

## Abstract

Plant breeding is a coevolutionary process between humans and edible plants that relies on the application of artificial selections by humans. This selection typically occurs in the form of generating improved lines or new varieties with desirable traits. This is done not only to increase the yield of crops, but also to combat the sort of evolutionary arms race against disease, pests, and other stresses. Considering this, the goal of this thesis is to develop a mutagenesis resource for sunflower by irradiating sunflower pollen with x-ray radiation. This project will potentially lead to the discovery of many new and exciting traits, including some that are desirable from an agricultural perspective.



**Figure 1 (above):** A heliotropism mutant found in our M2 isolation population in Puerto Rico

## Introduction

The development of a mutagenesis population resource for sunflower would allow for the technology to be more widely used in an effective and standardized manner. Among the goals that could be achieved is a variety of traits that would greatly assist in making sunflower a more reliable and prominent agricultural crop. The resistance to diseases such as Sclerotinia, Phomopsis stalk canker, downy mildew, and rust is certainly desired, along with resistance to insects, including but not limited to red sunflower seed weevil, sunflower head moth, and sunflower banded moth. As with many other crops, resistance to herbicide (e.g. imidazolinone resistance) is certainly a desirable trait as well. More recently, high-oleic, low-saturated oil sunflower has become desirable. The oil has a neutral taste and provides phenomenal stability without hydrogenation. It offers a “0 gram trans fat” oil solution to customers, and can be used in bakery applications, spray coating oils for cereal, crackers, dried fruit, oil for frying, and even in non-dairy creamers. As a result of the patent on high oleic sunflower oil and seed expiring recently, the use of mutagenesis could greatly help breeders in finding ideal sunflower lines for the production of such oil. Finally, mutagenesis could assist with the more common challenges of agriculture, including increased yield, oil content, and greater yield stability over environmental gradients.

## Methods

This mutagenesis resource project is nearing completion, and follows the below steps:

1. Green, male sterile plants (NMS) and red fertile plants were grown in Glyndon, MN in the summer of 2015
2. Pollen from red, fertile plants was collected daily and then taken to the Sanford Roger Maris Cancer Center in Fargo, ND where it received a 50 Gray (Gy) dose of x-ray radiation to cause genetic lesions
  - a. For reference, a 50 Gray dose is the equivalent of a multi-month cancer treatment in one single dose!
3. Control (0 Gy) pollen and experimental (50 Gy) pollen was applied to the green, male sterile plants in Glyndon, MN to produce seed for the F1/M1 population
4. The F1/M1 population was grown at winter nursery outside Santiago, Chile where we observed anthocyanin gene knockout as an indicator of successful mutation rate
5. Seed from Chile was then grown in a second winter nursery in Puerto Rico as the F2/M2 population
  - a. M1 lines with low seed yield had offspring grown in Fargo, ND instead of Puerto Rico

The first three field seasons have already been completed, but we do anticipate a fourth season occurring at our nursery in Glyndon, MN this upcoming summer. Genomic analysis on the anthocyanin and increased vigor trait will assist us with further understanding of what mutants are being observed in this fascinating population.

## Results

While the project is not yet complete, we are excited to have seen some positive results in both the M1 and M2 populations in Chile and Puerto Rico, respectively.

In the M1 population, 11 plants of the 1,127 plants in the winter nursery, had an anthocyanin gene knockout resulting in a green phenotype that still obviously resembled the parents. This results in a roughly 1% mutation rate (pending genomic validation) at that locus, which is actually a little higher than we anticipated.

In the M2 population, some very interesting mutations were observed, and include the following:

- A heliotropism mutant
- A dwarfism mutant
- A high vigor mutant

We anticipate that there are many more mutants present in our population, and look forward to using further genomic and agronomic information to decipher what could prove beneficial for the sunflower industry.

