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Mutagenesis of Raphanus sativus Using Ionizing Radiation

Adam Lyko

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Abstract

Mutagenesis of *Raphanus sativus* was conducted using 10kR, 20kR, and 30kR radiation in an attempt to determine the dosage which produces most mutants without being lethal. Pelargonidin production and physical characteristics of the storage root of the radishes were used as measures of mutagenicity. Plants which received 30kR of radiation exhibited the poorest development in every measured aspect except germination which was high. The results of this study suggest that at least 30kR of radiation should be used in experiments involving mutagenesis of *Raphanus sativus* plants.

Introduction

The role of ionizing radiation in mutagenesis was first described by H.J. Muller in 1927. Muller showed that ionizing radiation increases the normal mutation rate in a manner proportional to the radiation dosage (5). This finding is useful for genetic studies. The ability to mutate organisms allows scientist to map genes and alter traits. For example, novel varieties of durum wheat have been developed in Italy with the help of ionizing radiation, and in America verticillium wilt resistant peppermint plants have been developed in the same way (9).

Ionizing radiation is defined as a highly energetic electromagnetic radiation (x-rays, gamma rays, or cosmic rays) and particulate radiation (alpha particles, beta particles, neutrons, or heavy charged ions) (1). This radiation is ionizing because it is energetic enough to cause the molecules it hits to become ions. The process of ionization involves the loss of electrons. Energy from the radiation transfers to the electron which then uses the extra energy to leave its atom. This phenomenon is called the Compton effect (1).

Ionization of molecules usually results in a rearrangement of bonds, or simply put, it causes molecular damage. Ionizing radiation causes mutations by causing molecular damage to DNA. Any change in the nucleic acid sequence of DNA is defined as a mutation (11). Ionizing radiation has been recognized to cause several kinds of damage to DNA. The damage can be simply a point mutation or the loss of whole chromosomes. A broken DNA molecule may not reattach itself at all, may attach in the wrong place or orientation, or lose nucleotides before finally reattaching (3).

Ionizing radiation has especially been used in mutating plants. This has been done to try to develop new plant varieties which produce better crops, are sturdier, grow taller, exhibit new colors, or are resistant to disease (9). Mutagenesis of plants begins at the seed stage (11). Seeds are irradiated, germinated and grown. Resulting mutant plants are then screened for desirable mutants (9). One problem with this kind of work, however, is that preliminary experiments have to be done to determine the radiation dosage which kills the least number of plants and produces the greatest number of mutations (10). The purpose of this study was to try to find this special dosage for the radish plant *Raphanus sativus*.

Radish plants contain in their storage roots a red pigment called pelargonidin (2). This compound comes from the anthocyanin family which are the pink, red, mauve, violet, and blue pigments in fruits, flowers, and vegetables. Chemically, pelargonidin is a flavonoid, meaning its structure is based on the flavan nucleus (Appendix A) (2). In its functional form pelargonidin is glycosylated, and is produced primarily in the dermal cells of radish roots (Appendix B).

Biosynthesis of pelargonidin is carried out by several enzymes (Appendix C). This means that several genes are involved. Mutation of any of these genes leads to a decrease in the amount of pelargonidin synthesized in mutant plants. The concentration of pelargonidin was, therefore, used in this

study as a measure of the mutagenicity of the radiation. Physical characteristics, namely length, width, and mass of radish roots, were also used to measure mutagenicity.

Methods and Materials

Seeds

A packet of *Raphanus sativus* seeds was purchased from the Village Market, Collegedale, Tn. The seeds were examined for physical defects and size. Four groups of 30, large, intact seeds were selected from the lot.

Irradiation

The seeds were irradiated at Loma Linda University Medical Center using a cesium ionizing source. The seeds were exposed to 0kR, 10kR, 20kR and 30kR of radiation respectively. Radiation dosages were chosen according to previously reported data (7). Group 0kR served as the CONTROL.

Germination

The seeds were germinated in petri dishes on top of moist Ahlstrom Filter Papers (Pic. 1,3). The filter papers were used to evenly distribute moisture on the bottom of the petri dishes. Each group of 30 seeds was placed in a separate petri dish, and germination in each group was recorded.

Growth

Four 6" x 7" x 26" plastic pots and American Countryside Ready to Use top soil were purchased at Lowes, Chattanooga., TN (Pic. 5). Each pot had two small holes for water drainage. The soil was used to fill the each box 5 inches up from the bottom, and radish seedlings were planted in the soil about 2 inches apart. The 4 boxes were then placed on a metal table in the middle of a greenhouse room and left to grow. The plants were watered with 550 ml of tap water per pot whenever soil moisture dropped (4). The drop in soil moisture was determined subjectively by feeling the top soil layer with a finger. The growing boxes were periodically rotated, and the plants were grown for 36 days before harvesting (Pic. 6, 7).

Macroscopic Analysis

Each radish plant was loosened from the soil with a spoon. It was cut in two places to isolate the storage part of the root that contains perlargonidin (Pic. 10). This portion of the root is normally above the soil level so it did not need to be extensively cleaned from soil. The length, width, and mass of the isolated roots were then measured (Pic. 11). Data was analyzed with the ANOVA statistical test.

