

Creating Mutations in Plant Pathogen Resistance Genes

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ABSTRACT

One of the most effective means of studying a vital function like pathogen defense is to create mutations in the genes responsible for the function. By creating such mutant organisms, one can dissect the biochemical pathways leading to pathogen defense. To isolate a mutant plant lacking pathogen resistance genes, one must first disable the resistance genes. Radiation from a cesium 137 source was selected as the mutagen to create such mutant plants. Seeds were exposed to the radioactive source to determine the proper dose of radioactivity for mutagenesis. A kill curve has been generated using timed exposures ranging from 1 to 12 hours. The kill curve data was used to expose *Arabidopsis thaliana* seeds to enough radiation to create a survival rate of 50%. The surviving plants will be allowed to self-fertilize and set seed (M2 generation). The M2 plants will be exposed to the bacterial pathogen *Pseudomonas syringae* and scored for resistance to infection. All phenotypic plant mutations will be logged for future reference.

INTRODUCTION

Bacterial pathogens are a major cause of damage to plant crops around the world. For example, it is estimated that 50% of the potential world rice crop is lost to disease by pathogens every year (Ronald, 1998). Another example is *Phytophthora infestans*, the causative agent of late blight disease in potato and tomato, with its direct economic impact of \$5 billion each year (Howard Judelson, personal communication). All plants are susceptible to some sort of infection or disease brought about by microbes. Some plants, however, have been shown to have resistance to certain bacteria that infect other plants of the same species. These resistant plants have been found to contain resistance genes in their genomes. Currently there have been 25 resistance genes that have been located and successfully cloned (for example, Century et al., 1997; Martin et al., 1993). The products of these plant genes interact with a corresponding gene product from the pathogen. The complex formed by the two gene products confers pathogen resistance to the plant by an unknown mechanism, a phenomenon known as gene-for-gene resistance. To investigate the pathways responsible for pathogen resistance, a project was begun to create mutant *Arabidopsis thaliana* plants incapable of defending themselves against *Pseudomonas syringae*.

Mutations in the genome can occur in many ways. One of the most common ways is through exposure to gamma radiation. Gamma radiation works by breaking the DNA molecules, causing segments of the DNA to be lost during cell division. Mutations caused by lost or missing DNA are known as deletions. By causing deletions in DNA it is possible to map where various genes are located in the genome due to the loss of DNA markers associated

with the phenotype. Herein, the primary interest was determining the proper dose of radiation for mutating genes responsible for pathogen resistance in *Arabidopsis thaliana*. The ultimate goal of this experiment is to generate a mutant form of *Arabidopsis thaliana* lacking genes that give the plant resistance to the bacterial pathogen *Pseudomonas syringae*.

METHODS AND MATERIALS

Irradiation Process

Wild-type *Arabidopsis thaliana* (ecotype Columbia) seeds were placed in clear plastic straws plugged by a cork stopper at one end. The straws were then placed upright in port 3 of the Cesium 137 containment unit at the radiation center in the basement of Cowley Hall. Port 3 emits 13.2 Krads/hour of gamma radiation. Straws containing seeds were then removed at selected time intervals and seeds were planted.

Planting

Irradiated and non-irradiated seeds were planted ten seeds per pot in Premier Pro-Mix BX soil. 15 pots were placed in each flat. The pots were then watered from the bottom and fertilized using Peters Professional Water Soluble Fertilizer. Once planted, the pots containing the seeds were covered with plastic wrap and placed at 4°C for 48 hours. The flats containing the pots were then removed from the cold room and placed in a growth chamber at 20° C, 50% relative humidity and 100 microEinsteins of light with a standard photoperiod (16 hrs light/8 hrs dark). After the seeds germinated, the plastic was slashed over a period of days to allow the seedlings to acclimate to the humidity in the growth chamber. The plastic wrap was then removed and the plants were allowed to grow.

RESULTS

Cesium 137 served as the source of gamma radiation (Figure 1) for mutagenesis. In order to begin, a kill curve had to be developed so that the proper amount of radiation to create a 50% death rate could be administered to the seeds. According to Carolina Biological (1999), approximately 4,000 Krads of radiation from a Cobalt 60 source was necessary to kill all marigold, radish, and squash seeds. Cobalt 60 emits twice as many gamma rays as Cesium

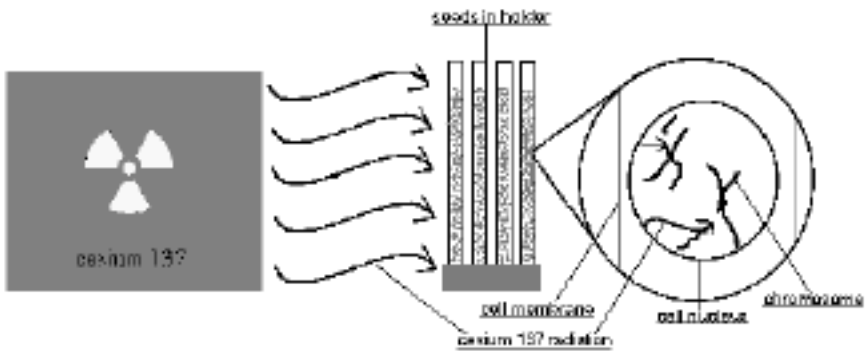


Figure 1. Illustration showing the effects of gamma radiation from cesium 137 source on chromosomes of *Arabidopsis* seeds.

