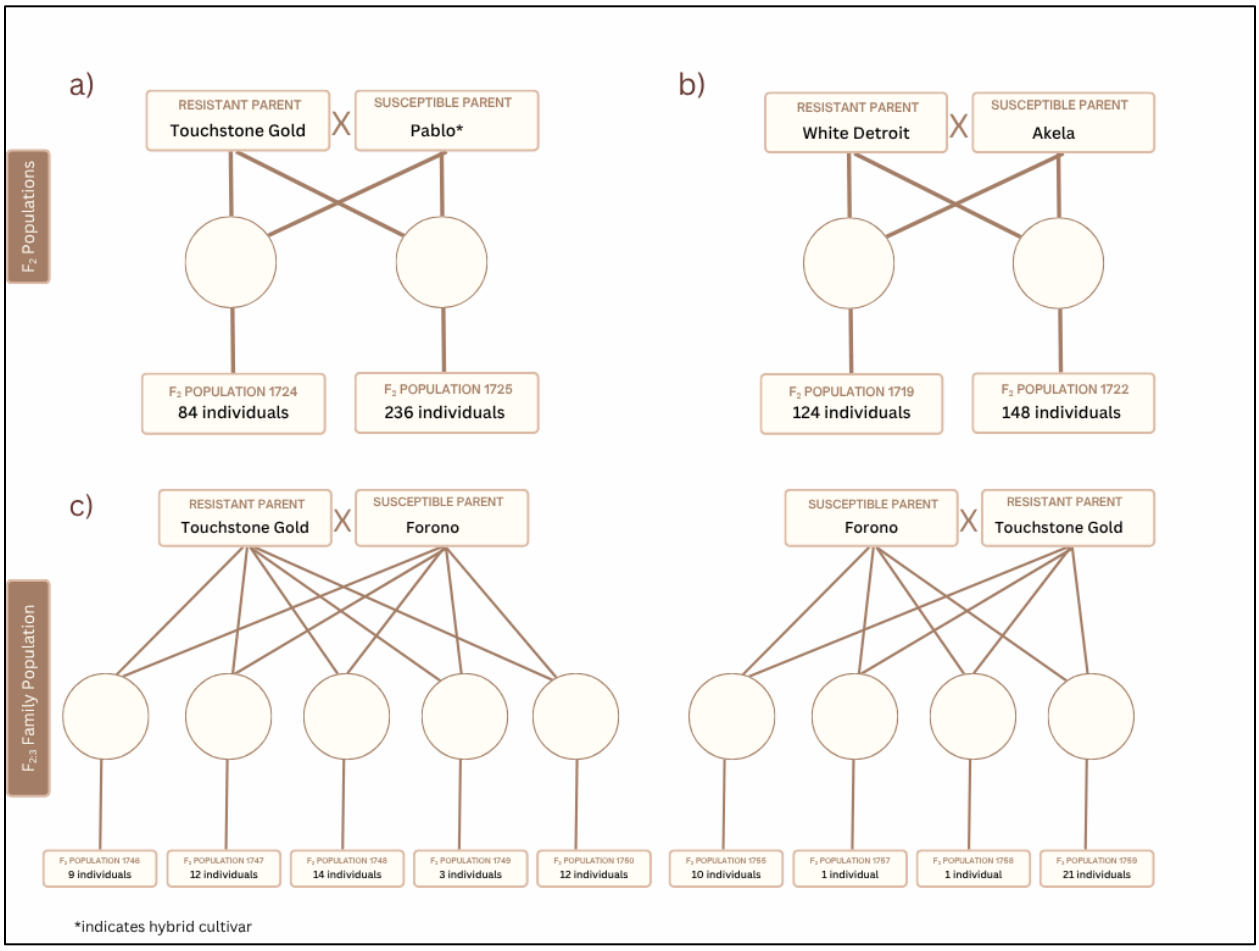
Beets are biennial crops, meaning that they require two "seasons" to produce seed (Figure 5.7). During their first season, they produce a storage root and leaves. If left in the field and exposed to cold temperatures, the beets will bolt, growing a long stem with floral development, and seed production can follow. If no cold temperatures are present, then the plant will only put on new vegetative growth and will not switch into its reproductive life stage. In breeding programs, breeders can speed up this natural requirement for vernalization by putting the roots in a cooler, rather than leaving them in the field to overwinter. Roughly twelve weeks at 0-15°C will satisfy the vernalization requirement.