Pigment Extraction and Analysis

Each radish root was placed in a separate 20ml test-tube, and an aliquot of distilled water equal to 1.2 x root length was added to that test-tube. All testiness from a group were placed in a metal rack which was then submerged in 50°C water bath and heated up to 90°C. Pelargonidin pigment was extracted in the 90°C water bath for 30 minutes.

At the end of extraction the metal rack was taken out and the testiness were transferred to another metal rack to cool for a minimum of 20 minutes. An aliquot of 2 ml of the extract from each test-tube was then placed in a 8ml test-tube used for photospectrometery. The absorbance of 535 nm light by the extract was then recorded from the Bausch & Lomb Spectronic 20 photospectrometer (8).

Results

Germination

No group had a 100% germination success (Fig. 1). Most seeds germinated in group 30kR, while in group 10kR the least number germinated.

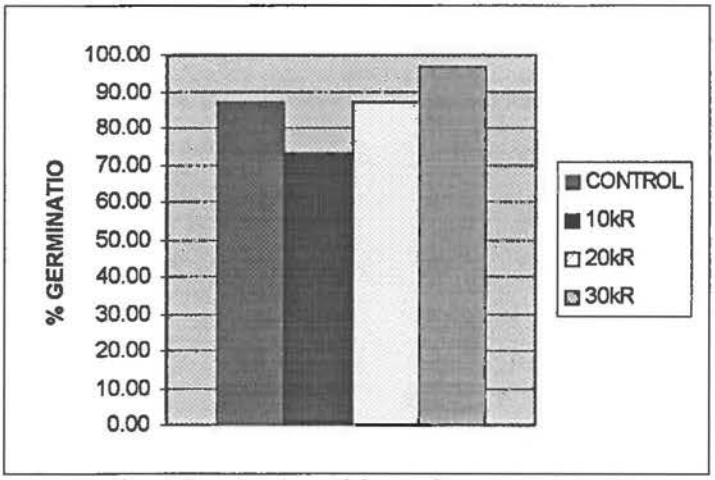

Figure 1. Seed Germination. CONTROL=26 seeds (87%), 10kR=21 seeds (73%), 20kR=26 seeds (87%), 30kR=29 seeds (97%).

Root Lengths

Roots in group 10kR were the longest, and roots in group 30kR were the shortest (Fig. 2A). The CONTROL had a similar average length to group 20kR (Fig. 2B).

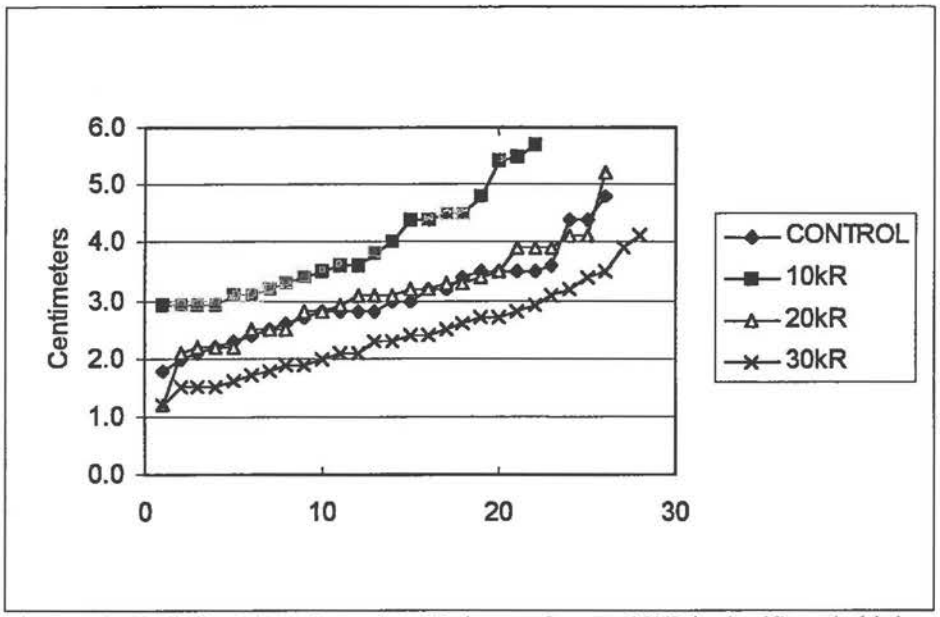

Figure 2A. Individual Root Lengths. Variance of group 10kR is significantly higher than variance of group 30kR ($\alpha<0.05$). Variance between all groups is also significant ($\alpha<0.05$).