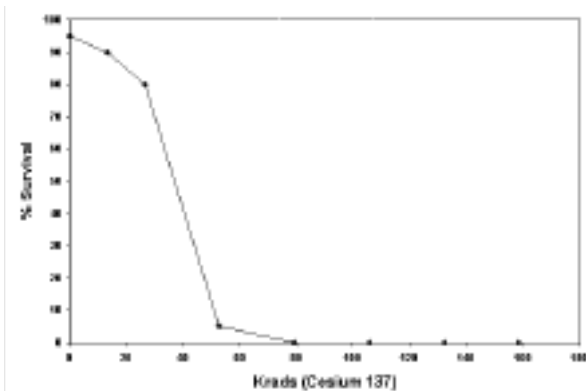


Figure 2. Percent survival of *Arabidopsis thaliana* subjected to varying amounts of radiation from cesium 137.

137, and the gamma rays are twice as powerful as those from Cesium 137 (Chase and Rabinowitz, 1967). Previous work with Cesium 137 showed morphological mutations of marigolds resulting from seeds exposed to 5,000 Krads of radiation (Dr. Thomas Weeks, personal communication). Approximately 13.2 Krads/hr of gamma radiation bombarded the Cesium 137 port used for seed exposure. Using the above information, a kill curve was designed to expose seeds over a time period of 38 hours to 190 hours (500 Krads to 2500 Krads).

Due to sensitivity to radiation, the original exposure times to Cesium 137 caused 100% death to *Arabidopsis thaliana* plants grown from irradiated seeds. After severely reducing the exposure time, another problem was encountered. After germination, all plants that appeared to be healthy suddenly died once they reached the cotyledon stage in development. To ensure that the observed death was not due to contaminated soil or toothpicks, seeds were grown in several soil mixtures using different planting methods. In all cases, the non-irradiated seeds grew into healthy plants while the irradiated seeds germinated and died at the cotyledon stage (data not shown). These results were consistent with previous research demonstrating plant death at the cotyledon stage as a result of overexposure to radiation. (Casarett, 1968). At this point, seeds were exposed to radiation for times ranging from 1 hour to 12 hours (13.2 Krads - 158.4 Krads). The kill curve (Figure 2) shows a death rate of 95% after (52.8 Krads) and a 50% death rate at approximately 40 Krads (3 hours).

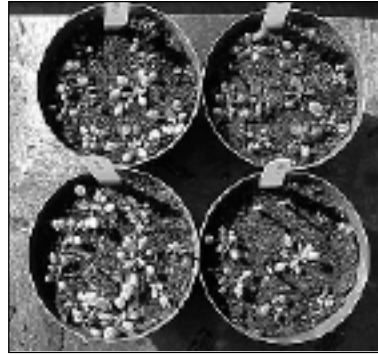
Growth and development of the surviving irradiated seeds was markedly slowed. (Figure 3) Wild-type plants are normally beginning to flower at six weeks under the growth conditions employed. Plants from seeds exposed for one hour are still in the rosette (leaf formation) stage, while plants from seeds exposed for two hours are even smaller (Figure 3). Plants (the few that survived) grown from seeds exposed for four hours are only beginning to develop true leaves (Figure 3).

DISCUSSION

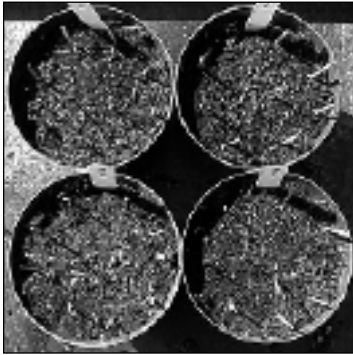
Compared to other dicotyledonous flowering plants, *A. thaliana* is remarkably sensitive to gamma rays. Using a less powerful isotope, *A. thaliana* seeds can only withstand about 1% of the radiation other plant seeds (e.g. squash, marigold, radish) can withstand. The sensitivity of *Arabidopsis thaliana* seeds may be due to their small size, their small genome size, or some other physiological constraint.



A



B



C

Figure 3. Growth of *Arabidopsis thaliana* after 6 weeks. Seeds were exposed to cesium 137 for various times and planted. (A) Plants from seeds exposed 1 hour. (B) Plants from seeds exposed 2 hours. (C) Plants from seeds exposed 4 hours.

Further research is to be performed using the above data by growing the plants exposed to enough radiation for 50% survival and then creating an M2 generation. Current data indicates the 50% lethality exposure is at approximately 3 hours (40 Krads). Experiments are underway to better understand the area of the curve between 26.4 Krads and 52.8 Krads, thereby increasing the accuracy of the exposure time necessary for 50% lethality. Once the mutant plants grow and self-pollinate, the M2 seeds will be collected. M2 plants will then be screened for susceptibility to infection by *Pseudomonas syringae*. Eventually, genes activating pathogen defense mechanisms may be cloned from *Arabidopsis thaliana*. The defense-activating genes may then be placed in plants lacking pathogen resistance to ultimately create pathogen resistant crops.