Figure 5.7. Illustration showing the biennial life cycle of beets. Vegetative growth occurs in the first year. When followed by a period of cold temperatures, satisfying the vernalization requirement for the storage organ, reproductive growth will be triggered, causing the root to bolt and flower to produce seed in the second year. This life cycle can be sped up utilizing cold chambers/coolers and conducting seed production in the greenhouse rather than keeping the roots outside during winter and spring/summer months. Figure produced by Audrey Morrison.



After the vernalization chilling requirement was met for these roots, they were potted in the greenhouse to await bolting and flowering. Once they started flowering, I self-pollinated them to produce F_2 (second generation) seed. I decided to produce one extra generation (F3, the third generation) with the Touchstone Gold x Forono crosses. When populations are taken to the F_3 generation, they are often said to utilize the $F_{2:3}$ family structure, as the F_3 offspring represent the F_2 parents they were produced from. These two crosses used the same cultivar types, but different individuals. I ended up combining them in later steps because I had small population sizes, which is why I consider them one big multi-parental population (Figure 5.8). Therefore, I generated three total populations to utilize for genetic mapping and QTL identification.

Figure 5.8. Illustration of the three mapping populations used in the linkage mapping project. Panels **a)** and **b**) show the generation of the two F_2 populations, and panel **c)** shows the two $F_{2:3}$ family populations that were combined to create one multi-parental population. Figure produced by Audrey Morrison.



Inoculation Screenings

Inoculation experiments for all three populations were managed in the greenhouse setting, keeping many aspects similar to the BLS screening done for the GWAS project. However, I did decide to change two major details of inoculation protocol. First, I decided to adjust my BLS scoring rubric. With the economic threshold of 5% being the benchmark for industry standards for blemishes present in baby leaf salad lots, I wanted to incorporate this

into my scoring rubric. Therefore, my scoring changed from a 0-7 scale to a 1-5 scale. The scale I used for the mapping phenotyping is included in Table 5.2.

Second, I needed to change my humidity chamber setup to something more adaptable for a high throughput operation. The individual bagged chambers worked very well in the GWAS, but were really time consuming for me, and anyone assisting, to de-bag, inoculate, then re-bag all while being gentle on the leaves so as not to damage them in the process. Since my experimental size in mapping experiments was quite large, and I wanted my inoculation window to be as short and consistent as possible, I decided to do a “humidity chamber” setup over the greenhouse table as a whole, encompassing each pot. Therefore, I constructed one large humidity chamber over each greenhouse table, rather than one over each pot. These were made with wood and plastic tubing to make a structure that resembled a mini hoop house. I then draped and stapled plastic sheeting over it to attempt to lock that humidity in over and around the table. This can be seen in Figure 5.9, which looks similar to the low tunnels used in baby leaf production as seen in Figure 5.4.

Figure 5.9. Humidity chamber construction used in linkage mapping inoculation screens. Photo taken by Audrey Morrison.

The experimental setup was very similar to that done in the GWAS screens. I used the same bacterial concentration for spray inoculation. Each individual was sprayed with inoculum. I again chose to inoculate the individuals when they were around the two true leaf stage. I utilized the humidity chambers over each greenhouse table 24 hours prior to inoculation, and 24 hours post inoculation before removal. I rated each individual seven days post inoculation based upon percentage of diseased leaf area (Table 5.2). In the inoculation screenings, I saw a distribution of BLS responses: some individuals were quite diseased, some were barely affected by the inoculation, and plenty of individuals that were right in the middle.

