X-RAY INDUCED MUTAGENESIS IN SUNFLOWER POLLEN

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Title

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The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

MASTER OF SCIENCE

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ABSTRACT

The identification of new sunflower hybrids that have desirable traits depends on the availability of adequate genetic resources. A critical part of sunflower breeding is to find and recombine genetic variability into inbred lines that address modern agricultural challenges. Such genetic variation can come from standing genetic variation, crop-wild relatives, or via mutagenesis, transformation, and gene editing. The use of one standard mutagenesis resource in sunflower has not yet been established but has the potential to enrich genetic variability in this crop, provide for collaborative study, and thus assist with breeding for desirable traits. This project aims to generate a mutagenesis population and methodology for sunflower using x-ray radiation. By using a 50-Gray dose of radiation from a medical linear accelerator, mutagenesis was successfully induced in sunflower at an effective, yet non-lethal level. This paper outlines the methodology behind this project and the resulting mutants.

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DEDICATION

To my beautiful, brilliant, and benevolent wife Taylor. You have shown me true love, from our strong initial connection, through my battle with cancer, and towards our future together. I love you more than I can express in words.

To Dr. Gerald Gross and the Sanford Roger Maris Cancer Center. You not only provided the means for a successful x-ray induced mutagenesis resource, but you also saved my life. I am so incredibly lucky to have found you and your care team, Dr. Gross.

To my mom, dad, brother, and sister. You have given me a beautiful, wonderful, happy, and meaningful life and supported me through all my challenges and successes. This thesis is a direct result of your love and support.

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LIST OF ABBREVIATIONS

USDA-ARS	United States Department of Agriculture – Agricultural Research Service
INRAE	National Institute of Agricultural Research (France)
CMS	Cytoplasmic male sterility
HIR	Haploid induction rate
SNP	Single nucleotide polymorphism
INDEL	Insertions/deletions of genetic code
IT PGRFA	International Treaty on Plant Genetic Resources for Food and Agriculture, Food and Agriculture Organization of the United Nations
NMS	Nuclear male sterility
LINAC	Medical linear accelerator
Gy	Gray, a unit of absorbed dose of ionizing radiation
MU	Monitor unit, a measure of machine output from a LINAC; 100MU = absorbed dose of 1 gray
TF	Transcription factor
CNV	Copy number variation
ANOVA	Analysis of variance
Gr	Green, a green plant sample in this project
Br	Branched, a branched plant sample in this project
LC	Low count, a low count row sample in this project
bp	Base pair, a fundamental unit of double-stranded nucleic acids paired together to form a “rung of the DNA ladder”

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INTRODUCTION

Sunflower Agricultural History

Plant breeding is a coevolutionary process between humans and edible plants that relies on the application of artificial selection 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 evolutionary arms race against pathogens, pests, and other biotic and abiotic stresses.

Sunflower (*Helianthus annuus L.*) is a native domesticated crop from North America that is an annual species grown for its edible oil and confectionery seeds. It is a broadleaf, dicotyledonous plant that grows quickly and puts out large leaves with a rough, scratchy texture and coarse hairs. In agriculture, it is observed as a single large inflorescence supported by an unbranched stem, with a total height averaging around six feet. Sunflower is a warm season crop that grows best in a fertile, moist soil that is exposed to full sun. The heads of sunflower demonstrate heliotropism, tracking the movement of the sun, during development before maturity. Most commercial varieties have heads that turn downward following bloom to reduce bird damage. Sunflower is grown in humid subtropics and temperate regions of developed countries, particularly in eastern Europe with Ukraine and Russia typically producing over half of the world's sunflower crop (Fischer et al., 2014).

Native Americans were responsible for domesticating sunflower from the wild, multi-headed type to a single-stemmed plant with a single large inflorescence. They used the crop for a variety of purposes, including milling for flour, roasted seed snacks, cooking oil extraction, skin and textile dye, sunscreen, and construction using the fibrous stem. In the modern world, confectionery sunflower seeds are sold as a snack food, raw or roasted, and often seasoned.

Meanwhile, sunflower oil is extracted from oilseed varieties and used for a wide range of cooking applications, as well as production of margarine, sunflower seed butter (Sunbutter®), and biodiesel. Following the removal of oil, oilseed sunflower hulls can be used as fuel for powering oil mills, for composting, or for low-quality ruminant livestock roughage. Sunflower stems and leaves also produce secondary compounds like latex.

Sunflower Breeding History

Sunflower is thought to have been first domesticated in the southeastern United States about 5,000 years ago (Blackman et al., 2011), but may have been domesticated in Mexico prior (Lentz et al., 2008). The earliest known record of a fully domesticated sunflower was in the eastern United States, circa 2300 BC, following a significant genetic bottleneck (Harter et al., 2004). Sunflower remained a staple in North America for over 4,000 years before being discovered by European explorers in 1510 with seed brought back to Europe. The unique foreign flowers were used primarily for ornamental purposes by Europeans during the 16th and 17th centuries, spreading across the European continent. In 1716, the first patent for oil extraction from sunflower seed was granted in England, followed by extensive cultivation of sunflower in Russia for oil production attributed to Peter the Great. Manufacturing of sunflower oil reached commercial scale by 1830. The Russian government funded the first research programs focused on breeding both oilseed and confectionary sunflower in the latter 19th century, resulting in the release of the enormous “Mammoth Russian” variety. Vasilii Stepanovich Pustovoit led a sunflower research lab in Krasnodar, Russia for the first half of the 1900’s and is attributed with producing the foundation of high-oil content, high-yielding modern varieties of sunflower (Schneiter, 1997). Of note, such breeding efforts brought the oil content of sunflower oilseed from 30% to over 45%.

Until the 1970s, open-pollinated varieties had been bred and cultivated as the primary genetic stock of sunflower due to sunflower's inadequate natural self-pollination among its male and female reproductive parts. Bringing new traits into sunflower varieties proved difficult because of open-pollinated varieties being multi-parent synthetic varieties from intermating in isolation. Breeding efforts in the late 1960's started to shift focus past just yield and oil content, adding further pressure for the creation of a hybrid sunflower system to introduce desirable traits more efficiently and capitalize on the effects of heterosis that had, by that time, already resulted in large increases in genetic gain in maize (*Zea mays L.*). The greatest breakthrough in sunflower breeding history occurred in 1969 when a researcher named Philippon Leclercq at France's National Research Institute for Agriculture, Food and Environment (INRAE, known at the time as the National Institute for Agricultural Research) discovered a method of producing "female" sunflower through cytoplasmic male sterility (CMS; Leclercq, 1969). By identifying more self-compatible lines and having a male-sterile "female" plant, breeders were now able to introduce pure pollen from another sunflower without any contamination from the plant's own genetics. This discovery was complemented in the following year when Murray Kinman of the United States Department of Agriculture's Agricultural Research Service (USDA-ARS) identified a fertility restoration gene for cytoplasmic male sterility, originally designated as *Rf1*. This pair of discoveries produced by the extensive collaboration between INRAE and the USDA-ARS facilitated commercial production and reduced costs of sunflower F_1 hybrid seed (Kinman, 1970).

Modern sunflower crops are largely hybrids, produced by cross-breeding lines derived from genetically and artificially isolated populations, called heterotic groups using the technology developed originally by Leclercq and Kinman. Hybrids of sunflower have a higher fitness, improved yield, and often have characteristics that are desirable in agriculture, like resistance to pathogens (Gontcharov et al., 2006). Modern sunflower breeding is aimed toward finding plants that exhibit the desirable traits in agricultural applications, such as increased productivity, disease resistance, and drought tolerance, among others. Many scientists have suggested genetic engineering as a promising approach to finding such lines of sunflower (Eapen and D'Souza, 2005; Krämer and Chardonnens, 2001; Grichko, Filby, and Glick, 2000). Yet, the development of genetically modified plants in sunflower is problematic for a variety of reasons including public perception (Vassilev et al., 2004). As mentioned previously, hybrid breeding has become the primary breeding tool in agricultural sunflower. However, classical mutation techniques have frequently been used to discover a variety of traits in many other crops (Maluszynski, Ahloowalia, and Sigubjornsson, 1995). For example, the use of mutagenesis has been successful in producing lines with salt tolerance in barley (Forster et al., 1995) and cold tolerance in rice (Maluszynksi et al., 1995).

Mutagenesis

The identification of new sunflower hybrids that have desirable traits depends on the availability of adequate genetic resources. A critical part of sunflower breeding is to find and recombine available genetic variability and create the most suitable recombinant inbred lines to face a variety of environmental and agricultural challenges. Such genetic variation can come from standing genetic variation, crop-wild relatives (Terzic et al., 2020), or via mutagenesis. The use of one standard mutagenesis resource in sunflower has not yet been established but has the potential to enrich genetic variability in this crop, provide for collaborative study, and thus assist with breeding for desirable traits. Encheva et al. (2003) have stated that significant changes in morphological and biochemical characteristics of sunflower have been noted in previous mutagenesis attempts, where plants were successfully regenerated from immature zygotic embryos using organogenesis (cell culture method), along with gamma irradiation. Although sunflower breeding has been successful in the past few decades, there is still a wide range of issues that have yet to be addressed, such as resistance to disease and the parasite *Orobanche*. It is important to the future of this agricultural crop that mutagenesis be utilized as an additional tool to add functional diversity to the sunflower gene pool.

While not a primary objective of this thesis, the research would ideally lead to the development of a doubled haploid inducer method to reduce inbred line production to one year, down from five to seven years in a traditional breeding system. One example of a useful mutant is one that induces haploidy in crosses, known as a haploid inducer. Other crops, including wheat (*Triticum aestivum L.)* and rice (*Oryza sativa L.*), have been vastly improved in recent history due to the creation of doubled haploid plants (Wan et al., 1989). Haploid plants can be directly found through x-ray treatment of pollen, inducing parthenogenesis (Todorova et al., 2017); however, it is not a goal to find haploids this way due to efficiency limitations of this method. Instead, an effort to discover a line of sunflower that will reject its own chromosomes when crossed with pollen from a normal plant was conducted. This is called paternal haploid induction. The ability to breed with a haploid-inducing stock allows breeders to skip the inbreeding steps by developing plants in one generation that are simultaneously homozygous at all loci of the genome, and thus be more successful in fixing, studying, and releasing desirable traits.

Maize is an excellent example of a crop that has had fantastic genetic gain with a doubled haploid system and serves as motivation for attempts to develop a similar technology in sunflower. There are two main approaches used to develop maize haploid inducer lines: conventional breeding using the Stock6-derived haploid inducer lines, and more recently through the genetic manipulation of the *Centromeric Histone3* gene (*CENH3*). Meng et al. (2022) manipulated the Stock6-derived inducer lines through overexpression of *CENH3*, successfully integrating two *in vivo* haploid induction methods and increasing the maternal haploid induction rate (HIR) by 6.1% (16.3%) compared to the Stock6-derived control lines (HIR ~10.2%). Maize doubled haploid lines are generated by (1) identification of an agronomically desirable line, (2) inducing haploidy through crossing with an inducer line, (3) selection of haploid lines based on a visual kernel marker, (4) growth and artificial duplication of chromosomes in haploids, and (5) self-pollination of successful plants. This process results in lines that are genetically pure and inbred, with remarkably high phenotypic uniformity among progeny of a single doubled haploid. Doubled haploid maize lines are used widely by larger maize breeding programs but have seen limited use in smaller breeding programs due to licensing costs and limited open-source inducers (Trentin et al., 2020).

It is hypothesized that a sunflower paternal haploid inducer (female plant with CMS to carry sterile cytoplasm but not the donor nuclear genome) could be produced by inducing large genomic lesions in or near key meiosis genes via x-ray mutagenesis. Producing doubled haploid material would allow the sunflower breeding community to select optimum progeny using genomic assisted or traditional selection methods and make the development of “female” cytoplasmic male sterile (CMS) inbreds just a single cross operation, as opposed to the more complex and work-intensive backcross approach. It would also reduce inbred line development time from five to seven years down to under two years, while improving the genetic purity of lines and hybrids. Being able to instantly produce “true breeding” material could allow for selection of optimum progeny using single nucleotide polymorphisms (SNPs). Such a haploid inducing stock has not yet been found in sunflower, and a secondary goal of this thesis was to find a stock capable of producing doubled haploid progeny. However, the development and analysis of a unique mutagenesis resource, where the pollen is the target of the mutagen, is a useful source of genetic diversity with direct utility that will benefit the plant science community.

Justification

This research project was significant for a variety of reasons. Sunflower is already regarded as a very hardy plant capable of growing in a wider range of environments than other oilseed crops. It is the only oilseed included in the list of crops covered under the Multilateral System in the International Treaty on Plant Genetic Resources for Food and Agriculture (IT PGRFA Secretariat, 2009; Annex I). This is a major international agreement made through the Food and Agriculture Organization of the United Nations aimed to "conserve, use and manage plant genetic resources for food and agriculture around the world for the benefit of people everywhere." The goal is to specifically ensure that both farmers and breeding programs have access to the raw genetic material necessary for development of new crop varieties capable of higher yields and climate change resilience.

Sunflower is the 13th most produced crop in the world as measured by harvested area (2008-10), experiencing an increase of over 50% since 1988-90. Fischer et al. (2014) determined that overall farm yield for all world food crops must increase by a rate of 1.1% per annum relative to 2010 yield to provide adequate food supply and argues that substantial investment in research, development, and extension to close the yield gap is the best way to address global food needs. With sunflower's unique hardiness and oil profile, ability to grow in a wide range of environments, and importance to the global community, it is imperative that the breeding community ensures that genetic gain remains at an adequate level to address the needs of the world. With the success of mutagenesis in other crops thus far and the constant need for new desirable genetics in sunflower, it appears worthwhile to identify an ideal mutagenesis method for sunflower to facilitate a novel approach to improve the crop and address the issues facing it now and in the future.

Objectives

1. Determine if radiation-induced mutagenesis of pollen is a useful mutagenesis method for sunflower.
2. Identify types of mutations (point mutations, small or large deletions, insertions) that are observed following x-ray radiation on pollen.
3. Diagnose any potential doubled haploid genomes present in the M_2 x yellow testcross populations as a proxy for identifying a novel haploid inducer via mutagenesis.

METHODS

Mutagenesis Experimental Process

Figure 1: A "red" plant derived from the "French" sunflower variety 83HR3. Observed with strong anthocyanin expression, most notably on the branches.

Figure 2: A "green" plant derived from the "French" sunflower variety 83HR3. Observed with no anthocyanin expression, even on the branches where it most strongly occurs.

This project began with the growth of two isogenic sunflower types at our Glyndon, Minnesota research field called the "French" variety, also identified as 83HR3. In this variety of sunflower, high expression of anthocyanin in the hypocotyl and nuclear male sterility (NMS) are not present in the wild type and are dominant and recessive, respectively. Two isolines were developed through backcrossing to allow the use of anthocyanin expression as a marker for male fertility: (1) a green hypocotyl (*r*), nuclear male sterile (*s*) "French" line and (2) a red hypocotyl (*R*), male fertile (*S*) "French" line plants. Pérez-Vich et al. (2005) conducted a molecular mapping study and found that high anthocyanin expression at the "T" locus, conditioning the high anthocyanin trait, was linked with the Ms_{10} NMS gene on linkage group 11. This insight allows for the use of high-anthocyanin expression as a phenotypic marker for male fertility in this study.

Seed for both types were counted, placed in labeled coin envelopes, and organized into our spring 2015 field planting boxes. Using an ALMACO 4 row cone plot planter, the seeds were planted at a depth of two inches with 30-inch spacing. Not long before the heads were developed, our nursery experienced a heavy thunderstorm with wind gusts that resulted in moderate lodging of these two types. Lodged individuals were rescued by buttressing the plants with reinforcing bars (rebars). Not long before flowering, all the plants were tagged and bagged with cloth head bags to isolate them for the duration of pollen production and fertility. Pollen from the red hypocotyl, fertile plants was then collected each morning by tapping the heads over paper bags. Paper bags were labeled and stored in a refrigerator until an adequate amount had been procured to pollinate the green hypocotyl, male sterile plants. At this point, induction of mutagenesis using x-ray radiation was pursued on the pollen.

A medical linear accelerator (LINAC) is a device most used for external-beam radiation therapy of cancer by delivering high-energy x-ray radiation directly to tumors. This method of x-ray radiation is highly precise and can destroy cancerous cells in the body while minimizing the negative impacts on surrounding tissue (Weissbluth et al., 1959). During the treatment of cancer, a standard therapeutic total dose typically ranges from as little as a few grays (Gy, a unit of radiation) to as much as 80 or more-Gy over the course of numerous treatments. Grays are a unit of radiation where a single gray is the absorption of one joule of radiation energy per kilogram of matter. Total doses can be given over just a few sessions or over as many as 50 sessions over a couple of months. Dosage rates of the newest research LINACs can range from about 3-Gy per minute (Gy/min) up to a staggering 3,500-Gy/min (Jang et al., 2020), with radiation therapy settings typically using LINACs with a much lower dose rate range. Medical LINACs often measure doses in monitor units (MU), with 100 MU being equivalent to an absorbed dose of 1-Gy (Srinivas, 2019).

Figure 3: The Varian 21EX linear accelerator used to provide x-ray mutagen dose to sunflower pollen. Located at the Sanford Roger Maris Cancer Center. Sunflower pollen was placed on top of two boxes and beneath a stabilizing plate to ensure proper exposure to high-energy x-rays.