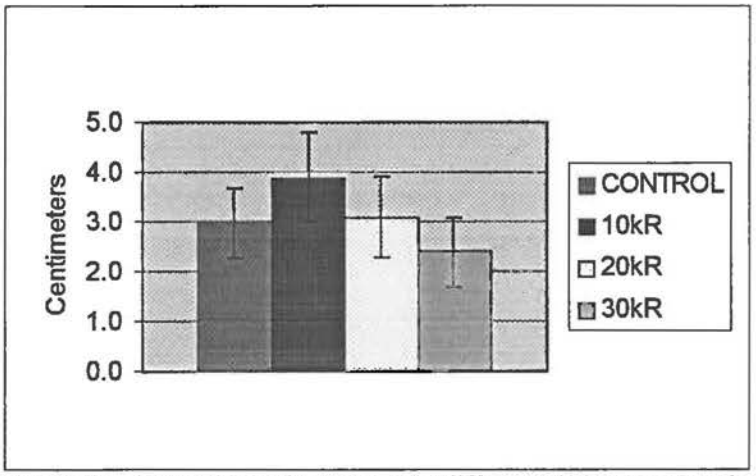

Figure 2B. Average Root Lengths. The difference between values in group 10kR and group 30kR is significant ($\alpha < 0.05$).

Root Diameters

The average width of the radish roots was slightly above 1 mm (Fig. 3). Group 30kR had the lowest average diameter, but the differences between the groups are not significant.

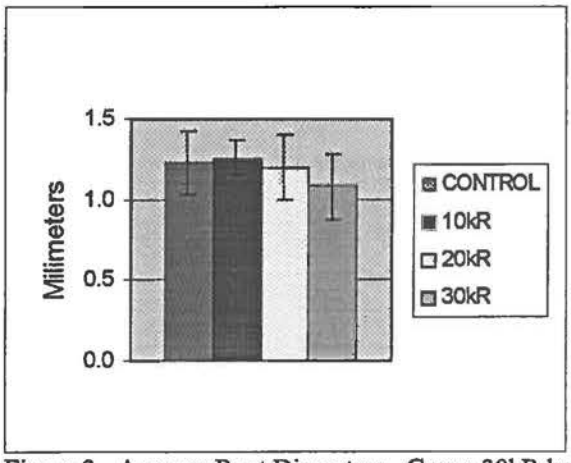

Figure 3. Average Root Diameters. Group 30kR had the smallest average diameter of the 4 groups.

Root Mass

Mass of radish roots correlated directly with the length of the roots. Regression analysis of length and weight values of each radish in each group revealed that radishes in the CONTROL had a fitted mass of 20.7mg/cm. This was the highest value of the 4 groups. Group 30kR had a fitted mass value of 15.7mg/cm (Fig. 4).

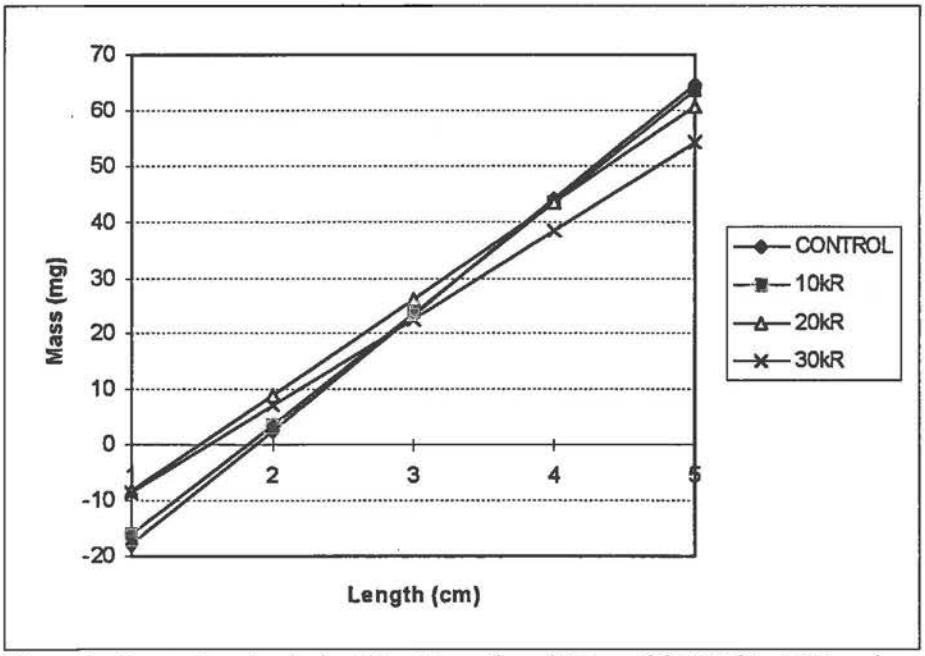

Figure 4. Regression Analysis of Root Length and Mass. CONTROL=20.7 mg/cm, 10kR=19.8mg/cm, 20kR=17.2mg/cm, and 30kR=15.7mg/cm.

Absorbance

Group 10kR exhibited the highest absorbance values of the 4 groups (Fig. 5A). Average absorption values show this difference (Fig. 5B) .