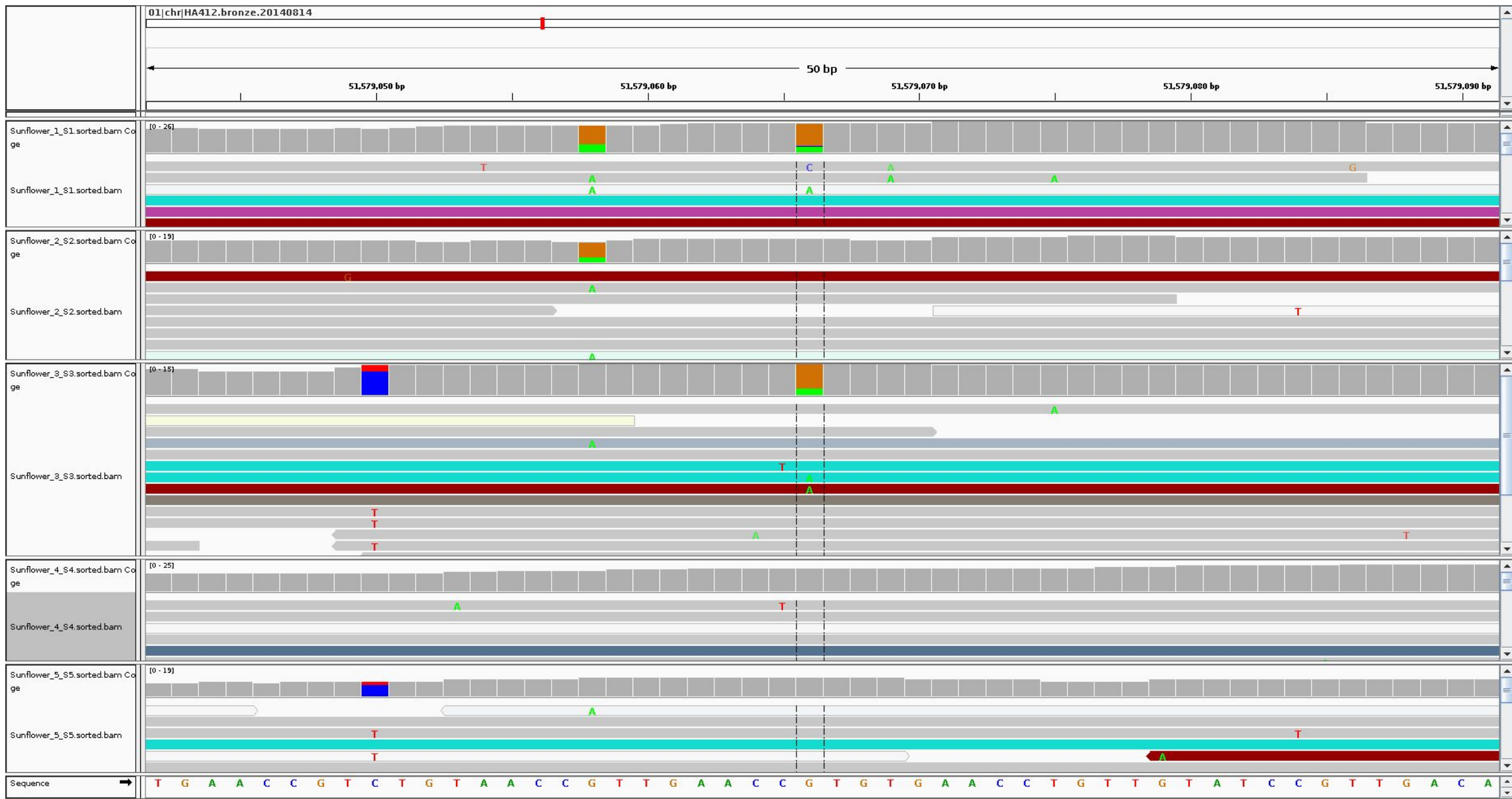


**Figure 2 (left):** A red, fertile plant  
**Figure 3 (right):** A green, male sterile (NMS) plant

## Discussion

As we move forward, we will be using a Variant Calling Pipeline to further understand what is going on in each of these mutations in terms of genomics. The below flow chart details what our plan is, and what we have done so far:

1. Upload data and QC (**complete**)
  - a. Sequence (fastq file)
  - b. Reference genome (fastq file)
  - c. Filter reads based on quality scores
2. Alignment with BWA (**complete**)
  - a. Align with BWA
  - b. SAM to BAM conversion
3. Variant calling (**in progress**)
  - a. Mpileup
  - b. Bcftools view



**Figure 2 (above):** Integrated Genomics Viewer (IGV) of sorted.bam files aligned to sunflower reference genome