For this study, a Varian 21EX Linear Accelerator at the Sanford Roger Maris Cancer Center in Fargo, North Dakota was used to induce mutagenesis on the sunflower pollen. The maximum dose rate on this machine is 600 MU/min. This machine is designed to provide a warning if a dose of 500 MU is exceeded, as that is beyond the dose a patient would receive in a single setting. This warning was overridden for this experiment. Previous test doses on this same machine included 3, 9, 15, 21, 27, 33, 50, 100, and 150-Gy (B. Hulke, pers. comm., 2015). The doses up to 33-Gy were found to be inadequate due to an insignificant number of genetic lesions and obvious phenotypic mutations, while doses of 100 and 150-Gy resulted in too much fatality for effective study. As a result, a single dose of 50-Gy was selected for this mutagenesis effort. The pollen collected from our sunflower nursery was taken immediately to the Sanford Roger Maris Cancer Center on the final day of pollen collection to receive a mock treatment of no radiation, or a 50-Gy dose of x-ray radiation to induce genetic lesions. After applying this dose to the experimental pollen, both the control and irradiated pollen were then brought back to the field and applied immediately to the green hypocotyl, male sterile “female” plants. These initial cross plants were then monitored for the rest of the season and the resulting seed was collected in isolation and organized according to plant number and pollen source (control or experimental).

As mentioned previously, the primary focus of this project was to create a mutagenesis method for sunflower, with the generation of lines with desirable traits or a doubled haploid inducer discovery being secondary. The objective of this study was to develop a process by which x-ray mutagenesis in sunflower was accomplished, evidence of its success, general mutation notes, notable knockouts of expression at the anthocyanin/male sterility locus, and any other results of interest, both across the genome and in phenotypic trait analysis. To provide these results, a variety of phenotypic and genomic analyses were conducted.

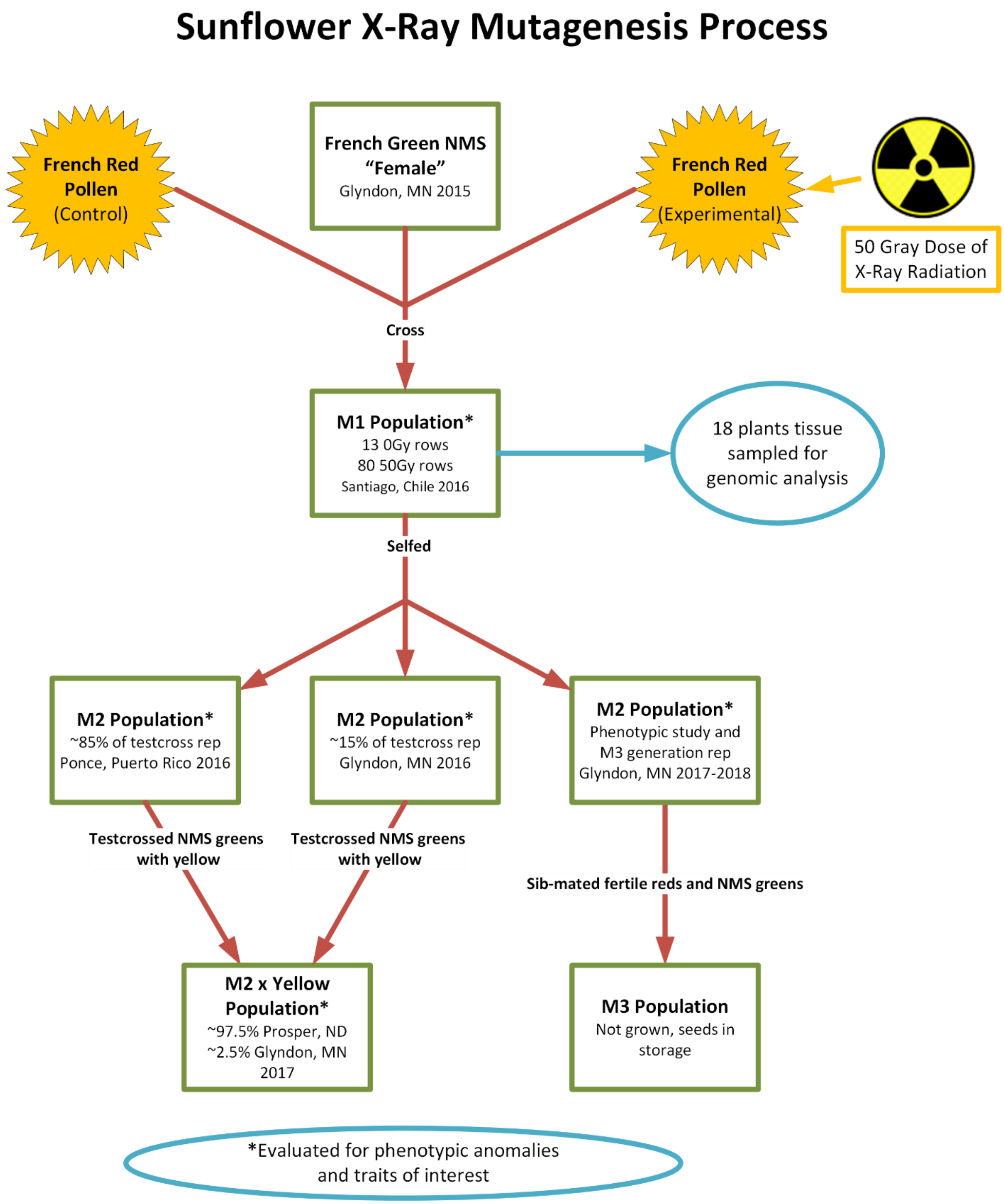

Figure 4: A flowchart of the sunflower x-ray mutagenesis process used in this project

Phenotypic Analysis

About 2,000 M_1 (similar to F_1, designates the first generation of progeny from the mutagenesis effort) seed obtained from the green hypocotyl, male sterile plants previously pollinated with the red hypocotyl, fertile pollen (both irradiated as well as a non-irradiated control) were grown as the M_1 population at a winter nursery site outside Rancagua, Chile in 2016. The M_1 population comprised of over 1,300 plants spanning 93 rows, with the vast majority having the dominant, red anthocyanin expression and a small portion being green plants likely the result of knocking out anthocyanin expression from the irradiated red hypocotyl pollen parent. Twenty seeds were manually stab-planted in each of the 13 0-Gy and 80 50-Gy rows, coming from two 0-Gy and 12 50-Gy pollen-treated mother plants. The proportion of green to red among the 50-Gy experimental M_1 plants allows for determination of a general mutation rate achieved from mutagenesis. Prior to flowering, all plants were bagged to ensure selfing (self-pollination) of each plant. While growing to maturity, field notes were taken to note any interesting mutants among the plants such as crinkle leaf, stunting or branching, as well as stand count and uniformity notes. Stand counts were conducted just before flowering and uniformity was collected based off an estimation ranging from 1-5. Following collection of phenotypic notes for this M_1 population, the plants were then monitored until maturity and harvested according to each row and treatment.

The harvested seed of each plant from the M_1 population in Chile was sent to a second winter nursery site outside Ponce, Puerto Rico to be grown as the M_2 population in 2016, except for plants with low seed count that were instead grown in our Minnesota nursery in 2016. In this first replication of the M_2 population, a pollen-producing (male) line with a homozygous recessive chlorophyll deficiency that results in a peculiar yellow appearance was utilized to aid in the search for a paternal haploid inducer. The Puerto Rico M_2 population included 744 rows of sunflower derived from individual M_1 plants and 276 rows of "yellow" male testers spaced in pairs after every six M_2 plants. The Minnesota nursery included 147 rows of M_1-derived M_2 rows and 11 rows of yellow male testers. Any red plants at the Puerto Rico M_2 winter nursery were removed by hand due to the anthocyanin gene being genetically linked with a male fertility gene. Removing such fertile individuals and having this winter nursery isolated from any other sunflower pollen sources (both wild and other agricultural plots) allowed for a specific cross to be conducted across the all-male-sterile M_2 population, avoiding outcrossing with undesirable plants. The yellow variety was sowed such that it would bloom simultaneously (nick) with the M_2 male-sterile population by using two planting dates to ensure pollen availability during M_2 flowering. Following pollination of the M_2 green male-sterile "female" plants with the yellow "male" plants, the population was monitored until maturity and each female head was then harvested individually and sent back to Fargo, North Dakota. To ensure the same cross occurred in the low seed count M_2 lines grown in our Minnesota nursery, all plants were bagged before flowering and instead testcrossed manually with the yellow male parent. Remaining seed from this population remains in cold storage at the USDA-ARS in Fargo, North Dakota.

The resulting M_2 x yellow testcross seed was grown in the form of 6,074 short observation rows in 2017 at the North Dakota State University Prosper Research Site, as well as a smaller group of 153 rows in our Minnesota Nursery in 2017. At the V4 stage, yellow leaf types were then identified as potential results of haploid or doubled haploid induction (Schneiter et al., 2019). Since the male parent's yellow expression is homozygous recessive, observation of a yellow in this testcross population could indicate the female green parent functioned as a haploid inducer. The use of genomic analysis and cytogenetic confirmation would then later allow for confirmation that a doubled haploid was indeed observed. Beyond looking for such yellow plants, any other phenotypic anomalies or traits of interest were documented. Seed from this population remains in cold storage at the USDA-ARS in Fargo, North Dakota.

Finally, the entire M_2 population was grown once again in our Minnesota nursery over the summers of 2017 and 2018. Instead of testcrossing with the yellow, the fertile red and NMS green plants of each row were sib-mated to produce M_3 seed. To accomplish this, all plants in the population were bagged prior to flowering and the heads were then sib-mated down the row, ensuring that all green plants contacted red fertile siblings. This process happened multiple times to ensure adequate pollination and seed set. During the growth of this population, phenotypic anomalies and traits of interest were again documented. Seed from this population remains in cold storage at the USDA-ARS in Fargo, North Dakota.

Genomic Analysis

To analyze this mutagenesis effort at the genome level, 18 plants were selected and subsequently tissue-sampled from the M_1 population at the winter nursery site outside Rancagua, Chile. Two control, non-irradiated plants were selected from the first and last rows of the M_1 population to allow comparison of the 50-Gy experimental lines. Two experimental, irradiated plants were selected from the first and last 50-Gy rows of the M_1 population with no phenotypic anomalies to function as representatives of the 50-Gy population. Ten green plants identified in experimental, irradiated rows were selected to potentially identify the exact cause of anthocyanin expression being knocked out. Of these 10 green experimental plants, seven were noticeably shorter/dwarfed. Three branched plants found in the experimental rows were also sampled because of the lineage being single inflorescence, with the second and third plants also demonstrating a green phenotype. Finally, one plant was selected from the three rows with uniquely low stand counts.

Sequencing efforts were completed using the methodology outlined by Barstow et al. (2022). Lyophilized leaf material for the 18 selected plants were ground using tungsten carbide bearings in a Qiagen 96-well plate shaker. Genomic DNA was extracted from the leaf tissue using a Qiagen DNeasy 96 plant kit. Qiagen's protocol for extraction was modified to include (1) the addition of 10mM sodium metabisulfite to the initial lysis buffer, (2) a 45-minute incubation of the ground material at 65°C in lysis buffer, (3) a 100% ethanol wash before final drying of the membrane prior to elution, and (4) an elution buffer that contained 10mM dithiothreitol for DNA storage. All these modifications have been shown to improve DNA concentration and purity (Gao et al., 2018; Pogoda et al., 2018). Prior to library preparation, samples were stored at -20°C. Genomic libraries were prepared according to standard protocols using Nextera® XT DNA library prep kits (Illumina®) and then barcoded with Nextera® adapters i5 and i7. Insert size was 450 base pairs (bp). Pools that passed quality control were processed for an average coverage depth of 3x and an average read length of 141 bp. Paired end HiSeq® 2000 reads were produced at the Novogene sequencing facility in Sacramento, California.

Demultiplexed data (separate files for each sample) was downloaded directly from Novogene's servers upon completion of processing. FASTQ data were trimmed using Trimmomatic version 0.36 with the following parameters: ILLUMINACLIP: AllAdapters.fa:2:30:10 HEADCROP:20 CROP:120 LEADING:3 TRAILING:3 SLIDINGWINDOW:4:15 MINLEN:60. FASTQ files were aligned with the most recent sunflower genome assemble, HA412-HO v2 (L. Rieseberg, pers. comm., 2019), using Burrows-Wheeler Aligner (BWA) MEM version 0.7.17 to produce aligned SAM files (Li, 2013). Next, the Genome Analysis Toolkit (GATK) SortSam function was used to produce sorted BAM files for each sample. GATK's MarkDuplicates, BuildBAMIndex, RealignerTargetCreator, IndelRealigner, and BuildBAMIndex functions were also used to prepare sorted BAM files for analysis (Auwera & O'Connor, 2020).

To investigate whether large insertions and deletions occurred in the mutagenesis samples, looking into copy number variation across the genome was selected as the best approach. Copy Number estimation by a Mixture Of PoissonS (cn.MOPS) is a data processing pipeline for copy number variations in next generation sequencing data (Klambauer et al., 2012). The program is written in C++ and runs using the statistical software R (version 3.6.3; R Core Team, 2020). The program is designed to convert BAM files into genomic ranges objects as the inputs for cn.MOPS analysis. By modeling the coverage depth across samples and position, this program can avoid read count biases. Ultimately, the program uses a Bayesian approach to identify copy number variation with a low false detection rate through the filtering of wrong detections indicated by high noise. Seventeen of the 18 samples in this project were used in this analysis. The 50-Gy sample from the first experimental M_1 row (50Gy1) was not included due to a corrupted BAM file with a size far smaller than the other samples.

Zhang et al. (2019) investigated the regulatory mechanism of anthocyanin biosynthesis in *Paeonia suffruticosa* and specifically focused on two little understood MYB transcription factors (TFs), PsMYB114L and PsMYB12L. They found that these two TFs enhanced anthocyanin expression through regulation of some anthocyanin biosynthesis-related genes (Figure 5). Such genes were identified as chalcone synthase (*CHS*), chalcone isomerase (*CHI*), flavanone 3-hydroxylase (*F3H*), flavonoid 3'-hydroxylase (*F3'H*), dihydroflavonol 4-reductase (*DFR*), anthocyanidin synthase (*ANS*), and flavonol synthase (*FLS*). To identify copy number variations in the sampled green mutants that could be responsible for knocking out the expression of anthocyanin, homologues of these genes and TFs had to be located in the HA412-HO v2 genome. Anthocyanin biosynthesis pathway and MYB gene protein sequences were previously identified in the XRQ *Helianthus annuus* genome annotation (Badoiun et al., 2017). These protein sequences were run against the HA412-HO v2 genome using TBLASTN to identify the locations of these genes on the HA412-HO v2 genome to allow for comparison with the copy number variation results (Altschul et al., 1990).

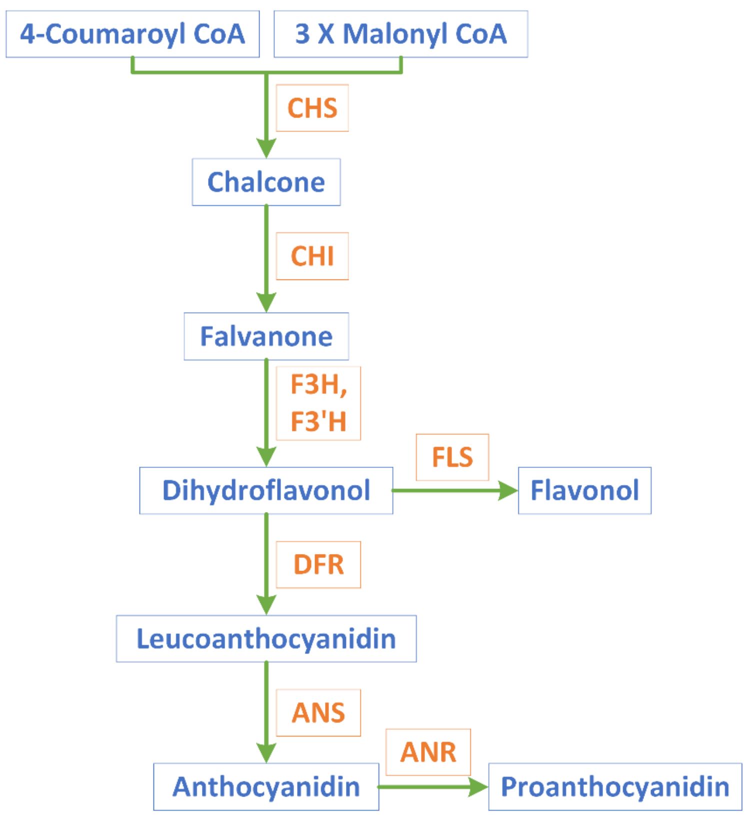

Figure 5: The general anthocyanin biosynthesis metabolic pathway found in plants. Adapted from Zhang et al., 2019.