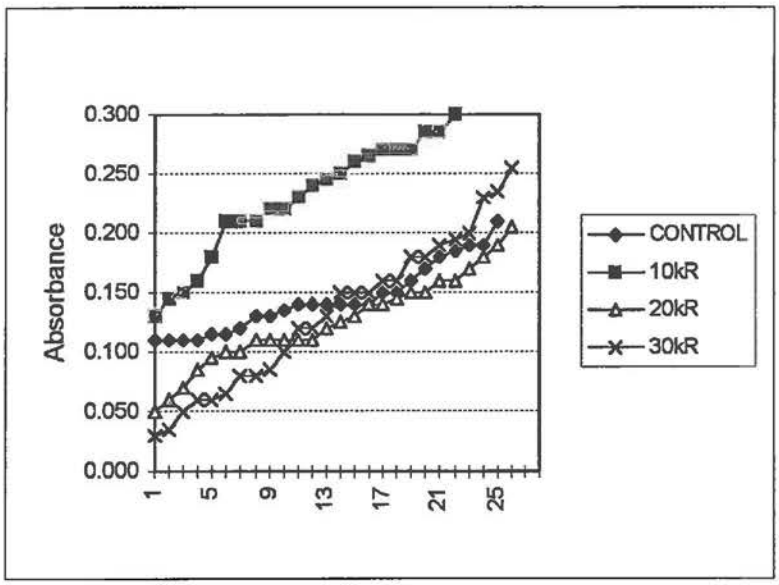

Figure 5A. Individual absorbance measurements of 2 ml samples of radish root extracts. Group 10kR had the highest values of absorbance. Groups 20kR, 30kR, and CONTROL had comparable values.

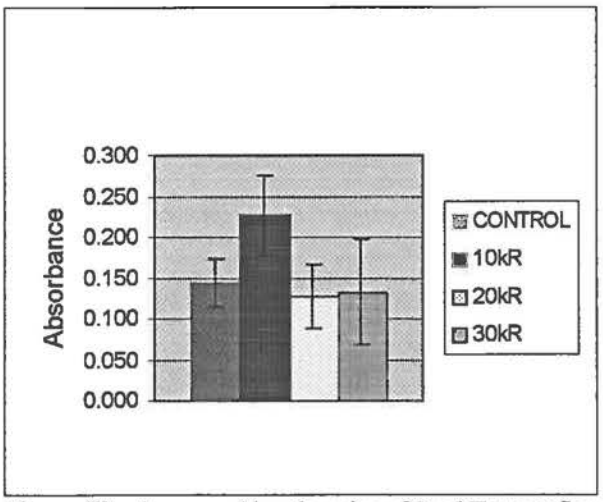

Figure 5B. Average Absorbencies of 2 ml Extract Samples. The difference between the average absorbance values of group 10kR and groups 20kR, 30kR&CONTROL is significant ($\alpha<0.05$). Differences between groups 10kR, 20kR, and 30kR are not significant ($\alpha>0.05$).

Discussion

Germination

The present germination results were not expected. In group 10kR 8 seeds did not germinate, whereas in group 30kR only 1 seed did not germinate. In groups 20kR and CONTROL 4 seeds did not germinate. These results suggest that germination in this experiment was dependent on something other than the radiation dosage.

Growth

The radish plants did not grow as well as was anticipated (Pic. 8). Radishes are usually ready for harvest in about 30 days. At 36 days the experimental radishes were still not mature. Two reasons can be suggested for this unanticipated poor growth: inadequate supply of light, and too short of a day length during March when the radishes were grown.

Comparison with an experiment previously conducted makes this inference likely. That previous experiment was conducted under timed fluorescent lighting and the radishes grew much better. This result suggests that experiments with plant growth during the Fall and Spring seasons should be conducted under timed fluorescent lights.

Root Length, Width, and Mass Values

The present length results were not expected. There is a significant downward progression in the average length of the radishes in groups 10kR, 20kR, and 30kR respectively which seems to correlate well to the radiation dosage (Fig. 2B). However, the CONTROL is similar to the 20kR group. This similarity suggests that 10kR and 20kR dosages do not greatly affect the growth of the root. In contrast, 30kR radiation does seem to have an effect because group 30kR had the lowest average root length. The difference between the CONTROL and the 30kR group was significant ($\alpha<0.05$).

The average width of the 30kR radishes was the smallest of the 4 groups although the differences between the groups are not significant (Fig. 3). This result suggests that 30kR radiation affected the radishes more negatively than did 10kR or 20kR radiation.

The mass results are dependent on the length results (Fig. 4). Linear regression analysis shows that the CONTROL and the 10kR group have similar mass to length correlations. Group 20kR has a lower mass to length correlation, and group 30kR has the lowest. These results suggest that 30kR radiation affected radish development the most.

Absorbance

The absorbance values show that group 10kR had the highest absorbance of the 4 groups (Fig. 5A). This result, though not expected, is at least consistent with the root length/width/mass results. Group 10kR has, with respect to the root length/width/mass and absorbance variables, the highest values of the four groups.

