Science Fair Resource Package

Teacher and Student Edition: Experiment Package

SASKATOON REGIONAL SCIENCE FAIR

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Science Fair Project Descriptions

There are three types of projects that may be presented at the Saskatoon Regional Science Fair:

- Experiment
- Study
- Innovation

Experiment: A practical, hands-on investigation undertaken to test a scientific hypothesis. Experiments follow the scientific method and are designed to investigate one measureable variable; other variables are controlled. Data is thoroughly analyzed using statistical methods. The best projects include original questions, to which the answer is not presently known, or new experimental methods.

Study: Analysis of, and possibly collections of, data using accepted methodologies from the natural, social, biological, or health sciences. This includes studies involving human subjects, biology field studies, data mining, observation and pattern recognition in physical and/or socio-behavioural data. The study correlates information from a variety of peer-reviewed publications and from systematic observations, and reveals significant new information, or original solutions to problems. Quantitative studies should include appropriate analysis of some significant variable(s) using arithmetic, statistical, or graphical methods. Qualitative and/or mixed methods studies should include a detailed description of the procedures and/or techniques applied to gather and/or analyze the data (e.g. interviewing, observational fieldwork, constant comparative method, content analysis).

If you are unsure of what category your study falls into, ask your teacher or a mentor!

Innovation: Development and evaluation of new devices, models, theorems, physical theories, techniques, or methods in technology, engineering, computing, natural science, or social science. Students may integrate several technologies, inventions, or social/behavioural interventions or design and construct an innovative application that will have human and/or commercial benefit. The best projects include a clear understanding of technological and scientific principles that guide the design and construction of the device.

Science Fair Outline & Information

Purpose:

This document is intended to serve as a guide in creating a high-quality science fair project. There are numerous components to be considered and included in a thorough project. Before beginning, set yourself up for success by reviewing this document carefully. This resource package provides guidance specifically for *experiment* projects. For guidance on *innovation* and *studies*, please see the respective resource packages.

The purpose of science fair projects is to develop ***real*** science skills with a topic that interests you. Science fairs give you the opportunity to complete an independent, hands-on, inquiry based project that addresses an important scientific question or problem. Consider reaching out to universities and local scientific organizations for possible mentorship opportunities to support your project requirements.

Timeline:

The Saskatoon Regional Science Fair is held late March or early April at the University of Saskatchewan, Education Building. Students can register at www.usask.ca/srsf by clicking on the 'register' tab by the beginning January. Registration, including a completed abstract, closes at the end of March. Please be aware of deadlines which can be found at https://secure.youthscience.ca/sfiab/saskatoon/important_dates.php

Groups:

Students must work alone or in a group of two. If working in a group of two, both students should be at the same level (junior or senior). Grade 7-12 students are eligible to compete at the Canada Wide Science Fair Event. These requirements are also firmly upheld at the National Science Fair level.

Mentorship:

If students are interested in pursuing a mentored project, they should contact the applicable colleges at the University of Saskatchewan and professional associations such as APEGS. This information can be found in the contact page of each college https://www.usask.ca/colleges.php. You may also contact the Science Fair Committee at chair.srsf@gmail.com

Getting Started

1. Decide if you will be working alone or with a partner. Either way, your teachers, parents, or others may provide appropriate assistance, however the work must be student produced. Pick a partner that you will work well with. This is a major project that requires lots of time and energy. Don't just pick someone because they are your friend – make sure you can trust them to handle half of the workload. You will need to schedule time to work on the project together, so pick someone that you communicate well with and are able to meet with after school or on weekends.

2. Decide if you are doing an experiment, innovation or a study. If you choose to do an experiment project, you are in the right place! If you want to do an innovation or study, look at the other resource packages on our website.

3. Choose a topic that interests you. Since this is a major project that takes up time and energy, you might as well do it on something that you like!

4. Choose a topic that is safe and legal – making bombs, fireworks, firecrackers, drugs etc. would not be considered safe or legal. If you are working with human participants or animals, please contact the Saskatoon Regional Science Fair Chair at chair.srsf@gmail.com. Your project must also follow Canada Wide Science Fair ethical standards. More information on safety requirements can be found on the Canada-Wide Science Fair website.
https://cwsf.youthscience.ca/
http://www.usask.ca/srsf/years/2019/ethics_and_safety.html

5. Design an experiment that requires materials and equipment you can easily access.

6. Develop a scientific question you want to answer. Experiments require that you first develop a quality, ***testable question*** with ***measureable variables*****.** Often students make the mistake of choosing a question that explores scientific phenomena through a demonstration or that does not have clearly measureable variables. From this scientific question you will develop your hypothesis.