Finally, to compare the 17 selected plant samples run through cn.MOPS with the locations of the anthocyanin biosynthesis pathway and MYB genes in the reference genome, the window length and prior impact parameters were modified in the cn.MOPS analysis. The author has stated that the ideal window length, or the length of the segments in which reads are counted, should be calculated by hand using the following equation (Klambauer, 2017):

$$averageReadCount = coverage * windowLength / readLength$$

Assuming you want to have 50 read counts per window, as suggested by the author, you can calculate the window length:

$$windowLength = readLength * 50 / coverage$$

In this project, the average read length was 141 bp and the average coverage was 0.89907 reads/locus.

$$windowLength = 141 * 50 / 0.89907$$

$$windowLength = 7{,}841$$

The prior impact parameter was changed from the default value of 1 to 0.5 by the suggestion of the algorithm's author because a high number of copy number variations (CNVs) were expected due to the mutagenesis of the sampled population (Klambauer, 2013). In addition to these two parameters being set, lower window lengths were also evaluated due to the low coverage of this project and because the smallest CNVs detected by the program are three times the window length. Window lengths of 7,500, 5,000, 1,000, and 500 bp were evaluated. Upon completion of these analyses, all copy number variations of 0 (complete deletions) were filtered out to locate where these knockouts occurred. The resulting locations were then compared with the TBLASTN results to identify if any anthocyanin biosynthesis pathway and MYB regulator genes fell in the same regions, potentially explaining the green phenotypes in the mutagenesis/experimental samples.

RESULTS

Phenotypic Results of the M_1 Population

Table 1: Dominant anthocyanin expression knockout in the M_1 population experimental rows

Treatment	Phenotype	Count
0-Gy	Red, anthocyanin expression	241
0-Gy	Green	0
50-Gy	Red, anthocyanin expression	1,116
50-Gy	Green	12

In the M_1 50-Gy experimental population at the winter nursery site outside Rancagua, Chile, there were 12 identified green plants out of a total of 1,128 experimental plants. This proportion of green to red among the 50-Gy experimental M_1 plants demonstrates a general mutation rate of 1.06% achieved from mutagenesis using anthocyanin expression as a marker, assuming there was only one gene that was heterozygous and affecting anthocyanin production. A low fatality rate with the 50-Gy dose of radiation and a satisfactory general mutation rate for a given trait were aimed for in this mutagenesis effort. This general mutation rate being satisfactory, along with about 70% of the planted seeds resulting in mature plants (1,128 plants from approximately 1,600 seed, 20 per row) appears to indicate a successful mutagenesis effort without too much lethality. Further evidence of lethality caused by mutagenesis was noted in stand counts of the M_1 population. This reduced stand count found in the 50-Gy experimental rows can be seen most profoundly in three rows planted using seed from the same parent (Figure 6) and looking at the population through a statistical lens.

Figure 6: Low stand count found in the M_1 50-Gy experimental rows. These three rows contained just six or seven plants and came from the same 50-Gy parent (16Ch_0800-0802).

A pair of two-way ANOVAs were performed using SAS software (version 9.4, Mixed procedure) to analyze the effect of the radiation and mother plant on stand count in the M_1 population (Tables 2 and 3). These analyses revealed that the treatment, 0 or 50-Gy, and mother plant had a significant effect on stand count, while also showing significant variation between rows of the population. This effect can be visually understood in the corresponding box plot visualization of stand count (Figures 7 and 8). The first two mother plants received 0-Gy control pollen and the other 12 mother plants received 50-Gy experimental pollen, leading to 13 and 80 total rows in the M_1 population, respectively. Stand count was measured just before flowering and at harvest with only viable seed-producing plants being counted to show further lethality effects.

Table 2: ANOVA of stand count prior to flowering in the M_1 population.

Component	df	Variance Estimate	Confidence Limit (0.05) Lower	Confidence Limit (0.05) Upper	F/Z Value	Pr > F/Z
Treatment (0 or 50-Gy)	1	–	–	–	F: 5.99	0.0307*
Mother Plant (Treatment)	12	7.6222	3.6387	24.8265	Z: 2.15	0.0157*
Residual	92	4.6372	3.4691	6.5166	Z: 6.26	<0.0001***

Table 3: ANOVA of seed-producing stand count in the M_1 population.

Component	df	Variance Estimate	Confidence Limit (0.05) Lower	Confidence Limit (0.05) Upper	F/Z Value	Pr > F/Z
Treatment (0 or 50-Gy)	1	–	–	–	F: 32.43	<0.0001***
Mother Plant (Treatment)	12	3.5612	1.5414	15.1911	Z: 1.84	0.0327*
Residual	92	5.7703	4.3150	8.1136	Z: 6.25	<0.0001***

Stand counts reflect plants that produced viable seed at harvest and produced M_2 progeny.

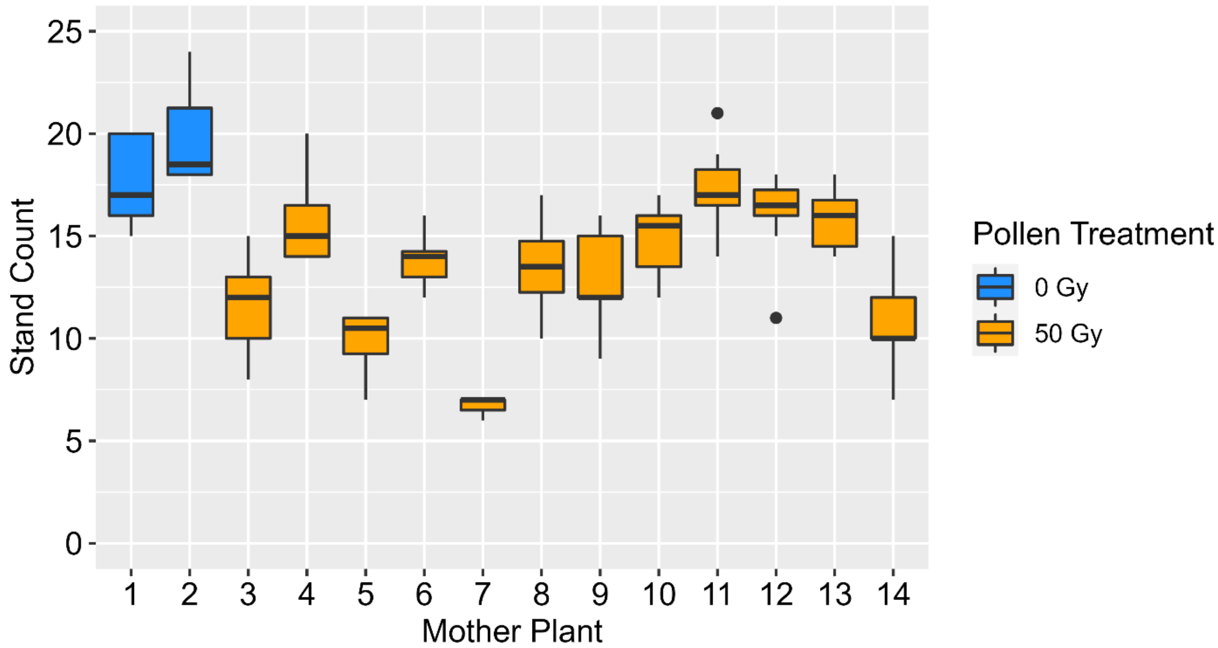

Figure 7: Stand count prior to flowering in the M_1 population. Average stand count per row was significantly lower in the 50-Gy derived M_1 plants, with significant variation between mothers and rows as well.

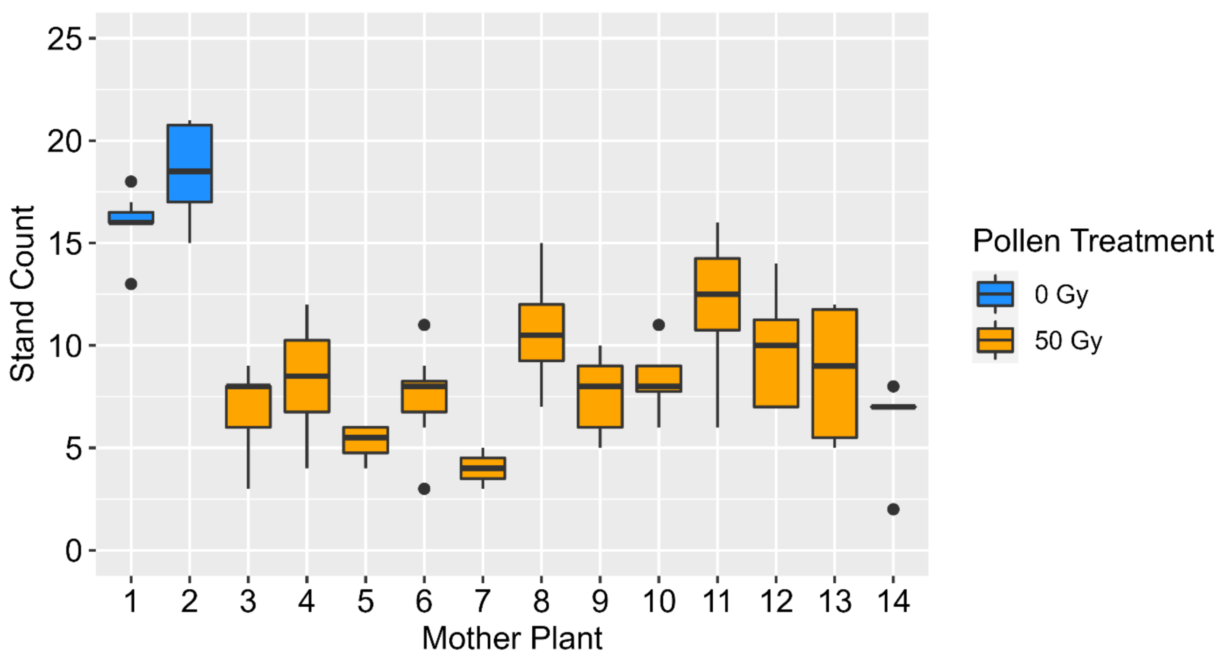

Figure 8: Seed-producing stand count in the M_1 population. In the 50-Gy row stand count averages, there was significantly less plants that resulted in viable seed, with significant differences present between mother plants and rows as well.

A third two-way ANOVA was performed using SAS software (version 9.4, Glimmix procedure) to analyze the effect of the radiation and mother plant on uniformity, scaled 1-5, in the M_1 population (Table 4). This revealed that the treatment, 0 or 50-Gy, had a significant effect on uniformity, while mother plant did not have a significant impact. This effect can be visually understood in the corresponding box plot visualization of uniformity (Figure 9).

Table 4: ANOVA of row uniformity in the M_1 population.

Component	df	F Value	Pr > F
Treatment (0 or 50-Gy)	1	21.18	<0.0001***
Mother Plant (Treatment)	12	1.37	0.1988

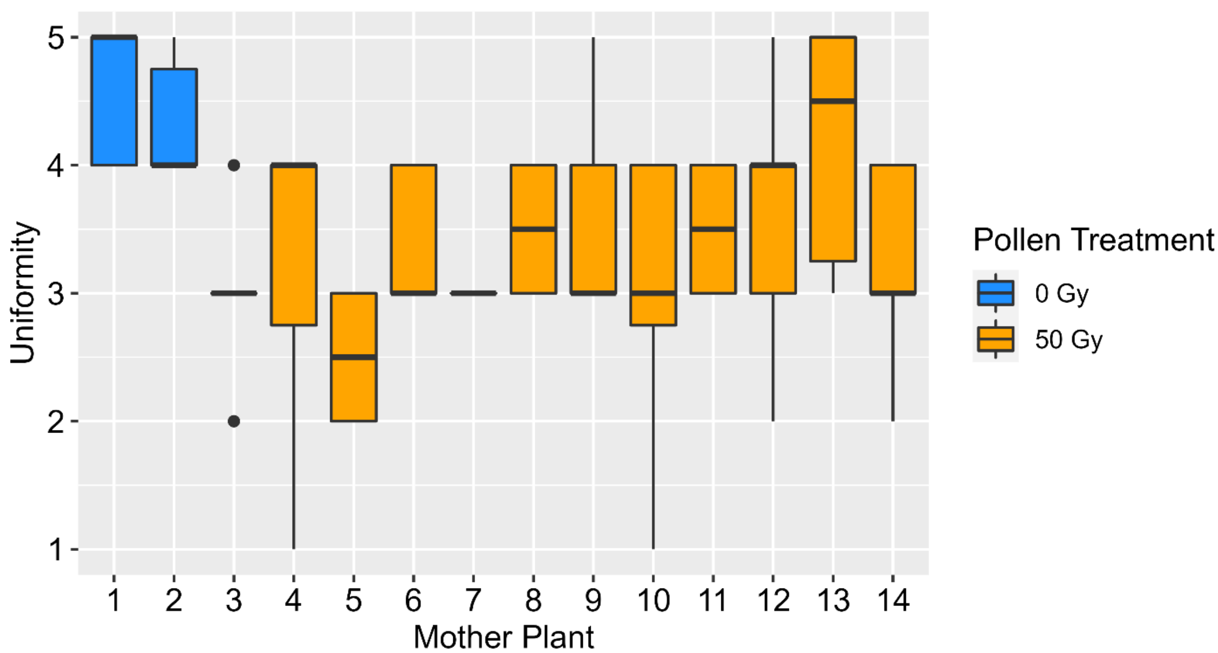

Figure 9: Row uniformity in the M_1 population. Uniformity was significantly lower in the 50-Gy derived M_1 row averages.

Beyond population scale analysis of the M_1 plants, further evidence of mutagenesis effects can be identified in individual plants within 50-Gy experimental rows. The anthocyanin expression knockout was one of the most obvious phenotypic mutations noted in this population as mentioned before, showing up in 12 of the 80 experimental rows (Figure 10). Beyond this notable mutation, other mutants of interest identified in this population included (1) leaf crinkle, (2) slender stem structure, (3) short stature, and (4) dwarfism (beyond short height). These mutations occurred in a small minority of the plants found in the experimental rows of the M_1 population (Figure 11). Finally, there were also five plants found in the experimental rows that were branched in some way (Figure 12).

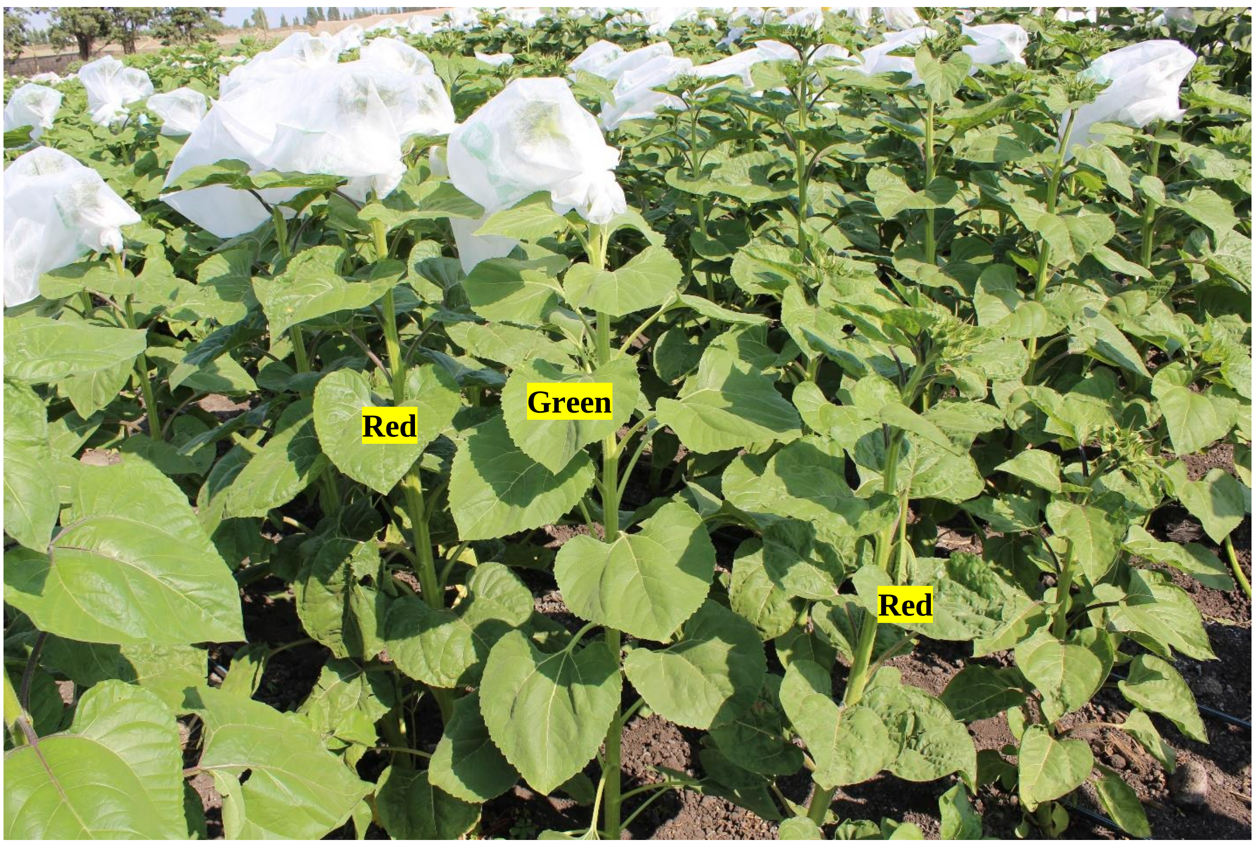

Figure 10: 50-Gy experimental row demonstrating anthocyanin expression knockout. One of the 12 experimental rows with a plant demonstrating the anthocyanin expression knockout found in the M_1 population. This phenotypic anomaly is labelled “Green” and is in contrast to the expected “Red” phenotype.