High absorbance was supposed to correlate with a high concentration of pelargonidin. Visually the CONTROL, 10kR and 20 kR did not differ much in coloration. Radishes in group 30kR were, however, more pale than the radishes in the other groups, but the visual result was not confirmed by the absorbance measurements. The CONTROL, group 20kR, and group 30kR had similar absorbance averages. There should have been differences.

One feasible reason may be given to account for the unexpected absorbance results. The extracts may have been too dilute for the photospectrometer to detect differences appropriately. Future experiments may have to determine a better way to quantify pelargonidin concentrations in roots, possibly by concentrating and purifying extracts beyond the initial extraction stage.

Conclusion

The results of this experiment suggest that 30kR radiation had the most significant effect in mutating radish plants. Radishes in group 30kR were consistently shorter, thinner and lighter than the other groups. Visually the radishes in group 30kR were the most pale, but the absorbance results did not confirm this (Pic. 8). Experiments with mutagenesis of *Raphanus sativus* should use at least 30kR of radiation.

Acknowledgments

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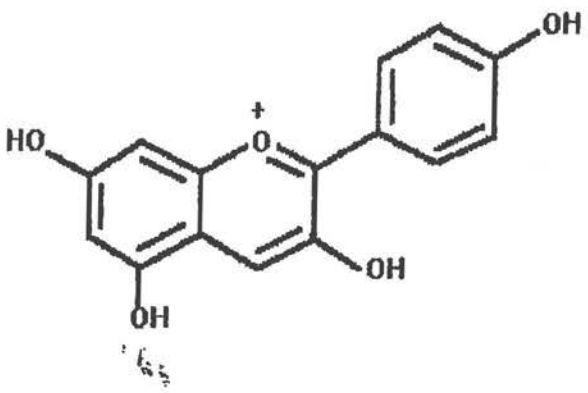
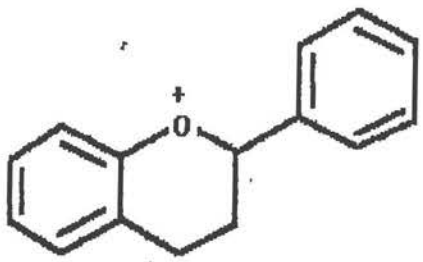
APPENDEX A