Table 5.2 Scoring rubric used to visually assess mapping population individuals seven days post inoculation with *Pseudomonas syringae* pathovar *aptata.*

Percentage of diseased leaf area (%)	Score
0-5	**1**
6-24	**2**
25-49	**3**
50-69	**4**
70-100	**5**

Genetic markers were successfully recovered for all individuals in the study: markers for the original parent plants, their first-generation offspring, and the second generation offspring. I then filtered the markers for data quality to ensure only trustworthy markers for these individuals would be used in my mapping analysis.

Making genetic maps

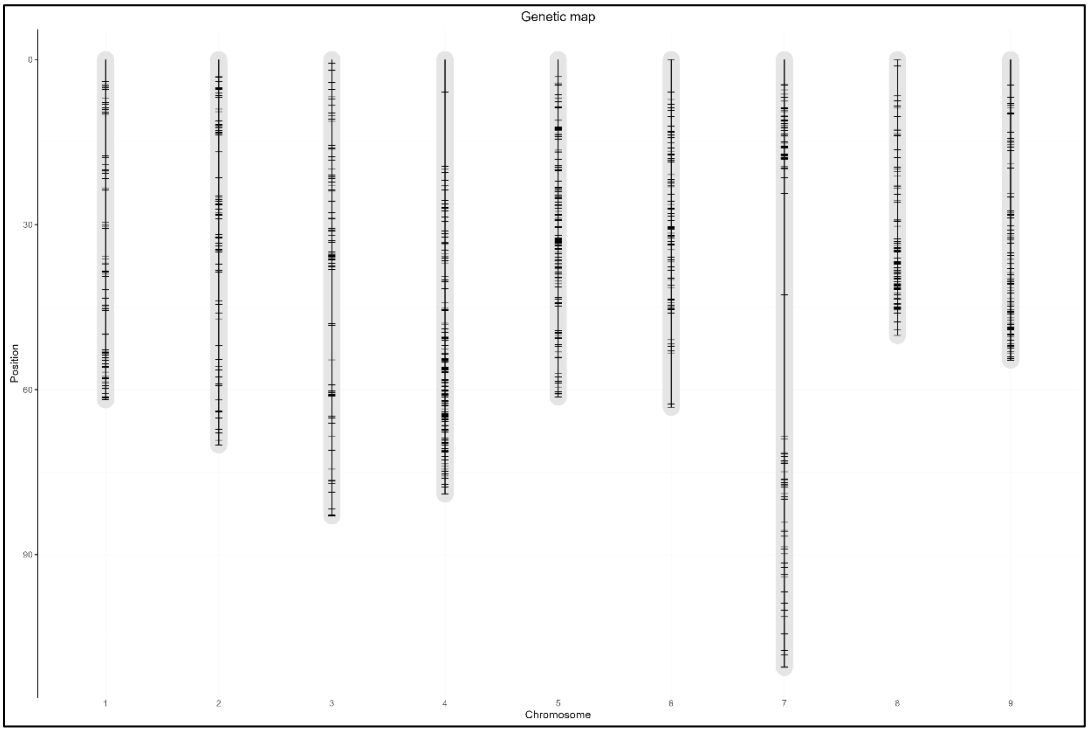
All individuals were genotyped as described above, and molecular markers allowed me to track which chromosome segments each offspring inherited from founder parents. I used a software program to make my genetic map for each population. As the first step in creating a genetic map, the software was used to calculate **recombination frequencies**, asking the question “how often do markers recombine in this population?”.

Going back to our roadmap analogy, recombination would be as if two highways (chromosomes) had to intersect. Normally, nearby exits or mile markers tend to stay together because they are physically close on the same stretch of the highway. But occasionally when highways cross over each other, they might exchange sections. I’m no civil engineer, but it

might make sense to put a highway overpass in an area of two highways that doesn't have a lot of present highway exits, to reduce congestion and traffic. Therefore, exits (or mile markers) that are very close together rarely get split apart by intersecting highways (recombination). In terms of biology, chromosomes work in a similar manner. Molecular markers that are far apart are more likely to have a chromosome crossover happen between them compared to markers that are very close together. By examining which exits (markers) stay close together and which are separated in offspring, geneticists can estimate how often recombination occurs between markers. Using the recombination frequency estimates, geneticists can then build a genetic map, putting markers in order along the chromosomes and estimating distances between the markers.