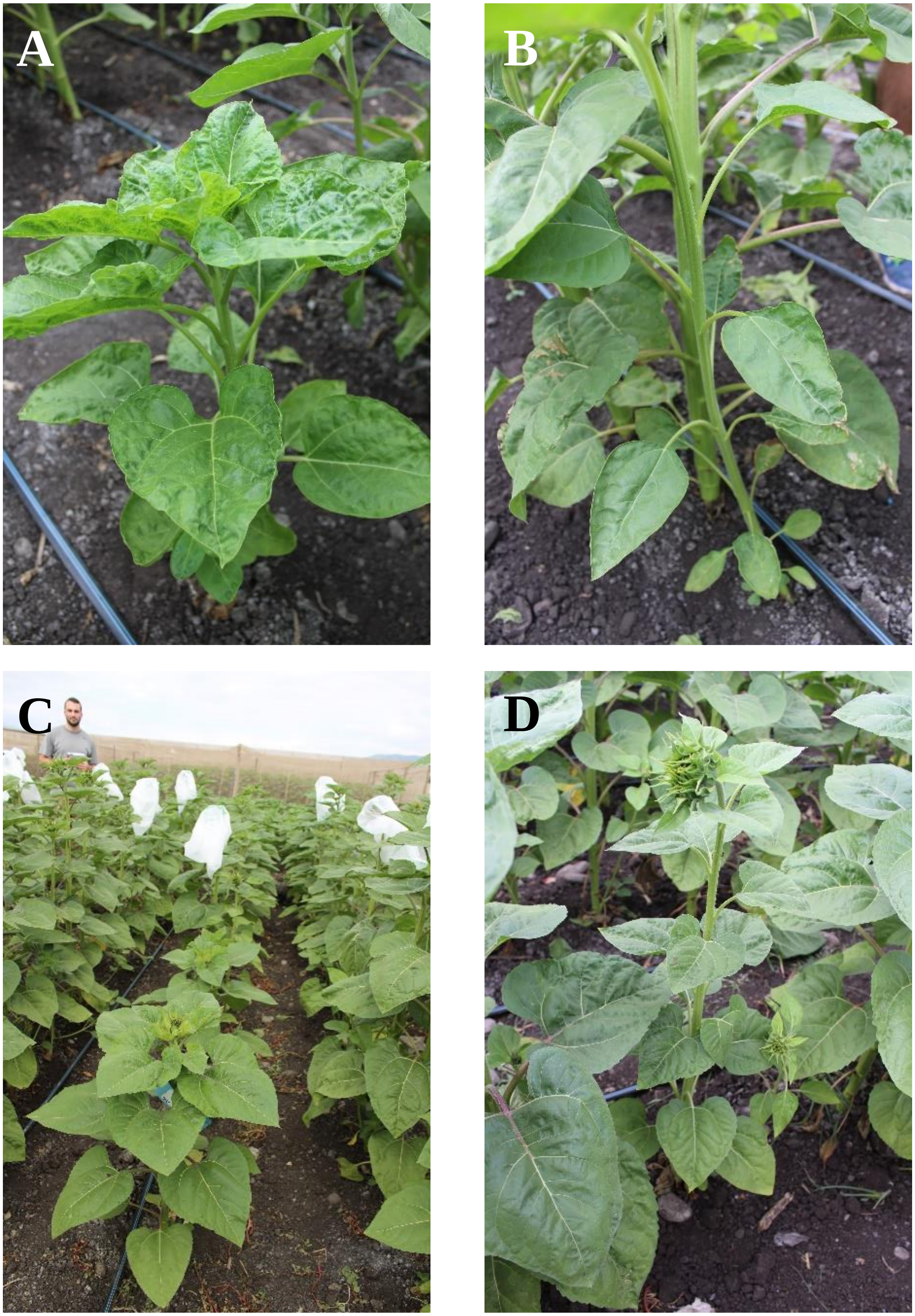

Figure 11: Mutants of interest found in the 50-Gy M_1 population. A. Green & leaf crinkle (16Ch_0782, sampled) B. Green & slender (16Ch_0804, sampled) C. Short row (16Ch_0822) D. Green & small (16Ch_0828, sampled)

Figure 12: Branching mutants found in the experimental M_1 population. Top: 16Ch_0815, bottom: 16Ch_0787.

Genomic Results of the M_1 Population

Copy number variation was detected by cn.MOPS (Figure 13). This data processing pipeline compared the genomes of the 17 samples and identified regions of their genomes where there was more or less than two copies, due to the reference genome used having one genome copy from each parent. The copy number variations visualized below were detected using cn.MOPS with a calculated window length of 7,841 bp and a prior impact value of 0.5. The samples consist of: 10 green plants ("Gr"), three branched plants ("Br"), a plant from a low count row ("LC") and the single 50-Gy sample taken from the last experimental row.

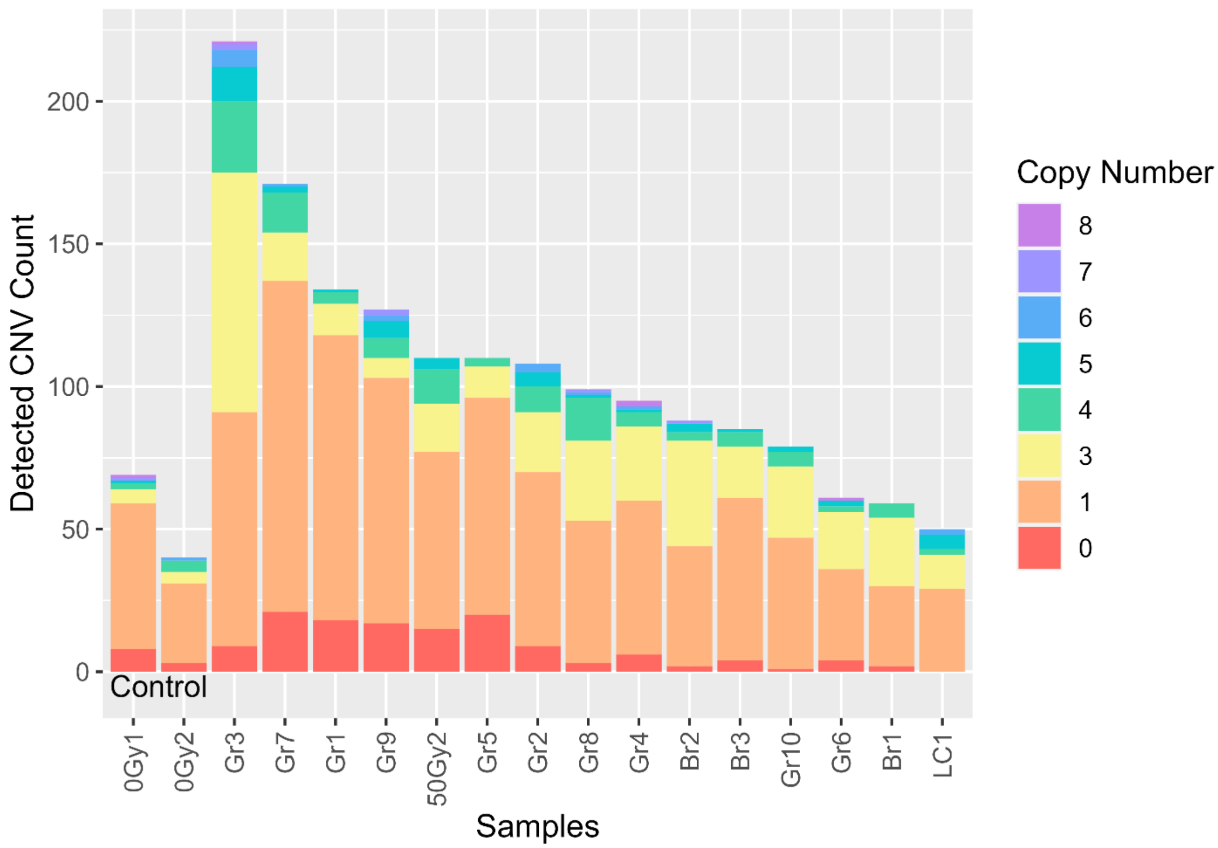

Figure 13: Copy number variation across samples in the M_1 population. The left two bars are control samples collected from plants in the M_1 population generated with pollen that did not receive radiation. The 15 bars to the right represent samples taken from the 50-Gy experimental rows of the M_1 population. Copy number variation was detected among these samples using cn.MOPS and with a minimum size of 23,523 bp, or three times the calculated window length.

On average, there was a higher number of copy number variations detected among the 50-Gy experimental samples compared to the control (0-Gy). However, due to the limited number of samples, particularly with just two control samples, there was not a significant difference in copy number variation found between or within the 0 and 50-Gy samples ($F = 2.56$, $p = 0.1302$; SAS software, version 9.4, Glm procedure). A full list of all copy number variations found across these 17 samples visualized in Figure 13 can be found in the Appendix Table A1. None of the copy number 0 variations detected in the green 50-Gy experimental samples overlapped the locations of genes responsible for anthocyanin biosynthesis identified by TBLASTN in the HA412-HO v2 reference genome, which would theoretically indicate an explanation for anthocyanin expression knockout identified in the phenotypes of the M_1 population.

Evidence of issues with this analysis can be found when interpreting the total count of copy number variations detected across chromosomes. In Figure 14, the total number of copy number variations detected in each chromosome per hundred million bp is visualized, with each bar broken down according to copy number. The average total count of copy number variations detected per hundred million bp in each chromosome using cn.MOPS (window length = 7,841 bp, prior impact 0.5) was 51.93. Notable outliers were chromosomes 11 and 17 (more than two standard deviation away from the mean). All other chromosomes in this analysis had a total number of copy number variations per hundred million bp equal to or less than 89.01.

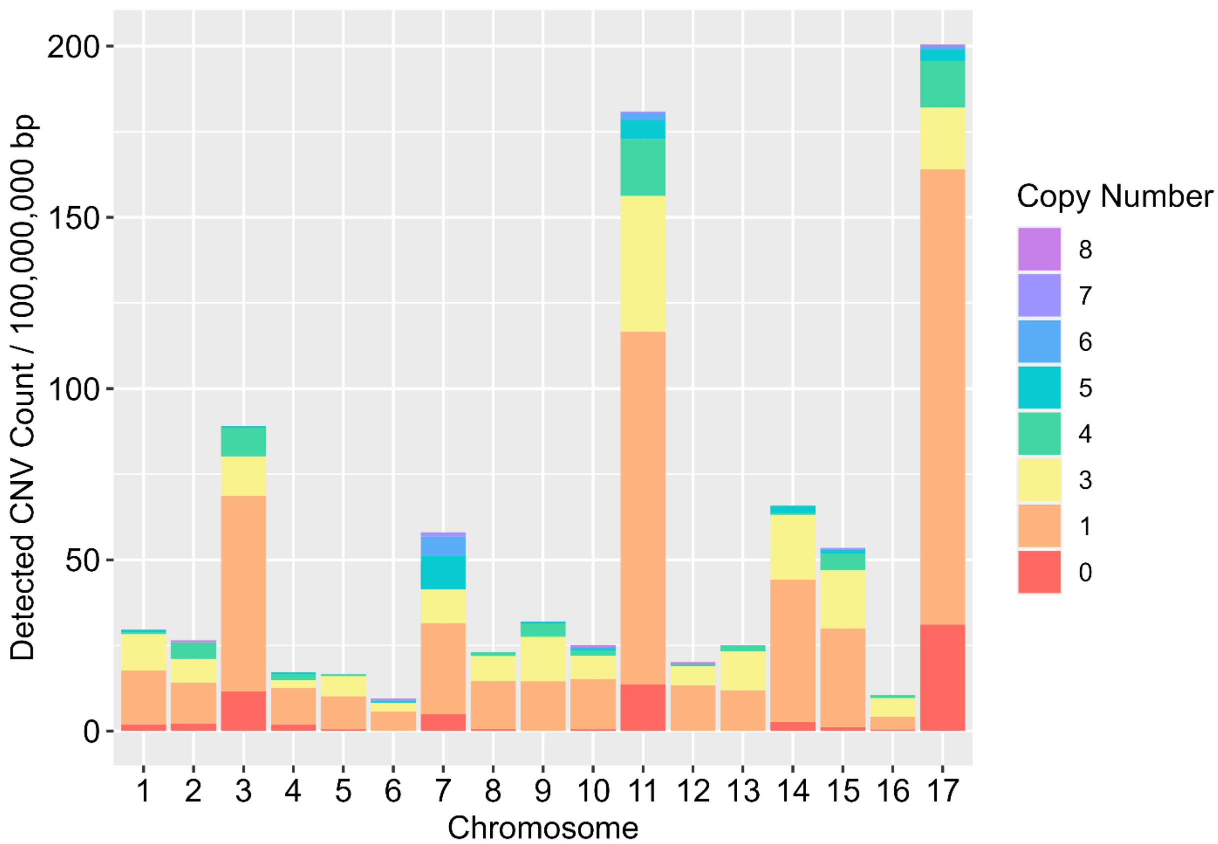

Figure 14: Copy number variation across chromosomes in the M_1 population.

Due to the low coverage of the sequenced samples, this analysis was limited to large copy number variations (23,523 bp width, or 3 times the window length of 7,841 bp). As a result, shorter window lengths were evaluated to determine if any smaller width 0 copy number variations could potentially explain anthocyanin expression knockout in the green 50-Gy experimental samples. Window lengths of 7,500, 5,000, and 1,000 bp did not detect any 0 copy number variations that overlapped with the locations of genes responsible for anthocyanin biosynthesis, but when reduced to 500, three matches were found (Table 5). The F3H gene location identified on chromosome 13 fits within the copy number 0 location identified by cn.MOPS and the two F3H gene locations on chromosome 17 fits within the copy number 0 window identified by cn.MOPS. As a result of using a window length of 500, which is much smaller than recommended by the author based on the average read depth (Klambauer, 2017), these results cannot be trusted as they could be false positives. They provide a possible explanation for the anthocyanin expression knockout found in the Gr2, Gr7, and Gr8 samples.

Table 5: Copy number 0 anthocyanin biosynthesis pathway gene matches

Copy Number 0 Identified by cn.MOPS (window length 500)						
Chr	Start	End	Width	Gr2	Gr7	Gr8
13	27319501	27321000	1500	0	0	2
17	57361001	57363500	2500	2	2	0
F3H Anthocyanin Biosynthesis Gene Locations*						
Chr	Start	End	Width	% Identity	Mismatches	Bitscore
13	27320537	27319605	490	100	0	601
17	57362769	57361822	522	100	0	536
17	57361747	57361118	522	98.1	4	433

*All three gene locations had an e-value of 0 as determined by TBLASTN.

Phenotypic Results of the M_2 Population

The M_2 population was produced by selfing all plants in the M_1 population, then planting the resulting seed in progeny rows in two replications: (1) ~85% near Ponce, Puerto Rico in 2016 and ~15% low seed count in Glyndon, Minnesota in 2016, and (2) in Glyndon, Minnesota over 2017 and 2018. The first replication was testcrossed with the yellow inbred line to assess for potential paternal haploid inducers and the second replication was grown out for further phenotypic analysis of the M_2 and to produce M_3 seed. The M_2 population demonstrated phenotypic evidence for a successful mutagenesis with a variety of mutations, including good vigor, low stand count, uniformity issues, crinkle leaf, chlorosis, dwarfism, and rounded leaf. An example of dwarfism and crinkle leaf can be seen in Figure 15. A summary of mutations found in the M_2 populations can be found in Table 6. Some of these mutations appeared to demonstrate Mendelian segregation, such as having approximately one quarter of the row having a given mutation present.

Figure 15: Leaf crinkle and dwarfism mutants found in the M_2 population. An example of the leaf crinkle trait being expressed in an M_2 plant (17_381, left). An example of dwarfism found in the M_2 population (17_351, below). Both traits demonstrate Mendelian segregation (1/4) in these examples.

Table 6: Mutants of interest found in the experimental M_2 population

Phenotype	Number of Rows
Short	40
Dwarf	24
Leaf Crinkle	23
Off Type	5
Chlorosis	2
Hypersensitive	1
Smooth leaf	1
Narrow leaf	1
Round leaf	1
Total Rows in M_2	892

These counts were taken from the 2016, 2017, and 2018 Glyndon, Minnesota fields that make up the M_2 population (rep 2) and does not include the M_2 population used for yellow testcrossing (rep 1).

Phenotypic Results of the M_2 x Yellow Population

The M_2 x yellow testcross population was produced by crossing all plants in the M_2 population with the yellow inbred line and then planting the resulting seed across the 2017 Glyndon, Minnesota field and 2017 and 2018 Prosper, North Dakota fields. This population was grown out to take further phenotypic notes of this mutagenesis effort but most importantly to look for potential paternal haploid inducers by observing testcross progeny. Such potential evidence would show up in this population as a yellow plant due to the recessive chlorophyll deficiency of the male parent passing to the offspring in this population. If found, it could mean the female parent induced paternal haploidy in the testcross offspring. Mutations found beyond yellow in this population included dwarfism, leaf crinkle, height variability, and leaf serration. A summary of mutations found in the M_2 x yellow population can be found in Table 7. Some of these mutations appeared to demonstrate Mendelian segregation, such as having approximately one quarter of the row having a given mutation present. Notably, none of the yellow plants in this population were considered "true" yellows with an appearance like the yellow inbred line.