7. Get yourself a binder for keeping all of your Science Fair work organized. This will make the process of writing your presentation and designing your display so much easier. Good science involves meticulous notes and careful descriptions of every step taken.

8. Be aware of the various deadlines. This project is ***very*** difficult (if not impossible) if you leave it to the last minute. By setting up a regular schedule and working on it in chunks, you will produce a high-quality science fair project and make your life easier!

Project Components

These are the components you are required to complete in order to partake in the Saskatoon Regional Science Fair:

1. **Proposing Your Idea to an Adult or Mentor and Researching Your Topic** – Once you decide upon your science fair topic, summarize your idea and propose it to an adult or mentor. Proposing your idea to an adult or mentor can help iron out kinks before beginning your experiment. After the proposal, you need to begin researching. Background research should include information such as any current research being done on your topic, scientific information about materials, etc.

2. **Science Fair Project Plan** – A report that goes into more detail. Work on your project, following the scientific method, by completing pages 8 to 11 in this resource package.

3. **Lab Book** – A bound book including all procedures, results and observations in their raw form. This includes all qualitative and quantitative data taken during the experiment (including the dates gathered), any calculations, and statistics such as averages as averages or percentages (this may include ranges, standard deviations, or error). Essentially, judges will look through this for more detailed data. The final report will include summarized charts and statistics to acquire only essential data. Please write the date one each page.

4. **Science Fair Project: Final Report and Reflection**– A two to six page final report based on the draft report you previously completed. You need to fully publish your findings and conclusions through a professionally written report. Review the requirements on pages 12 to 13 in this resource package.

5. **Display Backboard** – This is the final display that you will show to the world. You will need to present your project on a trifold backboard or poster display. Remember, this is what the audience gets to see, so make sure to pay attention to details! Display backboards require you to exercise skills in design aesthetics. Review the guidelines on pages 14 to 15 in this resource package.

6. **Abstract** – A 200 word maximum summary of your topic that includes:
    - purpose
    - methods (steps you are taking to complete your experiment)
    - some of your research
    - any results/analysis (if available)

The abstract should allow readers to understand the project without reading the entire science fair project report. The abstract is used to classify the type of science fair project and assign appropriate judges for the regional fair. For more guidance you can visit https://www.sciencebuddies.org/science-fair-projects/science-fair/how-to-write-a-science-fair-project-abstract

7. **Oral Presentation** – The scientific world emphasizes the value of share scientific findings with experts and members of the community. Make sure you are well rehearsed to share your project in various degrees of depth: 7 minutes, 5 minutes, 2 minute discussions. Review the guidelines on page 16 in this resource package.

Please note that Canada-Wide Science Fair has its own judging criteria for the presentation and project. http://cwsf.youthscience.ca/judging-criteria

Science Fair Project Plan

1. Project Title:

2. Investigative Question/Purpose: What is the purpose of your experiment? Is there a problem to be solved?

3. Research and Resources: In order to plan, analyze, and understand your results, you will need to complete research. Keep track of all sources below (books, websites, magazines, etc.). Write all research notes on separate pages.

Resource A:

Resource B:

Resource C:

Resource D:

4. **Hypothesis:** What outcome are you expecting and why? You may reference your research for evidence to support your hypothesis.

5. **Variables:** Identify the variables of your experiment.

Independent Variable: A variable that is intentionally changed to observe its effect on the dependent variable

Dependent Variable: What you are observing in your experiment.

Controlled Variable: Aspects which are maintained the same across all groups or trials

6. Procedure:

a. **Materials**: List any supplies utilized in the experiment (bullet points with quantities if possible). These are similar to the ingredients and equipment needed to cook a certain recipe.

b. **Method and Diagram:** Numbered step by step instructions. These should be detailed, yet simple instructions that ANYONE could follow. Your experiment needs to be replicable with reproducible results. You must include a diagram of you experimental set up. Think of this as the directions of a recipe and with how-to images.

7. Results:

a. **Data Tables:** Create data chart(s) you will use to record your data during the experiment. **Put this in your lab notebook.** Consider how you will record both qualitative and quantitative data. All tables containing numerical data should be clearly titled and include units and degrees of uncertainty.

