

Building a Genetic Foundation for the Biofortification of *Brassica rapa*

The promise of biofortification to combat micronutrient deficiencies worldwide:

Biofortification—the enhancement of crop nutritional quality through plant breeding—is potentially a sustainable and cost-effective strategy for addressing micronutrient deficiencies throughout the world. Success of biofortification in part depends on the identification of favorable genes associated with nutritional quality, followed by increasing the nutritional value of locally adapted crop varieties by selecting on favorable alleles of these identified genes in breeding populations¹. Such an approach requires the genetic dissection of nutritional traits with a range of simple to complex genetic architectures down to individual causative genes², but a tremendous amount of research is still needed in staple and vegetable crops before this phenotype-to-genotype connection comes to fruition for nutritional traits. Without connection of phenotype to genotype, biofortification is likely to be highly ineffective in the improvement of targeted nutritional traits³.

The need for biofortification of *Brassica rapa*:

Brassica rapa L. is an agriculturally important vegetable and industrial crop, with 100 million tons harvested globally in 2012⁵. Furthermore, it is an ideal candidate for biofortification through the application of statistical genetics and genomics approaches. A plethora of genomic resources are available for this genetically diverse species that consists of multiple morphotypes: oilseed, fodder, leafy and root vegetable types⁶. As a vegetable, it is a source of vitamin C, an essential nutrient that also enhances the uptake of iron—the most commonly deficient micronutrient in human populations^{7,8}. Plants in the Brassicaceae family also contain a unique class of sulfur-containing compounds called glucosinolates that show anti-carcinogenic activities⁹, but other chemically related plant defense compounds result in lower nutritional value¹⁰. Tremendous variation exists in the levels of vitamin C and glucosinolates among and within the germplasm pool of morphotypes^{6,11}, thus biofortification of these crops requires a strong balance between such beneficial and unfavorable metabolites as it relates to human nutrition and health. The biofortification of *B. rapa* could enhance health in developed and developing nations through increased glucosinolate levels, and improve nutritional security efforts in developing countries by improving iron uptake through greater vitamin C consumption.

Connecting phenotype-to-genotype through quantitative genetics and genomics:

I propose to apply quantitative genetics and genomics approaches in combination with analytical chemistry methods to dissect the genetic basis of vitamin C and glucosinolate levels in *B. rapa*. Currently, I am isolating total genomic DNA from a globally diverse panel of 364 *B. rapa* accessions that has been grown and maintained in a greenhouse under my care at the University of Missouri (MU). Through the implementation of a genotyping-by-sequencing (GBS) approach¹², the DNA samples will be sent for GBS library construction and sequencing on an Illumina HiSeq 2000 at the Institute of Genomic Diversity at Cornell University.

Throughout the summer of 2015, I will conduct the profiling of vitamin C and glucosinolates isolated from leaf tissue collected from the panel of 364 accessions that are to be grown in a replicated field trial at the USDA-ARS location in Geneva, NY. Vitamin C levels will be quantified using a commercially available kit. For generating the glucosinolate profiles, I am collaborating with members of Dr. Elizabeth Sattely's lab at Stanford University who have expertise in identifying and quantifying the levels of glucosinolates and their derivatives in Brassicaceae. Through their supervision in developing the liquid chromatography–mass spectrometry (LC-MS)-based assay and analysis of the results, I plan to measure glucosinolate levels and potentially identify novel derivatives.

Additionally, I will analyze the GBS data in consultation with Dr. Michael Gore's lab at Cornell University from the summer of 2015 to the spring of 2016 with the intent to score thousands of single-nucleotide polymorphism (SNP) marker loci across the genome. The GBS data will be cleaned and analyzed using the reference genome of *Brassica rapa* var. chiifu in the TASSEL software package¹³. For the collected vitamin C and glucosinolate data, a mixed linear model will be used to control for non-genetic field effects and obtain a best linear unbiased predictor (BLUP) for each accession. After removing low quality SNPs the resultant SNP data set will be used to test for associations with the trait BLUPs in a unified mixed linear model that accounts for population structure and relatedness^{14,15}. I will receive training in quantitative genetics and genomics and

learn how to conduct a GWAS in an R package called GAPIT¹⁶ as a summer 2015 intern in Dr. Gore's lab.

Integrating GWAS results into breeding practices using genomic selection (GS)

Unless the SNP markers found from the GWAS can accurately predict vitamin C and glucosinolate content they will not be useful for plant breeders. Genomic selection (GS) is a technique used to assign a genomic estimated breeding value (GEBV) to each accession, making it possible to establish how well a set of genome-wide markers can predict the trait of interest¹⁷. The ability to use GS would greatly reduce the cost and time normally required in breeding programs by allowing breeders to avoid the time consuming and expensive task of phenotyping and instead select breeding lines based on marker data¹⁸. I will compare the GEBVs against the observed vitamin C and glucosinolate BLUPs to assess the accuracy of GS models and support the utility of GWAS results for biofortification efforts in *B. rapa*. Rapid and inexpensive identification of candidate accessions for biofortification is key to successfully transitioning research results into field trials. GS models combined with a large number of genome-wide markers could create the foundation for a successful *B. rapa* biofortification program. The success of this program could result in a great improvement of current iron biofortification efforts with a vitamin C rich diet that increases iron absorption, and improved cardiovascular health and reduced cancer risk in developed and developing nations due to increased glucosinolates¹⁹.

Conclusion:

Plant genetic diversity is a topic that has captured my attention, and I want to use the latest molecular, computational, and statistical approaches to study how genes contribute to phenotypes and what this relationship can tell us about the inheritance of agriculturally important traits like nutrient density. This information will be invaluable for integrating the wealth of genomic data into breeding programs that are focused on biofortification. I believe that we have the technology available to start addressing these challenges, and I want to be at the forefront of the efforts to characterize these phenomena for solving pressing global issues.

Works cited:

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