The yellows in this population were likely caused by abiotic or biotic stresses, or had different types of chlorophyll deficiency than in the yellow inbred line used in this testcross population. This indicated that none of these plants demonstrated a successful haploid induction by an M_2 experimental parent.

Table 7: Mutants of interest found in the M_2 x yellow population

Phenotype	Number of Rows
Yellow	45
Short	26
Dwarf	22
High Vigor	14
Height Variation	9
Leaf Crinkle	8
Red	8
No Plants	5
Off Type	2
Chlorosis	1
Leaf Serration	1
Total Rows in M_2 x Yellow	6,227

These counts were taken from the 2017 Glyndon, Minnesota field and the 2017 and 2018 Prosper, North Dakota fields that made up the M_2 x yellow population.

DISCUSSION

The primary objective of this project was to determine if radiation induced mutagenesis of pollen is a useful mutagenesis method for sunflower. Induction of mutagenesis via radiation of pollen proved successful and useful in this project, demonstrated most clearly in the phenotypic results of the M_1 population, M_2, and M_2 x yellow populations. In the M_1 population, a general mutation rate of 1.06% was achieved from a 50-Gy dose of x-ray radiation on the parent pollen, measured through the knockout of anthocyanin expression (Table 1). Prior to the experiment, an adequate mutation rate was aimed for while also allowing for a low level of lethality in the resulting populations. In the stand count prior to flowering, the treatment (0 vs 50-Gy) and the mother plant resulted in significantly different stand counts ($p = 0.03$; $p = 0.02$; Table 2; Figure 7). In the stand count after harvest, the treatment and mother were again found to result in significantly different stand counts ($p < 0.0001$; $p = 0.03$; Table 3; Figure 8). The significant difference between mother plants is most easily explained by the significant lodging and pathogen stress experienced by the mother plants in the initial crossing effort. A significant storm caused severe lodging that amplified the *Phomopsis* infections found in the mother plants, which are notably susceptible. These numbers both prove that the mutagenesis effort was effective and that it was also not too severe of a dose, with every experimental mother plant resulting in enough M_1 plants to analyze. Doses in prior experiments higher than 50-Gy demonstrated unacceptable lethality while doses of 33-Gy and lower did not result in significantly noticeable mutations. It is estimated that 70% of M_1 seeds resulted in sexually mature plants. While the data was not specific enough in this population or in the non-irradiated “French” variety to statistically evaluate the germination rates, this rate fits in line with expectations because x-rays introduce random mutations. Most mutations are either harmful or neutral, with advantageous mutations being rare. This explains an overall reduction in fitness among the 50-Gy experimental rows as measured through uniformity, stand count, and germination rate.

Further evidence of successful mutagenesis found in the M_1 population is observed in the uniformity ratings of the control and experimental rows, as well as case studies of individual plants with notable phenotypic mutations. While uniformity ratings are subjective due to it being a human impression on a scale of one to five, an ANOVA did show a significant reduction in uniformity in the 50-Gy experimental rows compared to the 0-Gy control rows ($p < 0.0001$; Table 4; Figure 9). Beyond overall metrics, the mutagenesis effects were visually striking due to unique physical mutations being observed in the M_1 population. Figure 11 shows leaf crinkle, slender structure, and shorter height mutants, while Figure 12 showed a pair of branching mutants. Sometimes branching like the pair of plants in Figure 12 can be observed in any field setting that has experienced significant abiotic stress early in the sunflower life cycle, particularly flooding stress. It is impossible to prove whether or not this was the case in these examples, however it is important to note that (1) the soil had better drainage than soil in the author's region, (2) there was no rainfall during the lifecycle prior to phenotypic measurements (Weather Underground, 2016), and (3) the only water source was drip tape irrigation with no apparent leaks nearby. Mutations such as those mentioned here were not found in the 0-Gy control rows.

In the M_2 and M_2 x yellow populations, a wide variety of phenotypic mutants of interest were observed. In the M_2 population, short height, dwarfism, leaf crinkling, chlorosis, hypersensitivity, and leaf mutations were all observed with frequency of each phenotype ranging from just one row (~0.11%) to 40 rows (~4.48%; Table 6). In the M_2 x yellow population, chlorophyll deficiency, short height, dwarfism, high vigor, height variation, leaf crinkling, complete lethality, chlorosis, and leaf serration were all observed ranging from one row (~0.16%) to 45 rows (~0.72%; Table 7). It is important to note that the M_2 population included 892 rows and the M_2 x yellow population included 6,227 rows, which is a substantial number of plants to phenotype. As a result, it is reasonable to believe that some notable phenotypic mutations in this population were missed in the testcross population. Interestingly, multiple rows in the M_2 population appeared to demonstrate Mendelian segregation with a quarter of plants in each row presenting (likely recessive) phenotypic mutations of interest. Figure 15 shows this occurring in two rows; one with leaf crinkling and the other with dwarfism. Recessive mutants are the most common type of mutant produced with physical or chemical mutagens.

A secondary objective of this project was to identify the types of mutations that were observed in the experimental population following x-ray radiation on pollen. There were significant limitations in this project's ability to identify genetic evidence of mutations, particularly point mutations due to a relatively low average sequencing depth (average read number) of 3x and a low average coverage (average read coverage across reference genome) of 0.89907 reads/locus. The low average coverage particularly limited this project's ability to identify copy number variations that would indicate insertions or deletions because it required a rather large window length to be used in the cn.MOPS analysis. As seen in Table 5, if the window length was reduced from the appropriate 7,841 to just 500 bp, there were results detected that correlated with the location of anthocyanin biosynthesis pathway genes. Because there is some uncomfortably high chance of a false positive because of inappropriately reducing the window length to such a small size, such results cannot be considered dependable. False positives in a window length of 500 bp are much more likely since the coverage of these samples is too low, having a high chance of incorrect sequencing in such a short window. However, it was included in this analysis to demonstrate how replicating a similar experiment and analysis in the future with better genomic resources could greatly benefit the study of mutagenesis efforts and even lead to novel discoveries of genes of interest.

One additional limitation of this project's genomic analysis was the lack of a reference genome related to the parental line. By using the HA412-HO v2 genome as a refence for analysis, it made it impossible to detect SNPs and insertions/deletions (INDELs) through germline short variant discovery using software such as GATK. Because the SNPs and INDELs identified by GATK are in relation to a reference genome from a quite different variety, there is no reasonable way to know what is a novel mutation among the experimental samples. While the two control samples could act to filter out non-mutagenesis SNPs and INDELs (ones that are simply differences between the French Red parent line and the HA412HO reference line) such an analysis would not be dependable, particularly due to the limited number of control samples. That said, this analysis was still able to produce an interesting number of copy number variations identified using cn.MOPS, which can all be seen in Appendix Table A1 and are also visualized by sample in Figure 13 and by chromosome in Figure 14. Notably, the copy number variants detected per hundred million bp for each chromosome is not at all consistent and further indicates that the reference was not ideal for this project.

A third objective of this thesis was to diagnose any potential doubled haploid genomes present in the M_2 x yellow testcross populations as a proxy for identifying a novel haploid inducer via mutagenesis. Despite 45 of the 6,227 rows in the M_2 x yellow population containing plants with a yellow appearance, none were considered "true" yellows with an appearance similar enough to the yellow inbred line parent. The yellows in this population were likely caused by abiotic or biotic stresses, or had different types of chlorophyll deficiency than in the yellow inbred line used in this testcross population. As a result, there does not appear to be any successful haploid induction by an M_2 experimental parent.

CONCLUSION AND RECOMMENDATIONS FOR FUTURE WORK

Overall, the mutagenesis methodology presented here was found to be effective and successful, with strong evidence of mutations caused by x-ray radiation on pollen being demonstrated in the phenotypic results of the M_1, M_2, and M_2 x yellow populations as well as in the genomic study of the M_1 population. Induction of mutagenesis using a 50-Gy dose of x-ray radiation given to experimental pollen using a LINAC at the Sanford Roger Maris Cancer Center in Fargo, North Dakota was quite successful with an acceptable rate of lethality in the resulting populations. However, it was not possible to identify with confidence a direct cause of anthocyanin expression knockout in the M_1 experimental green plants, nor was this project successful in identifying a paternal haploid inducer. The mutagenesis methodology outlined here could certainly be used for further mutagenesis efforts with different variables (e.g. dosage, parent lines) and the resulting populations from this project could be further analyzed and utilized for inbred line development.

This project lays the foundation for a wide variety of future work. First, the germplasm generated through this effort could be analyzed in yield, disease, and herbicide trials to potentially identify novel mutations of interest. Second, more sequencing of experimental lines in this project could occur with higher coverage to allow for a stronger copy number variation analysis (e.g. smaller window length in cn.MOPS). Third, assembly of a parental reference (French Red) genome could provide greater insight into these mutagenesis populations, including superior detection of novel SNPs, INDELs, and CNVs. Alternatively, this mutagenesis methodology could be replicated with different genetics or LINAC doses. For example, the high anthocyanin expression version of the sunflower inbred line HA 467 (not available at the start of this project) could be used as a parental line because of its superior self-compatibility, imidazolinone herbicide tolerance, superior lodging resistance, high oleic oil content, and strong *Sclerotinia* head and stalk rot resistance (Hulke et al., 2018). Pairing this more agronomically relevant variety with more powerful genomic analyses and a potentially higher x-ray mutagen dose (e.g. 75 or 100-Gy) would likely have higher success in terms of mutants and paternal haploid induction.

Todorova et al. (1997) was able to produce agronomically useful doubled haploid sunflower lines at a rate of 8.6% compared to the total fertile resulting lines in their induced parthenogenesis study using much higher doses of radiation on pollen (300, 600, 900-Gy), though they observed far higher lethality. This approach of inducing parthenogenesis via severe genetic damage of the pollen is useful as a reference for x-ray mutagen doses but not beyond that due to the different overall approach. Beyond further genomic and yield trial analysis of the populations resulting from this mutagenesis effort, it would make sense to apply for grant funding and seek collaborations (such as INRAE) to conduct further mutagenesis projects that experiment with similar and higher doses of radiation and more agronomically interesting sunflower lines. Pairing such an effort with enhanced genomic analysis would lead to a much deeper understanding of mutagenesis outcomes and have a greater chance of identifying a useful paternal haploid inducer that would benefit the global sunflower community and their ability to meet the demands and challenges of the future.

REFERENCES

Altschul, S. F., Gish, W., Miller, W., Myers, E. W., & Lipman, D. J. (1990). Basic Local Alignment Search Tool. *Journal of Molecular Biology*, *215*(3), 403–410. https://doi.org/10.1016/s0022-2836(05)80360-2

Auwera, G. V. Der, & O'Connor, B. (2020). *Genomics in The Cloud: Using Docker, GATK, And WDL In Terra* (1st Edition). O'Reilly Media.

Badouin, H., Gouzy, J., Grassa, C. J., Murat, F., Staton, S. E., Cottret, L., Lelandais-Brière, C., Owens, G. L., Carrère, S., Mayjonade, B., Legrand, L., Gill, N., Kane, N. C., Bowers, J. E., Hubner, S., Bellec, A., Bérard, A., Bergès, H., Blanchet, N., … Langlade, N. B. (2017). The Sunflower Genome Provides Insights into Oil Metabolism, Flowering and Asterid Evolution. *Nature*, *546*(7656), Article 7656. https://doi.org/10.1038/nature22380

Barstow, A., Prasifka, J. R., Attia, Z., Kane, N. C., & Hulke, B. S. (2022). Genetic Mapping of a Pollinator Preference Trait: Nectar Volume in Sunflower (Helianthus Annuus). *Frontiers Plant Science (In Review).*

Blackman, B. K., Scascitelli, M., Kane, N. C., Luton, H. H., Rasmussen, D. A., Bye, R. A., Lentz, D. L., & Rieseberg, L. H. (2011). Sunflower Domestication Alleles Support Single Domestication Center in Eastern North America. *Proceedings of The National Academy of Sciences*, *108*(34), 14360–14365. https://doi.org/10.1073/pnas.1104853108

Bolger, A. M., Lohse, M., & Usadel, B. (2014). Trimmomatic: A Flexible Trimmer for Illumina Sequence Data. *Bioinformatics*, *30*(15), 2114–2120. https://doi.org/10.1093/bioinformatics/btu170

Eapen, S., & D’Souza, S. F. (2005). Prospects of Genetic Engineering of Plants for Phytoremediation of Toxic Metals. *Biotechnology Advances*, *23*(2), 97–114. https://doi.org/10.1016/j.biotechadv.2004.10.001

Encheva, J., Köhler, H., Friedt, W., Tsvetkova, F., Ivanov, P., Encheva, V., & Shindrova, P. (2003). Field Evaluation of Somaclonal Variation in Sunflower (Helianthus Annuus L) And Its Application for Crop Improvement. *Euphytica*, *130*(2), 167–175. https://doi.org/10.1023/a:1022881200649

Fischer, R. A., Byerlee, D., & Edmeades, G. (2014). Crop Yields and Global Food Security: Will Yield Increase Continue to Feed the World? ACIAR Monograph No. 158. Australian Centre For International Agricultural Research. *Canberra*.

Forster, B. P., Packniyat, H., Simpson, C. G., & Handley, L. L. (1995). Genetic Control of Salt Tolerance in Barley. *Induced Mutations and Molecular Techniques for Crop Improvement. Proceedings of An International Symposium*. http://inis.iaea.org/search/search.aspx?orig_q=rn:27028559

Gao, Q.-M., Kane, N. C., Hulke, B. S., Reinert, S., Pogoda, C. S., Tittes, S., & Prasifka, J. R. (2017). Genetic Architecture of Capitate Glandular Trichome Density in Florets of Domesticated Sunflower (Helianthus Annuus L.). *Frontiers in Plant Science*, *8*, 2227. https://doi.org/10.3389/fpls.2017.02227

Gontcharov, S. V., Antonova, T. S., & Saukova, S. L. (2006). Sunflower Breeding for Resistance to Fusarium / Selección De Girasol Por Resistencia a Fusarium / Sélection De Tournesol Pour La Résistance Au Fongus Fusarium. *HELIA*, *29*(45), 49–54. https://doi.org/10.2298/hel0645049g

Grichko, V. P., Filby, B., & Glick, B. R. (2000). Increased Ability of Transgenic Plants Expressing the Bacterial Enzyme ACC Deaminase to Accumulate Cd, Co, Cu, Ni, Pb, And Zn. *Journal of Biotechnology*, *81*(1), 45–53. https://doi.org/10.1016/s0168-1656(00)00270-4

Harter, A. V., Gardner, K. A., Falush, D., Lentz, D. L., Bye, R. A., & Rieseberg, L. H. (2004). Origin of Extant Domesticated Sunflowers in Eastern North America. *Nature*, *430*(6996), 201–205. https://doi.org/10.1038/nature02710

Hulke, B. S., Ma, G., Qi, L. L., & Gulya, T. J. (2018). Registration of Oilseed Sunflower Germplasms RHA 461, RHA 462, RHA 463, HA 465, HA 466, HA 467, And RHA 468 With Diversity in Sclerotinia Resistance, Yield, And Other Traits. *Journal of Plant Registrations*, *12*(1), 142–147. https://doi.org/10.3198/jpr2017.04.0023crg

IT PGRFA Secretariat. (2009). *Introduction to The International Treaty on Plant Genetic Resources for Food and Agriculture*. FAO, Rome, Italy. https://www.fao.org/3/i0510e/i0510e.pdf

Jang, K. W., Lee, M., Lim, H., Kang, S. K., Lee, S. J., Kim, J. K., Moon, Y. M., Kim, J. Y., & Jeong, D. H. (2020). Development of A Wide Dose-Rate Range Electron Beam Irradiation System for Pre-Clinical Studies and Multi-Purpose Applications Using A Research Linear Accelerator. *Korean Society of Medical Physics*, *31*(2), 9–19. https://doi.org/10.14316/pmp.2020.31.2.9

Kinman, M. L. (1970). New Developments in The USDA And State Experiment Station Sunflower Breeding Programs. *Proceedings of the 4th International Sunflower Conference*, 181–183. www.isasunflower.org/publications

Klambauer, G. (2013, May). *Cn.MOPS: Copy Number for Low-Coverage Bam Files?* https://support.bioconductor.org/p/52338/

Klambauer, G. (2017, January). *Call CNV In Population with Large Depth Variance*. https://support.bioconductor.org/p/91068/

Klambauer, G., Schwarzbauer, K., Mayr, A., Clevert, D.-A., Mitterecker, A., Bodenhofer, U., & Hochreiter, S. (2012). Cn.MOPS: Mixture of Poissons For Discovering Copy Number Variations in Next-Generation Sequencing Data with A Low False Discovery Rate. *Nucleic Acids Research*, *40*(9), E69. https://doi.org/10.1093/nar/gks003

Krämer, U., & Chardonnens, A. (2001). The Use of Transgenic Plants in The Bioremediation of Soils Contaminated with Trace Elements. *Applied Microbiology and Biotechnology*, *55*(6), 661–672. https://doi.org/10.1007/s002530100631

Leclercq, P. (1969). Une Sterilite Male Chez Le Tournesol (A Male Sterility in Sunflower). *Annales D, Amelioration Des Plantes*, *19*, 99–106.