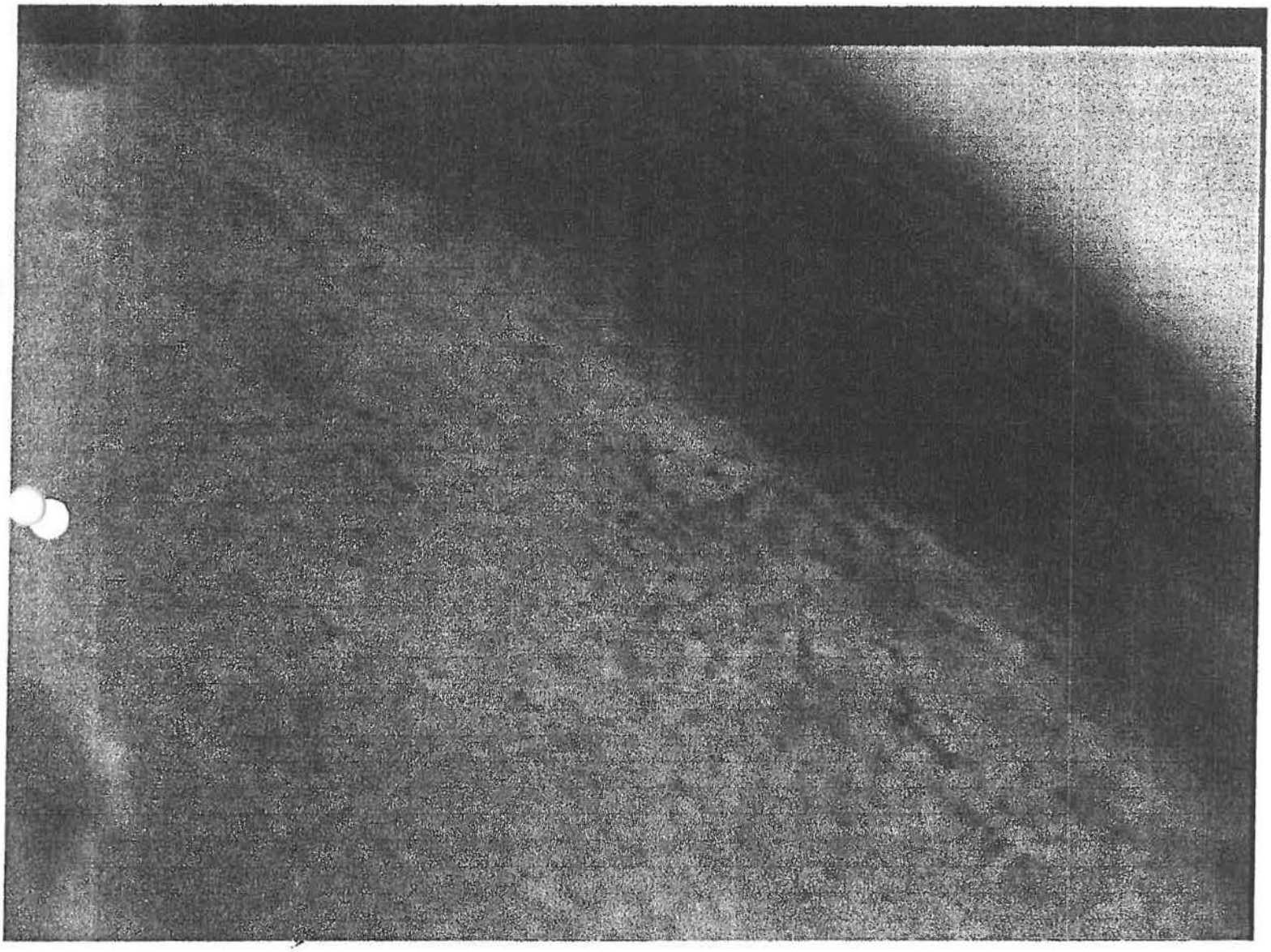
PELARGONIDIN



FLAVAN NUCLEUS

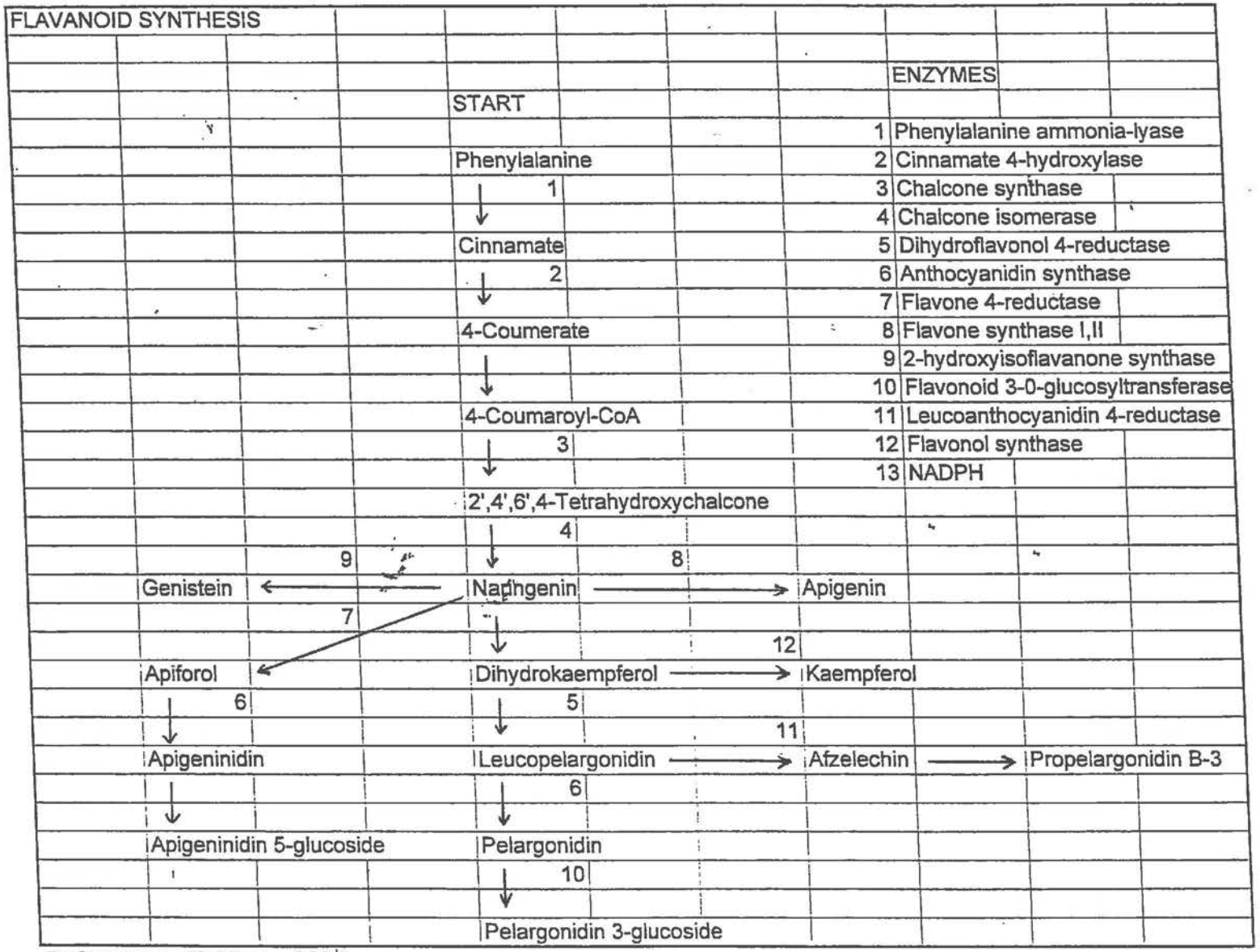
Reference: Coultate, 1989

APPENDIX B



Light microscope X100 magnification of a cross section of a Raphanus sativus root. Red pelargonidin pigment can be seen in the dermis of the root.