DO NOT RUSH THIS SECTION. THIS MAY BE ONE OF THE MOST IMPORTANT SECTIONS LATER WHEN YOU ARE WORKING ON YOUR EXPERIMENT!

Things to consider: How many trials do you need? What will the results look like?

i. Sample Calculation(s) – If you ever do an experiment that requires you to do a calculation after collecting your data, you always need to show one and only one example of how to perform the calculation. There should be one sample calculation for EACH different calculation performed.

A good experiment collects as much relevant data as possible. There are two types of data that you can collect in an experiment:
Qualitative Data is descriptive data that describes the qualities of an event. Could be things such as colour, smell, texture, etc. Does not include numbers.
Quantitative Data involves numbers and quantities and can be measured. Could be things such as mass, volume, length, temperature, time, speed, age, people, etc.

b. Graphs: Graphs allow for clearer communication. Quantitative results should be taken and made into a graph. As a rule of thumb, any graph being viewed should be understood without other information. The type of graph you utilize depends on the data being presented. Consider scatter plots or bar graphs.

Science Fair Project Report: Final Report and Reflection

Your final project report should be a typed two to five page report following the scientific method. Include the following sections in your final report:

1. Project Title
2. Investigative Question/Purpose
3. Background Research
4. Hypothesis
5. Variables
6. Procedure
    a. Materials
    b. Method and Diagram
7. Results– Results should summarize the outcome of the experiment. Results in this report should be in final form. Raw data is only included in the lab book. Viewers should be able to acquire all crucial data without needing to read through multiple pages of calculations. If more information is required the lab book can be accessed.
8. Conclusion – This is a summary of what you discovered from your experiment and what the implications of that are. To have a thorough conclusion:
    - Restate the purpose and your hypothesis.
    - Sum up the results of your experiment (if you found final numbers such as density, volume, etc. you should state them with units).
    - Say what your results mean in the context of the purpose of your experiment.
    - State whether these results support, verify, or falsify your hypothesis and why.
    - Discuss the implications of your experiment and what your results mean.
    - Reflect: What have you learned? Compare your results with your research.
9. Sources of Error – Every experiment has tiny errors, many of which you cannot control. You need to report all these errors to the best of your ability. This will help future scientists eliminate errors if possible.

Human Errors are errors that people make during an experiment. Could be things such as using the wrong amounts, not cleaning glassware well enough, accidently spilling, sneezing in your materials, etc. Human errors are always preventable.

Systematic Errors are errors that cannot be avoided. They are built into the experiment and the experimental procedure itself. Systematic errors are always present and always predictable. Examples include the markings on your ruler being incorrect, the temperature of the room changing over time, the air flow in the classroom changing, impurities in chemicals used, etc. Figuring out what these errors are can be challenging but if you think critically about your experiment you can figure them out.

10. Future Improvements – List what could be done about the design of the experiment in order to improve it in the future.

- Improvements should usually focus on reducing the impact of the errors listed
- This should never be about what you could change. Don't list things like "I could have been more careful when I measured" or "I could have retried it to make sure I did it right".

11. References – be sure to write a bibliography or list of sources you used in your background research.

12. Acknowledgments – You should also include acknowledgments of any mentorship or guidance you've received.

Display Guidelines

The display is a vital component to your science fair project. Essentially, your display showcases your work to viewers and judges and can often separate a superior project from a mediocre project. The display board should be well organized, include information allowing viewers to get a good understanding of your overall project, and be visually appealing.

Backboard Materials:

At the Saskatoon Regional Science Fair you may choose to construct a display board by utilizing a tri-fold cardboard display or a 4ft. by 3 ft. poster. If you would like to construct a poster please see requirements below. Regardless of your choice of materials, the display must be sturdy enough to stand alone on a table. At the Canada Wide Science Fair the display guidelines are extremely stringent. Please refer to the Canada Wide Science Fair website for more information https://cwsf.youthscience.ca/

If you used special equipment, the set-up should be placed in front of your display or in a place to enhance the exhibit—not to overwhelm it. Remember that you must follow the rules and regulations for items displayed at the Saskatoon Regional Science Fair (reference ethics manual). Please note that the Canada Wide Science Fair have different rules and regulations surrounding items at display tables. You can find this information at the Canada Wide Science Fair website https://cwsf.youthscience.ca/

Poster Display – These are to be exact digital replicas of a real poster board display. Imagine a trifold display board filling up your computer screen. These can be printed on large plotter sized paper. For more information about poster prints, please contact your local print shop.

poster displays must:

-be in a one page, poster format (including all information)
-be able to be viewed in a single screen shot (not in multiple slides such as a PowerPoint).