Lentz, D. L., Pohl, M. D., Alvarado, J. L., Tarighat, S., & Bye, R. (2008). Sunflower (Helianthus Annuus L.) As A Pre-Columbian Domesticate in Mexico. *Proceedings of The National Academy of Sciences*, *105*(17), 6232–6237. https://doi.org/10.1073/pnas.0711760105

Li, H. (2013). *Aligning Sequence Reads, Clone Sequences and Assembly Contigs with BWA-MEM* (Arxiv:1303.3997). Arxiv. https://doi.org/10.48550/arxiv.1303.3997

Maluszynski, M., Ahloowalia, B. S., & Sigurbjörnsson, B. (1995). Application Of In Vivo And In Vitro Mutation Techniques for Crop Improvement. *Euphytica*, *85*(1), 303–315. https://doi.org/10.1007/bf00023960

Maluszynski, M., Zanten, L. Van, Ashri, A., Ahloowalia, B., Weck, E., Brunner, H., & Zapata, F. J. (1995). Mutation Techniques in Plant Breeding. *Induced Mutations and Molecular Techniques for Crop Improvement. Proceedings of An International Symposium*. http://inis.iaea.org/search/search.aspx?orig_q=rn:27028566

Meng, D., Luo, H., Dong, Z., Huang, W., Liu, F., Li, F., Chen, S., Yu, H., & Jin, W. (2022). Overexpression of Modified CENH3 In Maize Stock6-Derived Inducer Lines Can Effectively Improve Maternal Haploid Induction Rates. *Frontiers in Plant Science*, *13*. https://www.frontiersin.org/articles/10.3389/fpls.2022.892055

Pérez-Vich, B., Berry, S. T., Velasco, L., Fernández-Martínez, J. M., Gandhi, S., Freeman, C., Heesacker, A., Knapp, S. J., & Leon, A. J. (2005). Molecular Mapping of Nuclear Male Sterility Genes in Sunflower. *Crop Science*, *45*(5), 1851–1857. https://doi.org/10.2135/cropsci2004.0684

Pogoda, C. S., Keepers, K. G., Lendemer, J. C., Kane, N. C., & Tripp, E. A. (2018). Reductions in Complexity of Mitochondrial Genomes in Lichen-Forming Fungi Shed Light on Genome Architecture of Obligate Symbioses. *Molecular Ecology*, *27*(5), 1155–1169. https://doi.org/10.1111/mec.14519

R Core Team. (2020). *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing. https://www.r-project.org/

Schneiter, A. A. (1997). *Sunflower Technology and Production*. American Society of Agronomy.

Schneiter, A. A., Miller, J. F., & Berglund, D. R. (2019). *Stages of Sunflower Development* [Review of *Stages of Sunflower Development*, By H. Kandel]. NDSU Extension. https://www.ndsu.edu/agriculture/ag-hub/publications/stages-sunflower-development

Srinivas, C. (2019). A Primer on Theory and Operation of Linear Accelerators in Radiation Therapy. *Journal of Medical Physics*, *44*(4), 298. https://doi.org/10.4103/jmp.jmp_68_19

Terzić, S., Boniface, M.-C., Marek, L. L., Alvarez, D., Baumann, K., Gavrilova, V., Joita-Pacureanu, M., Sujatha, M., Valkova, D., Velasco, L., Hulke, B. S., Jocić, S., Langlade, N., Muños, S., Rieseberg, L., Seiler, G., & Vear, F. (2020). Gene Banks for Wild and Cultivated

Sunflower Genetic Resources. *OCL Oilseeds and Fats Crops and Lipids*, *27*(9), 1. https://doi.org/10.1051/ocl/2020004

Todorova, M., Ivanov, P., Shindrova, P., Christov, M., & Ivanova, I. (1997). Doubled Haploid Production of Sunflower (Helianthus Annuus L.) Through Irradiated Pollen-Induced Parthenogenesis. *Euphytica*, *97*(3), 249–254. https://doi.org/10.1023/a:1002966824988

Uliana Trentin, H., Frei, U. K., & Lübberstedt, T. (2020). Breeding Maize Maternal Haploid Inducers. *Plants*, *9*(5), 614. https://doi.org/10.3390/plants9050614

Vassilev, L. T., Vu, B. T., Graves, B., Carvajal, D., Podlaski, F., Filipovic, Z., Kong, N., Kammlott, U., Lukacs, C., Klein, C., Fotouhi, N., & Liu, E. A. (2004). In Vivo Activation of The P53 Pathway by Small-Molecule Antagonists of MDM_2. *Science (New York, N.Y.)*, *303*(5659), 844–848. https://doi.org/10.1126/science.1092472

Wan, Y., Petolino, J. F., & Widholm, J. M. (1989). Efficient Production of Doubled Haploid Plants Through Colchicine Treatment of Anther-Derived Maize Callus. *TAG. Theoretical and Applied Genetics. Theoretische Und Angewandte Genetik*, *77*(6), 889–892. https://doi.org/10.1007/bf00268344

Weather Underground. (2016). *Pudahuel, Chile Weather History*. https://www.wunderground.com/history/monthly/cl/pudahuel/scel/date/2016-12

Weissbluth, M., Karzmark, C. J., Steele, R. E., & Selby, A. H. (1959). The Stanford Medical Linear Accelerator. *Radiology*, *72*(2), 242–265. https://doi.org/10.1148/72.2.242

Zhang, X., Xu, Z., Yu, X., Zhao, L., Zhao, M., Han, X., & Qi, S. (2019). Identification of Two Novel R2R3-MYB Transcription Factors, Psmyb114l And Psmyb12l, Related to Anthocyanin Biosynthesis in Paeonia Suffruticosa. *International Journal of Molecular Sciences*, *20*(5), Article 5. https://doi.org/10.3390/ijms20051055

APPENDIX A. TABLE OF COPY NUMBER VARIATION

Table A1. Copy number variation in the M_1 population. CN2 was omitted due to the plant being diploid, particularly the reference genome. Detected using cn.MOPS with a window length of 7,841 and prior impact value of 0.5.

Chr	Start	End	Width	0Gy1	0Gy2	50Gy2	Gr1	Gr2	Gr3	Gr4	Gr5	Gr6	Gr7	Gr8	Gr9	Gr10	Br1	Br2	Br3	LC1
1	4367438	4390960	23523					1												
1	5049605	5073127	23523				3	3												
1	8476122	8515326	39205								1									
1	12820036	12843558	23523						3											
1	13102312	13125834	23523										1							
1	29607617	29631139	23523				1								1					
1	37142818	37166340	23523			0					0				0					
1	37479981	37503503	23523						5											
1	37903395	37934758	31364			1		1	1										1	
1	38522834	38546356	23523															3		
1	45697349	45720871	23523					3												
1	47696804	47736008	39205							1							3			
1	47971239	48002602	31364			1														
1	48010444	48033966	23523								1									
1	48159423	48229991	70569															3		
1	48426017	48457380	31364				1													
1	52534701	52566064	31364										1							
1	54463587	54494950	31364																3	
1	57364757	57388279	23523								1				1					
1	61269575	61293097	23523						3											
1	61340144	61363666	23523			4														
1	67471806	67503169	31364		1															1
1	70780708	70835594	54887						1											
1	81452309	81483672	31364								1									
1	81812995	81836517	23523						3											
1	92053341	92092545	39205						3											
1	99486609	99517972	31364																3	
1	102168231	102191753	23523						3											
1	107586362	107617725	31364													1				
1	114517806	114549169	31364						1											
1	116744650	116768172	23523						1											
1	130160601	130191964	31364				1													
1	132356081	132379603	23523																1	
1	135657142	135680664	23523				3													
1	140150035	140181398	31364														3			
1	145176116	145199638	23523						3											
1	158019674	158051037	31364											3						
2	1928887	1952409	23523										1							
2	3246175	3269697	23523						1											
2	4202777	4241981	39205													1				
2	5582793	5621997	39205						1				1							
2	5669044	5692566	23523									8								
2	6602123	6641327	39205								1									
2	6853035	6892239	39205							3										
2	9589544	9613066	23523						3											
2	10342280	10365802	23523																	1
2	10624556	10655919	31364												3					
2	14333349	14356871	23523													1				
2	28556923	28580445	23523		0															
2	31379683	31403205	23523				1													
2	37174182	37197704	23523				1													
2	44913249	44936771	23523						3											
2	50456836	50480358	23523				1													
2	52762090	52785612	23523						0											
2	60697182	60720704	23523						4											

Chr	Start	End	Width	0Gy1	0Gy2	50Gy2	Gr1	Gr2	Gr3	Gr4	Gr5	Gr6	Gr7	Gr8	Gr9	Gr10	Br1	Br2	Br3	LC1
2	61598897	61630260	31364				1													
2	69087052	69110574	23523				1													
2	70819913	70851276	31364				1													
2	70976733	71000255	23523									3								
2	71721628	71745150	23523														1			
2	78707959	78731481	23523															3		
2	79139214	79186259	47046				4	4		4	4	3					4		4	4
2	79241147	79264669	23523			1														
2	81021054	81052417	31364				1													
2	99290584	99329788	39205				4	3		3						3				
2	102709260	102740623	31364								1									
2	123095860	123119382	23523												0					
2	125887256	125910778	23523												1					
2	131266182	131289704	23523												1					
2	149927762	149959125	31364						3											
2	150617770	150641292	23523						3											
2	151895853	151919375	23523						3											
2	152248698	152280061	31364														1			
2	154099174	154138378	39205												1					
2	158607749	158631271	23523												0					
2	159148778	159180141	31364												1					
2	162857571	162888934	31364												1					
2	165711695	165735217	23523												1					
2	167225008	167248530	23523												1					
2	167609217	167632739	23523												1					
2	169969358	170000721	31364												1					
2	170047768	170079131	31364												1					
2	174250544	174289748	39205												1					
2	175450217	175481580	31364												1					
2	178453320	178476842	23523						3											
2	179166851	179190373	23523											3						
2	183212807	183244170	31364						3											
2	184106681	184130203	23523						3											
3	1238879	1262401	23523															3		
3	5049605	5073127	23523						3											
3	12655375	12678897	23523		1															
3	19845572	19869094	23523											3						
3	20072961	20096483	23523																1	
3	20582626	20613989	31364					3												
3	32657766	32681288	23523					3												
3	53891194	53914716	23523										1	1						
3	77147600	77171122	23523											1						
3	78159089	78190452	31364															1		
3	89497175	89520697	23523					1												
3	90963442	90986964	23523									1								
3	91096739	91128102	31364	1					1						1					
3	91277082	91308445	31364	1								1			1					1
3	92029818	92892327	862510	1					1			0		1	0					1
3	92962897	93017783	54887	1					1						1					1
3	93213809	93347105	133297	0					1			1			1					1
3	93425516	93637222	211707	0					1			1		1	1					1
3	93668587	93856770	188184	1					1			1		1	1					1
3	93943022	93974385	31364						1											
3	94162570	94186092	23523												0					
3	94256662	94303707	47046	1					1			1			1					
3	94335072	94382117	47046						1						1					
3	96020887	96067932	47046				3	3					3							
3	96130661	96389413	258753	0					1			1		1	0					1
3	96507029	96530551	23523												1					
3	96569757	96608961	39205	1											1					
3	96648167	96679530	31364	1											1					
3	96718736	96742258	23523												1					
3	96852033	97040216	188184	1											1					

Chr	Start	End	Width	0Gy1	0Gy2	50Gy2	Gr1	Gr2	Gr3	Gr4	Gr5	Gr6	Gr7	Gr8	Gr9	Gr10	Br1	Br2	Br3	LC1
3	97071581	97424425	352845	1											0					
3	97455790	97612609	156820	0											0	3				
3	97643974	97667496	23523	0											1					
3	98318300	98341822	23523													5				
3	98396710	98420232	23523					1												
3	99055354	99078876	23523					0												
3	99227856	99259219	31364					1												
3	99541496	99572859	31364	1																
3	99698316	99737520	39205	1																
3	108840922	108887967	47046	1		1	1	4					4							
3	108911491	108966377	54887	1		0	1	4					4		0					
3	108989901	109091833	101933	1		1	1	4					4		1	4				
3	109115357	109178084	62728	1				4					4							
3	109185926	109585816	399891	1	1	1	1	4		1			4		1	4				
3	109695591	109750477	54887					3					3							
3	109781842	109828887	47046	1		1	0								1		1			
3	109907298	109970025	62728	1									3			3				
3	110024913	110056276	31364	0		0	0								1					
3	110244461	110464008	219548	1		1	1						3			3	1			
3	110471850	110558100	86251	1		0	0						4		0	3				
3	119269452	119300815	31364					1												
3	124319056	124350419	31364						3								4			
3	130435036	130458558	23523					1												
3	130787881	130811403	23523										1							
3	132175738	132222783	47046															3		
3	134841678	134912246	70569										1							
3	135743393	135774756	31364										1							
3	136088397	136127601	39205						3					3						
3	138362287	138385809	23523										1							
3	142941431	142972794	31364								4									
3	151864489	151888011	23523										1							
3	158215699	158239221	23523										1							
3	158490134	158521497	31364														1			
3	162802684	162826206	23523													1				
3	170330044	170353566	23523										1							
3	170447659	170471181	23523													1				
3	176155907	176187270	31364										1							
3	177339898	177363420	23523														3			
3	178696391	178719913	23523										1							
4	1968092	1999455	31364			0			5										1	1
4	4390961	4422324	31364			1							1			1		1		1
4	14968470	14999833	31364																	1
4	33786870	33810392	23523								3									
4	47610553	47634075	23523								3									
4	54792909	54816431	23523														3			
4	132544265	132567787	23523								1									
4	137491936	137523299	31364								1									
4	142737565	142768928	31364								1									
4	144705656	144737019	31364								1									
4	158913548	158937070	23523								1									
4	160183790	160215153	31364								1									
4	162191086	162214608	23523								1									
4	163359395	163382917	23523				4	4								3				
4	163782809	163806331	23523						4											
4	168158087	168181609	23523								0									
4	168793208	168824571	31364								1									
4	169632195	169655717	23523								1									
4	179260943	179300147	39205											3						
4	183024623	183048145	23523								1									
4	201819500	201866545	47046								1									
4	202062571	202086093	23523								1									
4	202721215	202752578	31364								1									
4	203136788	203168151	31364								1									

Chr	Start	End	Width	0Gy1	0Gy2	50Gy2	Gr1	Gr2	Gr3	Gr4	Gr5	Gr6	Gr7	Gr8	Gr9	Gr10	Br1	Br2	Br3	LC1
4	209135153	209158675	23523														4			
4	212875310	212898832	23523																1	
4	216638990	216662512	23523									0								
4	217328998	217352520	23523			1					0									
5	70570	94092	23523									3								
5	117616	156820	39205															3		
5	3575497	3606860	31364			1														
5	3646066	3685270	39205																1	
5	7511679	7543042	31364																1	
5	9213176	9236698	23523																1	
5	11385133	11416496	31364																1	
5	12114346	12145709	31364																1	
5	14270621	14301984	31364																1	
5	16168143	16191665	23523						3											
5	20331714	20355236	23523														1			
5	32430377	32453899	23523						3											
5	42545267	42568789	23523								1									
5	48402494	48426016	23523																	1
5	89850020	89873542	23523													1				
5	116862265	116885787	23523									3								
5	120727878	120751400	23523				3	3	3	3										
5	121856982	121880504	23523			1									1					
5	134771109	134794631	23523											1						
5	137382162	137405684	23523						4											
5	149582758	149606280	23523		1															
5	155949650	155973172	23523			1														
5	156333859	156357381	23523			1														
5	162747797	162779160	31364						3											
5	166542841	166566363	23523					0												
5	185612153	185651357	39205						3											
5	186325684	186349206	23523					1												
6	462620	493983	31364						3											
6	1654452	1693656	39205						3											
6	6758943	6790306	31364		1															
6	9244540	9268062	23523						3											
6	21476500	21500022	23523						3											
6	58697727	58729090	31364													1				
6	61246052	61269574	23523													1				
6	64100176	64131539	31364													1				
6	69322282	69385009	62728							5										
6	89912748	89944111	31364						1											
6	92265048	92312093	47046				1				1									
6	92680621	92711984	31364															7		
6	96412937	96436459	23523																1	
6	141710394	141749598	39205			1														
7	5841546	5865068	23523											1						
7	9032833	9056355	23523									3								
7	11910480	11934002	23523										1							
7	15642796	15674159	31364									3								
7	16967925	16991447	23523									3								
7	18238167	18261689	23523									3								
7	38005328	38036691	31364									3								
7	40718314	40741836	23523														1			
7	45509165	45532687	23523									5								
7	47210662	47234184	23523									3								
7	52087764	52111286	23523									3								
7	53757897	53993126	235230	1				5	7		1			1	5				1	5
7	54008809	54259720	250912	1		1		5	6		1		1		5					5
7	54291085	54573360	282276	0		0		5	5	1	0		0		5		0		0	5
7	54628248	54706657	78410					5	5						5					5
7	54722340	54792908	70569					6	6						6					6
7	54824273	54887000	62728	1		1		6	7	1					6					6
7	54894842	55232004	337163	1		1		6	3		1		1		5					3