APPENDIX C



Reference: Heller, 1994

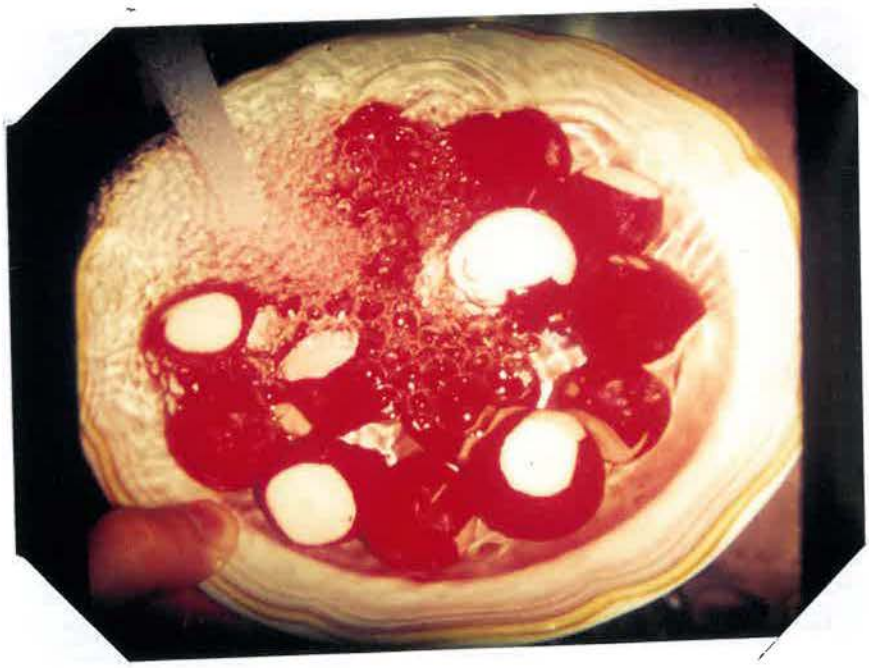
Pictures*

1. Germinating Radish Seeds.

2. Germination Experiment. On the left are radish seedlings whose seeds weighed 4 or 5 mg, and on the right are radishes whose seeds weighed 16 or 17 mg.

* These pictures are prints of slides. The picture quality of the slides is better.

3. 5-day-old Seedlings. These seedlings were planted on the 6th day of the experiment.

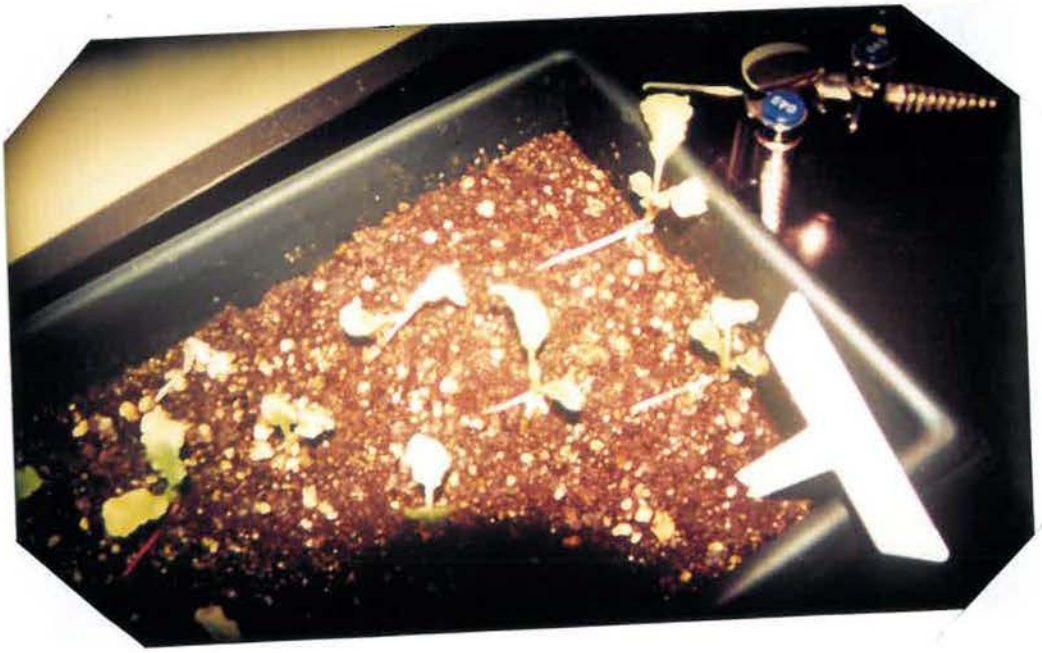


4. Store-bought Radishes. Experimental radishes were supposed to lose the red color exhibited by these store-bought radishes.

5. 3rd. Day of Growth. From left to right are groups 10kR, 20kR, 30kR, and CONTROL.

6. 17th. Day of Growth. From left to right are groups 10kR, 20kR, 30kR, and CONTROL. The pots were rotated so that the white tags are shown towards the incubator in the back.