An example of a genetic map is shown below in Figure 5.10. This is the map for the Touchstone Gold x Pablo mapping population. This map tells us where recombination happens and where the molecular markers are on the chromosomes.

Figure 5.10. Genetic map generated for the Touchstone Gold x Pablo F_2 mapping population. X axis represents the nine chromosomes in table beet, and the Y axis is the genetic position of markers. Each horizontal slash mark on the chromosome indicates a marker position.



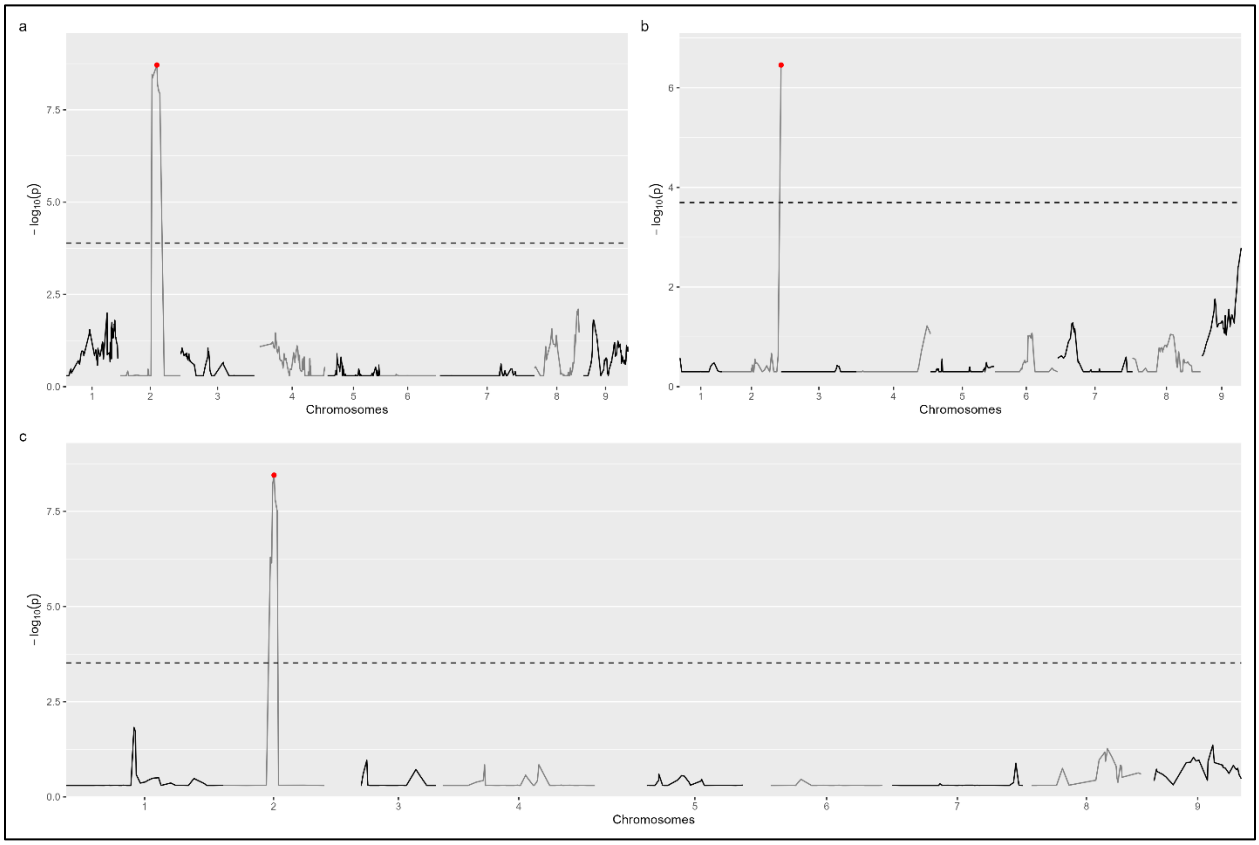
For each population, I had to create a pedigree file, similar to a family tree, that identified founder parents, F_1s, and F_2s and noted which samples were related to whom. After generating a genetic map with the formed nine chromosomes, the software determines **which chromosomal segments were inherited from which founder parent**. The question now is asked: **"which founder did this chromosomal segment come from?"**. This step tells us which parent each chromosomal piece came from, and tangibly, produces probabilities at each location indicating which founder likely contributed that chromosome segment to the offspring. With this step, we can visualize which blocks were transmitted from parent to progeny.