Chr	Start	End	Width	0Gy1	0Gy2	50Gy2	Gr1	Gr2	Gr3	Gr4	Gr5	Gr6	Gr7	Gr8	Gr9	Gr10	Br1	Br2	Br3	LC1
7	60508998	60556043	47046										1							
7	61324462	61355825	31364										1							
7	63308235	63347439	39205										1							
7	63378804	63410167	31364										1							
7	64688251	64711773	23523									1								
7	64986209	65009731	23523									1								
7	65088142	65111664	23523						1											
7	67142484	67189529	47046										1							
7	68310793	68334315	23523										1							
7	71031620	71055142	23523										1							
7	71713787	71737309	23523										1							
7	72129360	72168564	39205										1							
7	80472184	80495706	23523				3													
7	82385388	82408910	23523									3								
7	86407821	86431343	23523									3								
7	92288571	92319934	31364													1				
7	109907298	109962184	54887														0			
7	110871741	110895263	23523									1								
7	121770731	121809935	39205									1								
7	129392183	129415705	23523						3											
7	135186682	135218045	31364							1										
7	139358094	139381616	23523												0					
7	149778783	149810146	31364								1									
7	153879626	153903148	23523						3											
7	156239767	156271130	31364															1		
7	156608294	156631816	23523						3											
7	158105925	158129447	23523						3											
7	160050493	160081856	31364						1											1
7	160152426	160199471	47046			1			1	1									1	
8	3018786	3042308	23523								3									
8	5582793	5606315	23523												1					
8	5927797	5951319	23523											1		1				
8	9079879	9103401	23523												4					
8	12326053	12349575	23523												3					
8	13902094	13925616	23523						3											
8	18324418	18347940	23523		1															
8	23444591	23468113	23523						3											
8	25302908	25334271	31364										1							
8	26016439	26039961	23523				1													
8	33559481	33583003	23523															4		
8	36970316	36993838	23523												1					
8	39785235	39816598	31364								1									
8	43180388	43211751	31364															3		
8	49421824	49453187	31364							3										
8	49884443	49915806	31364									1								
8	51186049	51209571	23523						1											
8	56282699	56306221	23523												1					
8	61457759	61489122	31364															1		
8	72003904	72027426	23523												1					
8	76575207	76598729	23523														1			
8	82730392	82753914	23523												0					
8	86603846	86627368	23523											1						
8	90289116	90312638	23523												1					
8	90994806	91018328	23523						3											
8	92233684	92272888	39205												1					
8	112243916	112267438	23523								1									
8	114494283	114517805	23523															3		
8	118712741	118744104	31364												1					
8	127894552	127918074	23523			1								1						
8	151691987	151715509	23523						1											
8	154483383	154514746	31364						1											
8	161156074	161179596	23523								1									
8	163610307	163633829	23523															3		

Chr	Start	End	Width	0Gy1	0Gy2	50Gy2	Gr1	Gr2	Gr3	Gr4	Gr5	Gr6	Gr7	Gr8	Gr9	Gr10	Br1	Br2	Br3	LC1
8	169545944	169569466	23523															3		
8	171396420	171419942	23523															3		
8	173670310	173693832	23523											1						
8	176892961	176916483	23523															3		
8	177606492	177637855	31364															3		
9	1097741	1121263	23523																	1
9	3504928	3536291	31364												1					
9	4939831	4979035	39205										1		1				1	
9	5841546	5880750	39205														3			
9	16097574	16121096	23523									3								
9	17132586	17156108	23523						4											
9	19610342	19633864	23523														3			
9	29270454	29293976	23523		3															
9	44889726	44913248	23523						1											
9	47626235	47649757	23523								1									
9	55043821	55067343	23523														1			
9	59662170	59685692	23523											3						
9	64006084	64045288	39205								3									
9	64711774	64774501	62728											1						
9	64954845	64986208	31364							3										
9	65064619	65095982	31364															3		
9	67024869	67048391	23523							3										
9	67722718	67746240	23523			3														
9	67903061	67950106	47046					3		3										
9	70231838	70263201	31364															3		
9	79296034	79319556	23523				3	3		3						3				
9	84761211	84784733	23523					3												
9	86164750	86188272	23523					1												
9	90108773	90140136	31364													1				
9	95981682	96013045	31364														1			
9	102246641	102270163	23523			1		1												
9	106237710	106261232	23523						1											
9	114180643	114204165	23523								1									
9	116634876	116666239	31364		4	5					4		4							
9	117034767	117081812	47046	4										3						
9	117128859	117207268	78410	1	4	3			3				4		1	1	4		3	
9	127259431	127282953	23523															3		
9	128270920	128294442	23523														1			
9	135006339	135037702	31364					1												
9	138127057	138150579	23523				3													
9	139350253	139373775	23523							3										
9	141161524	141185046	23523						1											
9	156388746	156412268	23523														1			
9	164731570	164762933	31364		1															
9	171561081	171592444	31364															1		
9	173066553	173097916	31364														1			
9	184318388	184365433	47046														1		1	
9	194339186	194370549	31364								3									
9	197624565	197655928	31364													1				
10	2179799	2203321	23523											4						
10	27459183	27482705	23523											3						
10	27835551	27866914	31364				1													
10	28470672	28494194	23523	1																
10	30556378	30595582	39205															1		
10	37503504	37589754	86251							3										
10	37691688	37746574	54887													1				
10	38875679	38907042	31364																1	
10	39118750	39173636	54887										1		1					
10	42231627	42262990	31364						3											
10	42513903	42537425	23523			1														
10	44466312	44489834	23523											1						
10	45344504	45368026	23523				0													
10	66013380	66036902	23523						1											

Chr	Start	End	Width	0Gy1	0Gy2	50Gy2	Gr1	Gr2	Gr3	Gr4	Gr5	Gr6	Gr7	Gr8	Gr9	Gr10	Br1	Br2	Br3	LC1
10	66805321	66828843	23523									1	1							
10	72568456	72591978	23523						4											
10	86588164	86611686	23523				3													
10	87717268	87740790	23523								1									
10	107249199	107272721	23523										3							
10	115889981	115913503	23523						1											
10	117818867	117850230	31364						3											
10	118461829	118493192	31364														3			
10	125103156	125126678	23523												1					
10	131266182	131313227	47046						1											
10	133069612	133100975	31364								1									
10	135421912	135445434	23523						1											
10	138291718	138323081	31364															1		
10	141318344	141341866	23523						3											
10	147904784	147936147	31364													1				
10	158882184	158905706	23523								1									
10	159681966	159713329	31364																3	
10	167311259	167334781	23523											1						
10	167350464	167389668	39205							8										
10	173662469	173685991	23523						6											
10	173733038	173756560	23523																1	1
10	174140770	174164292	23523						3											
10	175787380	175810902	23523													3				
10	178492525	178523888	31364						3											
10	178962985	178986507	23523			1								1			1			
10	183800882	183832245	31364																1	
10	185251467	185274989	23523						3											
10	185721927	185745449	23523										4							
10	187831156	187854678	23523						5											
11	2446393	2469915	23523							1										
11	2477757	2524802	47046										3							
11	2901171	2924693	23523											4						
11	3732317	3755839	23523										3							
11	6680533	6704055	23523										3							
11	13274814	13298336	23523						3											
11	15909390	15940753	31364								1									
11	18967380	18990902	23523						1											
11	19147723	19179086	31364														1			
11	25663594	25702798	39205										3							
11	28690220	28721583	31364													3				
11	28948973	28972495	23523							3										
11	29670345	29709549	39205											3		3				
11	29787960	29819323	31364											4						
11	30297625	30321147	23523											4						
11	31324796	31356159	31364									5		6		4				
11	31944235	31975598	31364									3		3						
11	32657766	32681288	23523						1											
11	33535958	33590844	54887											4		3				
11	34351422	34382785	31364											4						
11	34861087	34884609	23523			0														
11	34970861	34994383	23523						3											
11	35017907	35049270	31364									3								
11	35174727	35213931	39205											4						
11	35480526	35504048	23523											3						
11	35833371	35864734	31364																	3
11	36045078	36084282	39205													3				
11	36531220	36554742	23523											3						
11	36719404	36750767	31364											4						
11	37479981	37503503	23523							0				0						
11	37550550	37589754	39205						1											
11	38240558	38326808	86251							1		1		1		1				1
11	39393185	39432389	39205						3		1									
11	42725610	42749132	23523														1			

Chr	Start	End	Width	0Gy1	0Gy2	50Gy2	Gr1	Gr2	Gr3	Gr4	Gr5	Gr6	Gr7	Gr8	Gr9	Gr10	Br1	Br2	Br3	LC1
11	42756974	42811860	54887										3							
11	43666530	43697893	31364										3							
11	49296368	49343413	47046						5											
11	49594326	49617848	23523											3						
11	51233095	51272299	39205							1						1				
11	52636634	52660156	23523									3								
11	53067889	53107093	39205						6											
11	53256073	53279595	23523											3						
11	53358006	53389369	31364											4						
11	53601077	53632440	31364													3				
11	54385177	54408699	23523										3							
11	55224164	55255527	31364									3								
11	55710306	55749510	39205						1					1						1
11	55882808	55914171	31364						1	1				1						
11	56031787	56141560	109774						1			1		1		1				1
11	56463042	56486564	23523						0											
11	56706113	56768840	62728						0	1		1		1		1				1
11	56933502	56957024	23523						0											
11	56996230	57019752	23523																	1
11	59724898	59748420	23523						3											
11	61857650	61881172	23523						3											
11	63370963	63418008	47046						1	1			1							
11	65997698	66021220	23523										3							
11	67463965	67487487	23523						4											
11	70090700	70114222	23523											4						
11	75352011	75375533	23523							1										
11	75704856	75736219	31364											3						
11	77045667	77069189	23523										0							
11	83373354	83396876	23523										1							
11	94562461	94585983	23523						1											
11	97573405	97596927	23523							4										
11	101611520	101642883	31364										1							
11	109774001	109797523	23523					3												
11	111969481	111993003	23523						5											
11	113835639	113859161	23523							3										
11	113961095	113984617	23523							3										
11	118242281	118273644	31364			1		3						1						
11	120625945	120649467	23523											1						
11	121339476	121362998	23523						4											
11	129062861	129086383	23523															3		
11	132999043	133053929	54887	1			1		3		1		4			1				
11	133116658	133140180	23523												1					
11	133273478	133430297	156820	1				3	6		1		5	4	4			1		
11	133469503	133524389	54887					0						4	3					
11	133547913	133579276	31364				1									1			1	
11	133657687	133689050	31364						3						4					
11	133743938	133790983	47046	1							0					1			1	
11	133814507	133845870	31364	1							1							1		
11	133869394	133900757	31364						3		1									
11	133947804	134081100	133297	1	1		1		4		1		3		4	1			1	
11	134104624	134308489	203866	1	1	3	0	3	3		1				3	1	3		1	
11	134324172	134355535	31364	1	1						1									
11	134535879	134567242	31364			3														
11	134825996	134927928	101933	0	1	3	0		4		1		4			1			1	3
11	135123954	135147476	23523	1				5					5							
11	135304297	135327819	23523												5					
11	135421912	135735551	313640	1	1	4	1	4	3		1	1	4	3	4	1	3	1	1	4
11	136041351	136064873	23523						3								4			
11	137586028	137609550	23523								3									
11	138315241	138338763	23523					1						0	1					
11	139648211	139671733	23523				1													
11	148555587	148586950	31364												0					
11	149214231	149237753	23523			3														

Chr	Start	End	Width	0Gy1	0Gy2	50Gy2	Gr1	Gr2	Gr3	Gr4	Gr5	Gr6	Gr7	Gr8	Gr9	Gr10	Br1	Br2	Br3	LC1
11	150186515	150257083	70569						1					1	1					
11	153056321	153079843	23523					1												
11	153934513	153958035	23523				1					1								
11	154099174	154216788	117615				1			1		1								
11	154310881	154334403	23523				1													
11	154804864	154836227	31364				1													
11	154891115	154930319	39205				1					1								
11	155314529	155432143	117615				1					0								
11	155494872	155518394	23523				1													
11	155526236	155557599	31364							0										
11	155612487	155636009	23523				1													
11	155847717	155871239	23523				0					1								
11	156051583	156090787	39205			3				1							3			
11	156310336	156333858	23523						5											
11	156443633	156474996	31364																3	
11	156725909	156749431	23523																	3
11	156796478	156835682	39205										1				3			
11	156843524	156882728	39205				1						1							
11	157055231	157086594	31364							1			1							
11	160371974	160411178	39205										1							
11	160426861	160450383	23523				1			1										
11	160473907	160497429	23523																5	
11	160528794	160583680	54887				1													
11	160599363	160622885	23523												1					
11	160677773	160709136	31364										1							
11	160795388	160834592	39205																3	
11	160928685	160999253	70569				1			1			1							
11	161461873	161540282	78410				1			1			1	1						
11	161571647	161603010	31364							1			1							
11	161673580	161846081	172502			3	1			1			1						3	3
11	161924492	162253813	329322				1			1			1							
11	162316542	162363587	47046				1			1			0							
11	162394952	162426315	31364				1													
11	162590977	162614499	23523										1							
11	162732115	162779160	47046			1									1				1	
11	162787002	162818365	31364					1						1	1				1	1
11	162826207	162849729	23523										0							
11	162881094	162975185	94092				1			1			1							
11	162998709	163045754	47046				1			1			1							
11	163171211	163218256	47046				1													
11	163288826	163351553	62728				1			1			1							
11	163382918	163586783	203866				1			1			1							
11	163625989	163688716	62728										1							
11	163751445	163892582	141138				1						1						4	3
11	163916106	163947469	31364										0							
11	163978834	164010197	31364																3	
11	164080767	164151335	70569			3	1			1			1							
11	166848640	166911367	62728				1						1							
11	167123075	167146597	23523																4	
11	167342623	167373986	31364				0			0										
11	167421033	167460237	39205							1			1							
11	167726832	167758195	31364										0							
11	168103200	168150245	47046			4	1													
11	168252179	168307065	54887			3							1							
11	168377635	168416839	39205			3	1			1			1							
11	168557978	168581500	23523							0			0							
11	168605024	168644228	39205				1						1							
11	168675593	168754002	78410			3	1			1			1							
11	168793208	168848094	54887				1			1			1							
11	168879459	168934345	54887				1													
11	168973551	169004914	31364				0													
11	169240145	169295031	54887							1			1							
11	169334237	169389123	54887							1			1							

Chr	Start	End	Width	0Gy1	0Gy2	50Gy2	Gr1	Gr2	Gr3	Gr4	Gr5	Gr6	Gr7	Gr8	Gr9	Gr10	Br1	Br2	Br3	LC1
11	169553785	169624353	70569										1						4	
11	169647877	169734127	86251				1						1							
11	169741969	169781173	39205			3													3	
11	169820379	169859583	39205							1										
11	170079132	170134018	54887				1			1										
11	170392772	170424135	31364										1							
11	170455500	170510386	54887				1													
11	170604479	170628001	23523				1			0										
11	170659366	170690729	31364																3	
11	171027893	171145507	117615	5	3															
11	171223918	171270963	47046	7	6					3										
11	172564729	172588251	23523						5											
11	173740879	173764401	23523												1					
11	176187271	176210793	23523						1											
11	180468457	180499820	31364						1											
11	184859417	184898621	39205					1												
11	186898077	186937281	39205									1								
11	188552528	188576050	23523	3																
11	189775724	189799246	23523												1					
11	193900090	193923612	23523											1						
11	194198048	194221570	23523																3	
11	196550348	196573870	23523								3									
11	197467745	197499108	31364			4				1										
11	198667418	198690940	23523					1												
12	2924694	2948216	23523						3											
12	10444213	10467735	23523					1												
12	11055811	11079333	23523						3											
12	17736343	17759865	23523								1									
12	21837186	21868549	31364																	1
12	24565854	24597217	31364								1									
12	25216657	25248020	31364	1																
12	27035769	27059291	23523		1															
12	32054009	32085372	31364		1															
12	32163783	32187305	23523						1								1			
12	34555288	34578810	23523						3											
12	34923815	34947337	23523							8										
12	35982350	36005872	23523						4											
12	45164161	45187683	23523			1														
12	52260266	52283788	23523							1										
12	58415451	58438973	23523						3											
12	59317166	59340688	23523						3											
12	64453021	64476543	23523								3									
12	70765026	70788548	23523								1									
12	87191921	87215443	23523											3						
12	89152171	89175693	23523															1		
12	89810815	89842178	31364			3														
12	90022522	90053885	31364								1									
12	92735508	92766871	31364	1																
12	104873376	104904739	31364		1															
12	105555543	105586906	31364				1													
12	112588920	112612442	23523				3													
12	143098251	143129614	31364				1													
12	160136744	160160266	23523											3						
12	169671400	169694922	23523											1						
12	169898789	169930152	31364					1												
12	170134019	170173223	39205											1						
12	171890403	171921766	31364											1						
12	172000177	172047222	47046											1						
12	178304341	178327863	23523										1							
13	8491804	8515326	23523						4											
13	11079334	11110697	31364						1											
13	19382953	19406475	23523											4						
13	20480693	20504215	23523				3			3						3				