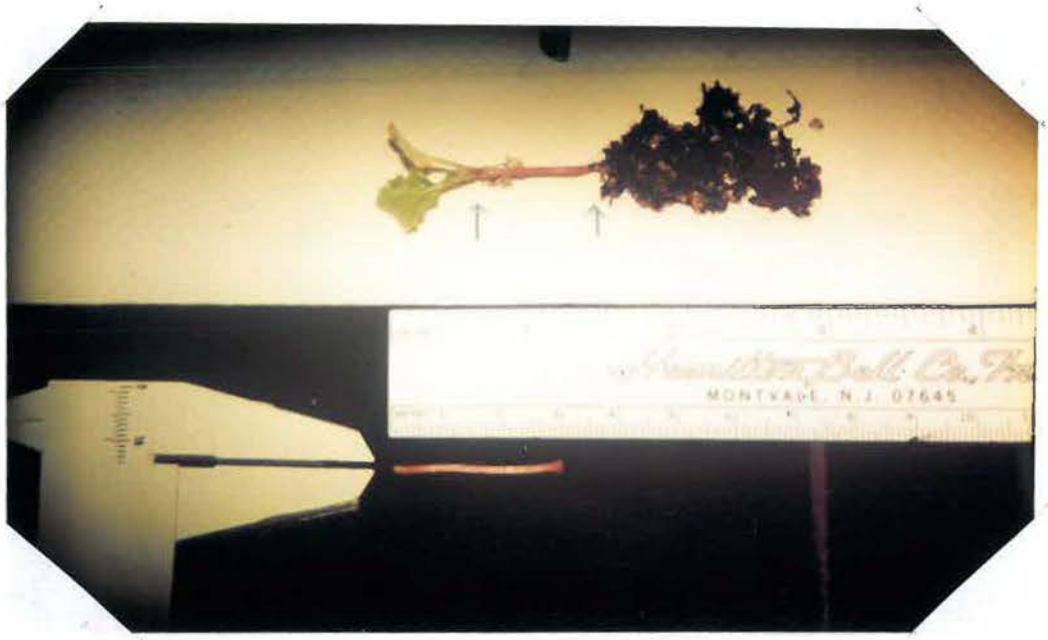
7. 25th. Day of Growth. The white tags are towards the camera. From left to right the groups are 10kR, 20kR, 30kR, and CONTROL.



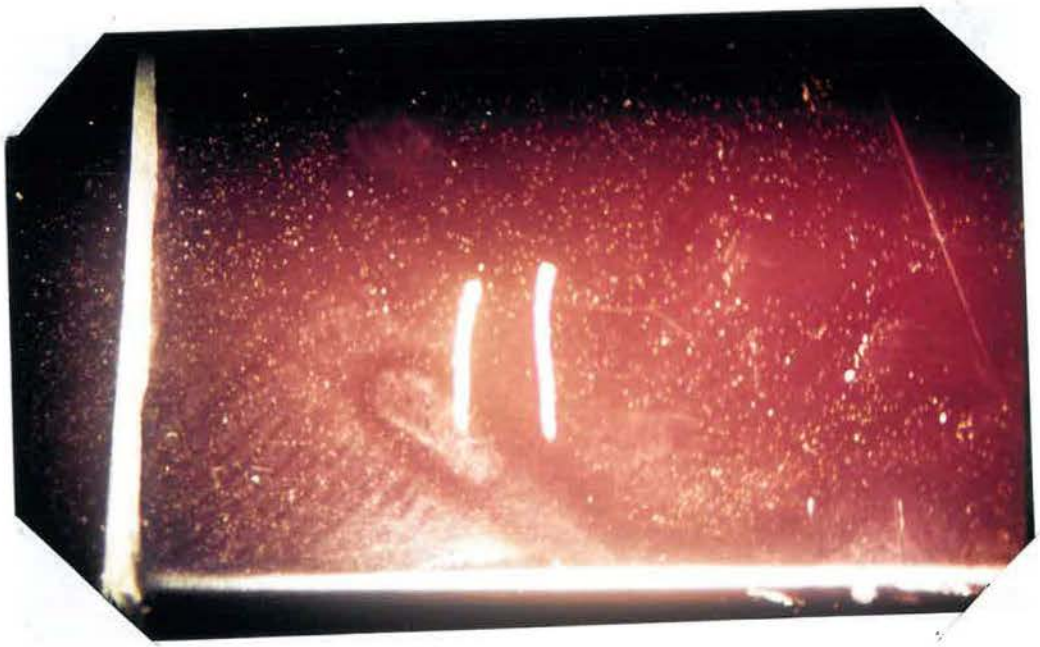
8. Group 30kR. Center top radish is pale. Bottom left radish is redder.

9. Group 30kR. The left radish is redder than the right radish and the radish in the back.

10. Analysis. Storage part of the roots was cut and measured.



11. Analysis. A ruler and a caliper were used to make measurements.



12. Group 30kR. The radish on the left is more pale than the radish on the right. The slide shows a better image.