QTL analysis and interpretations

Once the genetic map was made and founder chromosomal segments were tracked through the offspring, I utilized another software program to conduct association analysis. At this step, the software runs statistical tests at each genomic location on the genetic map, again asking the question **"at this genomic position, which founder chromosomal segment influences the phenotype?"**.

With all three mapping populations, I identified a QTL on **chromosome two**. Two of the three populations identified genetic regions that seemed to be in similar locations on chromosome two. These two populations share a common parent, Touchstone Gold, which may have passed down the same BLS-response region to the offspring studied. The third population still identified a region on chromosome two, but was a separate location from the others. Figure 5.11 shows examples of the QTLs identified in the three populations. Using our roadmap analogy, identifying a QTL would be similar to identifying a highway exit or nearby mile marker to our destination we are trying to get to.

Figure 5.11. QTL identification plots for **a)** Touchstone Gold x Pablo F_2 mapping population, **b)** White Detroit x Akela F_2 table beet mapping population, and **c)** Touchstone Gold x Forono $F_{2:3}$ multi-parental mapping population. X axis of each plot indicates chromosome (1-9) and Y axis represents how significant the molecular markers are.



These genetic regions explained **33%** of the variation in the BLS response trait seen in all the individuals inoculated. This indicates that I identified strong regions that hold a lot of responsibility for how the plant responds to BLS.

Instead of including leaf color in the analysis, I used a method that looks for multiple genetic signals one at a time. The process works by first finding the strongest genetic region linked to the trait, then accounting for it and repeating the search to find additional regions. I chose this approach because it can naturally separate different influences. For example, if a major highway exit was identified to lead to a destination (say, 'Exit 12'), then the method I

used here asks the question: "**Given that Exit 12 leads to our destination, are there any other exits that could get us there?**". What this does is reanalyze the highway to search for additional exits that still could get us where we need to go, beyond Exit 12. It considers multiple options- and by removing Exit 12 from consideration (as it was already identified), then we can see if there are other exits that lead us to our destination. So, if leaf color truly affects disease response, this method would identify one (or more) genetic signal related to disease response, and one (or more) related to leaf color.

Both researchers and farmers have seen the connection between leaf color and BLS response, and these two genetic studies presented here barely scratch the surface. Further work would need to be conducted to see if there is a true causal relationship between leaf color and BLS response, or whether they are simply two traits that often go together.

Conclusion

By two different genetic studies, one that surveyed the *Beta vulgaris* crop complex as a whole, and the other that utilized three table beet populations**, I found that chromosome one and chromosome two are associated with BLS response**. The GWAS captures a broader picture- many varieties, many possible genetic variants- whereas the linkage mapping approach is limited to the genetics of the parent plants used to create the offspring plants. In both studies, I found that leaf color plays a complex role in BLS response. Future experiments are needed to not only identify causal genetic regions that confer resistance to BLS, but to also delve into the relationship leaf color has with BLS response. The results presented by this dissertation are the first step in fully understanding the complex relationship and genetic architecture in *Beta vulgaris*.

These two BLS dissertation research projects not only provide insight into the genetic regions that might confer BLS response, but also provide information such as cultivar performance and reproducible inoculation procedures. We hope that industry seed companies or other public institutions can utilize this information to conduct further experiments or influence their own breeding program decisions.

References

1. Grahn, C. M., Benedict, C., Thornton, T., & Miles, C. A. (2015). Baby-leaf salad green production guide for western Washington. Accessed from: https://wpcdn.web.wsu.edu/extension/uploads/sites/25/EM095E.pdf