Chr	Start	End	Width	0Gy1	0Gy2	50Gy2	Gr1	Gr2	Gr3	Gr4	Gr5	Gr6	Gr7	Gr8	Gr9	Gr10	Br1	Br2	Br3	LC1
13	56267017	56290539	23523						3											
13	63441532	63465054	23523																1	
13	72325385	72356748	31364						3											
13	81420945	81452308	31364						3											
13	87976021	88007384	31364																1	
13	91488789	91512311	23523											3						
13	93088353	93119716	31364																1	
13	96162025	96185547	23523							1										
13	97134309	97165672	31364																1	
13	98114434	98137956	23523																1	
13	101878114	101909477	31364																1	
13	102074139	102097661	23523				1													
13	103360063	103383585	23523														3			
13	105798614	105822136	23523																1	
13	106425894	106457257	31364																1	
13	107837274	107860796	23523																1	
13	114729513	114760876	31364																1	
13	119598774	119630137	31364					1												
13	124452353	124483716	31364												1					
13	128302284	128325806	23523						3											
13	140204922	140236285	31364							3								3		
13	141584938	141702552	117615			1		3		3				1			3		3	3
13	142188695	142212217	23523			1														
13	142549381	142572903	23523												1					
13	146101354	146132717	31364															3		
13	150664816	150688338	23523						3											
13	156906252	156929774	23523											3						
13	165743059	165766581	23523								1									
13	168510932	168534454	23523											1						
13	172337340	172368703	31364															3		
13	174376000	174407363	31364						4											
14	3159924	3191287	31364															1		
14	7880206	7935092	54887							1										
14	16223030	16246552	23523							0										
14	16630762	16654284	23523									1						1		
14	17085540	17116903	31364																	1
14	30352512	30383875	31364	1		1					1			4	1					
14	30462286	30493649	31364	1		1								3						
14	30525014	30564218	39205	1		1		3			1				1					
14	30611265	30681833	70569	1		1					1	3		3	1					
14	30760244	30807289	47046	1										3						
14	30862177	30901381	39205					3						3		3				
14	30971951	31034678	62728	1		1					0			3	0					
14	31128771	31167975	39205	1		1		3			1				1		1			
14	56133720	56157242	23523							1										
14	58658522	58713408	54887				3	3		3						3				
14	63653239	63676761	23523							1										
14	73917108	73940630	23523															3		
14	74003359	74034722	31364											3						
14	74066087	74097450	31364															3		
14	74262112	74309157	47046			1														
14	74379727	74403249	23523							3										
14	74560070	74638479	78410															5		
14	74685526	74740412	54887					1					1		1	1				
14	75061894	75108939	47046						1				1							
14	75783266	75814629	31364				1								1					
14	76120429	76151792	31364												1					
14	76583048	76614411	31364							3										
14	76669299	76700662	31364													1				
14	76826119	76849641	23523							3										
14	77084872	77108394	23523				1													
14	77657265	77712151	54887										1							
14	77719993	77782720	62728	1	1												1			

Chr	Start	End	Width	0Gy1	0Gy2	50Gy2	Gr1	Gr2	Gr3	Gr4	Gr5	Gr6	Gr7	Gr8	Gr9	Gr10	Br1	Br2	Br3	LC1
14	79421490	79476376	54887							3								3		
14	79648879	79672401	23523							1										
14	81554242	81577764	23523															3		
14	86525436	86548958	23523				1									1				
14	87662381	87701585	39205						3											
14	89787292	89810814	23523														3			
14	91041852	91073215	31364				1	1											1	
14	91904362	91935725	31364												1					
14	92625734	92657097	31364					1												
14	93049148	93080511	31364								1									
14	93472562	93503925	31364				1													
14	97636133	97675337	39205						1											
14	98059547	98083069	23523					0												
14	105767250	105790772	23523						3											
14	122774379	122797901	23523											3						
14	124460194	124483716	23523						3											
14	131360274	131383796	23523													1				
14	132850064	132873586	23523			5														
14	135476799	135500321	23523					0												
14	136245217	136276580	31364																	1
14	137366480	137397843	31364														3			
14	137609551	137640914	31364														1			
14	139122864	139146386	23523				1													
14	139162069	139185591	23523						5											
14	145231003	145254525	23523											1						
14	147120684	147144206	23523						1											
14	152311426	152334948	23523				1													
14	154201107	154240311	39205														3			
14	154506906	154530428	23523						3											
14	155094981	155118503	23523											1						
14	156043742	156067264	23523					1												
14	164896231	164919753	23523										1							
14	166832958	166856480	23523		1															
14	168573660	168605023	31364												1					
14	169146053	169169575	23523													3				
14	171208236	171231758	23523																1	
14	172666662	172690184	23523								1									
14	174603389	174650434	47046								1									
14	175348284	175379647	31364								1									
14	176281363	176320567	39205								1									
14	177371262	177394784	23523						3											
14	180319478	180343000	23523						3											
14	181338808	181362330	23523													1				
14	182201318	182232681	31364							1										
14	183299058	183330421	31364								1									
14	184318388	184341910	23523						3											
14	185008396	185031918	23523															5		
14	187023533	187047055	23523								1									
14	187164671	187188193	23523														3			
14	187454788	187478310	23523								1									
14	189524812	189548334	23523								1									
15	2477757	2501279	23523														3			
15	3222652	3246174	23523								1									
15	4093003	4124366	31364														1			
15	5982684	6006206	23523										1							
15	12733785	12757307	23523						4											
15	18120552	18151915	31364						3											
15	20206258	20229780	23523												3					
15	28549082	28596127	47046											4						
15	28823517	28854880	31364											3						
15	29882052	29913415	31364											1						
15	32830268	32869472	39205											7						
15	34759154	34782676	23523																	3

Chr	Start	End	Width	0Gy1	0Gy2	50Gy2	Gr1	Gr2	Gr3	Gr4	Gr5	Gr6	Gr7	Gr8	Gr9	Gr10	Br1	Br2	Br3	LC1
15	38679654	38703176	23523						3											
15	39455913	39479435	23523								3									
15	41800372	41823894	23523				1													
15	54628248	54659611	31364													3				
15	54722340	54761544	39205													3				
15	57944991	57984195	39205						1				1							
15	60195358	60218880	23523								3									
15	60367860	60399223	31364			1														
15	65127347	65158710	31364		3		3						3							
15	65166552	65299848	133297		1		1	1	1				3					1		
15	66044744	66068266	23523				1													
15	66750434	66813161	62728		1		1		4				0				1	3		3
15	66954300	66977822	23523						5											
15	67118961	67314985	196025		1		1		3				1				1			
15	67573739	67628625	54887										1							
15	67722718	67808968	86251				1		4				1							
15	67942266	67973629	31364										1							
15	68012835	68036357	23523				0													
15	68052040	68083403	31364		1								1				1			
15	68122609	68153972	31364										1							
15	68193178	68373520	180343		1		1						1					4		
15	68546023	68640114	94092				1										1	3		
15	68742048	68773411	31364															3		
15	69000801	69141938	141138				1											5		
15	69188985	69275235	86251		1		1			3			1				1			
15	69290918	69392850	101933		1		1			4			1					3		
15	69400692	69447737	47046							4								3		
15	69518307	69596716	78410				1			3			1					3		
15	69777060	69871151	94092				1			4			1					4		
15	70082859	70184791	101933		1		1						1							
15	70200474	70263201	62728		1		1						1					3		
15	94484051	94507573	23523					3												
15	103148356	103171878	23523													1				
15	113145631	113169153	23523										3							
15	118054097	118077619	23523								3									
15	119912414	119935936	23523						3											
15	134747586	134771108	23523														3			
15	154381450	154420654	39205						3											
15	175575673	175607036	31364											1						
15	177245806	177269328	23523															3		
16	20253304	20284667	31364		1															
16	22417420	22440942	23523				1													
16	127980803	128004325	23523												1					
16	139381617	139412980	31364									1								
16	145419187	145450550	31364															3		
16	151441075	151472438	31364						3											
16	157023867	157047389	23523													3				
16	161454032	161477554	23523																	1
16	172133474	172188360	54887													4				
16	172282453	172313816	31364													3				
16	172525524	172549046	23523													4				
16	175018962	175050325	31364						1											
16	181472105	181495627	23523						3											
16	183761677	183793040	31364						3											
16	183934179	183957701	23523															3		
16	185941475	185964997	23523						3											
16	195570223	195593745	23523						3											
16	197601042	197624564	23523						3											
16	203458269	203489632	31364														3			
16	205645908	205669430	23523						3											
16	214474874	214498396	23523													1				
16	218089575	218113097	23523												0					
16	218167985	218191507	23523													1				

Chr	Start	End	Width	0Gy1	0Gy2	50Gy2	Gr1	Gr2	Gr3	Gr4	Gr5	Gr6	Gr7	Gr8	Gr9	Gr10	Br1	Br2	Br3	LC1
17	2587531	2634576	47046					1												
17	20167053	20190575	23523												1					
17	30611265	30642628	31364																1	
17	32108896	32132418	23523		1															
17	45595416	45618938	23523							1										
17	46199173	46222695	23523													1				
17	52534701	52566064	31364										0							
17	70184792	70208314	23523										0							
17	70239679	70271042	31364										1							
17	79139214	79201941	62728						8											
17	80777983	80809346	31364										1							
17	81138669	81162191	23523										1							
17	81319012	81350375	31364										1							
17	81405263	81734584	329322										1							
17	81765949	81867881	101933										1							
17	81907087	81930609	23523										1							
17	81961974	82009019	47046										1							
17	82079589	82134475	54887										1							
17	82314819	82338341	23523										0							
17	82628459	82848006	219548										1							
17	82887212	82918575	31364										1							
17	83396877	83428240	31364										0							
17	83592902	83624265	31364										0							
17	83875178	83898700	23523										0							
17	83922224	83945746	23523										1							
17	85866792	85898155	31364										1							
17	87631017	87654539	23523										0							
17	87693745	87725108	31364										0							
17	87976021	88015225	39205										1							
17	88046590	88077953	31364										1							
17	88211251	88242614	31364										1							
17	89120807	89144329	23523										1							
17	89222740	89261944	39205										1							
17	89277627	89301149	23523										0							
17	109515248	109538770	23523												3					
17	115411680	115435202	23523						3											
17	124177918	124209281	31364					3		3						3			1	
17	124907131	124938494	31364													3				
17	125628503	125652025	23523			1			1					1		3			1	
17	125714754	125761799	47046	4			5		1						1	5				5
17	125793164	125824527	31364											1					1	
17	125840210	125895096	54887	3		1														
17	126106804	126185213	78410			1			1			1	1	1	1			1	1	
17	127024201	127047723	23523			1												1		
17	127196703	127243748	47046	3																3
17	127353523	127431932	78410			1		4				1			1				1	
17	127643640	127667162	23523												1					
17	129501957	129533320	31364						1											
17	143333481	143357003	23523											1						
17	144548836	144650768	101933			1												1	1	
17	144666451	144705655	39205			1			1				1					1		
17	145583848	145607370	23523						4											
17	148367403	148398766	31364			1														
17	148853545	148884908	31364						1											
17	149339687	149371050	31364										1							
17	150484473	150515836	31364															1		
17	151182322	151205844	23523															1		
17	151237209	151268572	31364						1											
17	151339142	151409710	70569			0			1				0					0	1	
17	151456757	151723350	266594			1			1				1					1	1	
17	151762556	151801760	39205			1							1					1	1	
17	151997786	152091877	94092			1			1									1	1	
17	152123242	152146764	23523										0					0	0	

Chr	Start	End	Width	0Gy1	0Gy2	50Gy2	Gr1	Gr2	Gr3	Gr4	Gr5	Gr6	Gr7	Gr8	Gr9	Gr10	Br1	Br2	Br3	LC1
17	152209493	152311425	101933			0			1				1					1	1	
17	152632907	152672111	39205						1										0	
17	152750522	152774044	23523			0														
17	159870150	159901513	31364															1		
17	160293564	160324927	31364					1												
17	160662091	160709136	47046			1			1											
17	160889480	160928684	39205						1				1							
17	161297212	161391303	94092			1			1				1		4			1	1	
17	161485396	161516759	31364																0	
17	161524601	161548123	23523						1											
17	161563806	161642215	78410			1												1	1	
17	161995061	162112675	117615			1			1				1					1	1	
17	162128358	162198926	70569			1			0				1					1	1	
17	162943822	162975185	31364			1			1				1					1	1	
17	163312349	163335871	23523					1												
17	163625989	163673034	47046					1												
17	163900424	163939628	39205					1												
17	164002357	164025879	23523					1												
17	164159177	164276791	117615					1												
17	164339520	164370883	31364					1												
17	164613955	164645318	31364					1												
17	164692365	164747251	54887																3	
17	164802139	164849184	47046					1										3	3	
17	164864867	164904071	39205																3	
17	164998164	165021686	23523					0												
17	165531352	165562715	31364					1												
17	165617603	165641125	23523															3		
17	165758741	165790104	31364					1												
17	166864322	166903526	39205					1												
17	167256372	167311258	54887					1												
17	167436715	167468078	31364					0												
17	167617058	167679785	62728					1											3	
17	167899334	167962061	62728					1										3		
17	167985585	168009107	23523					1												
17	168040472	168071835	31364					1												
17	168667752	168691274	23523					1												
17	169099007	169130370	31364	3					1											
17	169208781	169396964	188184			1			1				1					1	1	
17	169467534	169498897	31364					1												
17	171161190	171192553	31364		0	1														
17	171247441	171286645	39205			0			1											
17	171490512	171710059	219548			4	1	1			0									
17	171913926	171953130	39205				1				0									
17	172219725	172243247	23523								1									
17	172549047	172619615	70569			4	0		5		0									
17	172925415	172956778	31364						1											
17	173309624	173489966	180343			3	0	1			0									
17	173552695	173725196	172502			4	0	1			0									
17	173811448	174195656	384209			4	0	0			0									
17	174219180	174297589	78410				0	1			0									
17	174744527	174799413	54887			1			1											
17	174893506	174979756	86251				1		4		1									
17	174995439	175018961	23523				0				0									
17	175160100	175199304	39205				1				0									
17	175332602	175418852	86251			1			1											
17	175458058	175481580	23523						0											
17	175512945	175544308	31364						1											
17	175756016	175787379	31364				1								1					
17	176053974	176461705	407732	3		3	0	0	4	1	0		0	5	0		3			
17	177128191	177151713	23523			0			1											
17	177324216	177402625	78410			3	1		4		1				1					
17	177488877	177967177	478301		3	5	1	1	3	1	1				1		3	1	3	
17	177998542	178037746	39205				1	1			1				1					

Chr	Start	End	Width	0Gy1	0Gy2	50Gy2	Gr1	Gr2	Gr3	Gr4	Gr5	Gr6	Gr7	Gr8	Gr9	Gr10	Br1	Br2	Br3	LC1
17	178061270	178657185	595916			4	0	1	4	1	0				0			1	3	
17	178680709	178915938	235230			4	1	1		1	0				1			1		
17	178931621	178994348	62728			4	1	1	4	1	1	4			1					
17	179010031	179072758	62728				1	1	4											
17	179159010	179182532	23523				1													
17	179206056	179582423	376368			5	1	1	4	1	1				1		3	1		3
17	179950951	180005837	54887			1			1								1			
17	180029361	180060724	31364			1														
17	181856314	181879836	23523									3								
17	182028816	182091543	62728			0			1											
17	182138590	182546321	407732			1			1											
17	182593368	182624731	31364			1			1											
17	183393150	183440195	47046			1												1		
17	183471560	183502923	31364																	3
17	183589175	183738153	148979			0			0											
17	183785200	183808722	23523												1					
17	183887133	183918496	31364				1				1				1					
17	184083158	184114521	31364								1					1				
17	184310547	184365433	54887						1											
17	184608505	184632027	23523					1							1					
17	184639869	184663391	23523														3			
17	184796689	184835893	39205			0									4					
17	184898622	184929985	31364						6											
17	185188739	185604311	415573	1						3										
17	185839542	185870905	31364												1					
17	186035567	186066930	31364												1					
17	186176705	186333524	156820			4	1	1	4	1	1				1					
17	187227399	187266603	39205					1												
17	188505482	188544686	39205						1											
17	189124921	189179807	54887					1			1				1			1		
17	189728678	189767882	39205							1	1				1			1		
17	189838452	190050158	211707					1	4		0				1			1		
17	191163581	191202785	39205															1		
17	191390970	191438015	47046															1		
17	191994727	192033931	39205						3											
17	192104501	192128023	23523													3				
17	192245639	192292684	47046															1		
17	192598484	192661211	62728	1					0					1	3	1				1
17	193264969	193296332	31364										1							
17	193829521	193853043	23523										1							
17	194103956	194151001	47046	1																
17	194480324	194543051	62728	1				1	1				4	1						1
17	194966466	194989988	23523										6							
17	196173980	196228866	54887	1								1	4	1		1				1
17	196464097	196495460	31364						0					0		0				
17	196542507	196573870	31364		4									1						
17	196589553	196620916	31364											1		1				
17	196644440	196762054	117615		4		4					1				1				
17	196769896	196816941	47046		0		1	1		1	0		0							
17	196942398	196973761	31364									0				1				
17	197020808	197193309	172502									1		1		1				
17	197216833	197287401	70569									1		1		1				
17	197295243	197365811	70569									1		1						
17	198236163	198298890	62728									1				1				