Backboard Approaches:

There are many different ways to approach the organization, content, and graphic design of a science fair display board. Your project will be surrounded by many others, so it should be aesthetically appealing. Remember the following when designing display boards:

- colour appeal (select a few complementary colours, avoiding florescence colours, glitter, or shiny elements)
- contrast (colours selected should have a high contrast)
- clear, concise statements (write better not more)
- organization (information should be square to readers, not tilted, and in a logical layout)
- completeness (include all necessary information)
- professional (pay attention to details)

Your display board should tell the story of your project. Be sure to include information that allows viewers to get a good enough understanding to summarize your project. You MUST include:

Introduction:

- investigative question/purpose
- hypothesis

Methodologies:

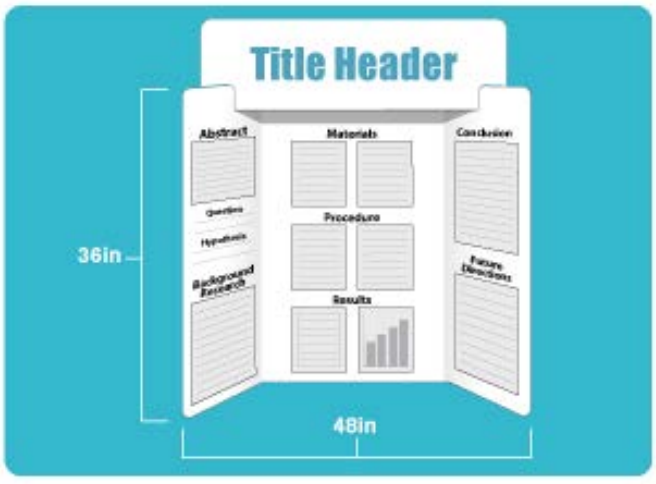
- variables
- procedure

Results & Analysis:

- data charts and/or graphs
- results analysis

Conclusion:

- conclusion
- future improvements



References

Acknowledgements

Consider including only pertinent background research, materials, and sources of error.

Presentation Guidelines

The ability to clearly communicate the purpose, methodology, and findings of your science fair project is critical to your success and enjoyment of sharing your work to viewers and judges. This portion refers to the oral communication of your project. Scientists and researchers in various vocations and industries attend conferences to share their work and to learn and listen to others. Similar to writing a science fair report or designing a display board, presenting your project orally requires specific skills and preparation. Typically, you will first have an opportunity to present your project to judges and then engage in a question and answer session.

Preparation:

The first time you present your project to viewers should not be at the regional science fair; you need to practice presenting your project to others before attending. Prepare the flow of your presentation to follow the scientific method: plan, predict, carry out design, analyze findings, and conclude. Each person who views your project at the Saskatoon Regional Science Fair will wish to understand your project at different levels: quick summary, detailed summary, in-depth. In order to prepare for all viewers, create a 2 min, 5 min, and 7 min oral presentation.

Speaking/Presenting Criteria – The "Do's" and "Don'ts"

- speak with a clear loud voice
- be confident: remember, you are the expert because it's your project!
- make eye contact with the viewer (do not read straight off your board or cue cards: these are references only)
- smile and introduce yourself before talking about your project
- speak at a moderate pace (too fast—viewers cannot comprehend the information, too slow and you will not be able to explain all information to viewers)
- watch and listen to your viewers—if questions are asked during your presentation, pause, breathe, and respond to the question before continuing your speech

Appendix: Experiment Resource Package

Appendix 1: Experiment Science Fair Project Idea

1. Will vitamins affect the growth of a plant?
2. Do weed killers affect house plants?
3. Does the amount of light on plants affect their growth?
4. Does the amount of water given plants affect their growth?
5. What is the effect of detergent on bean seeds?
6. Under what color light do plants grow best?
7. In what kind of material (sand, clay, etc.) do seeds grow best?
8. Will plants grow better in soil or water?
9. What is the effect of heat when dissolving sugar? Salt?
10. How fast do fabrics burn?
11. How is the strength of a magnet affected by glass, cardboard and plastic?
12. What is the best shape for a kite?
13. Which holds two materials together better, a screw or a nail?
14. Which detergent breaks up oil best?
15. How does the absorption rate of various paper towels differ?
16. Which detergent makes the most bubbles?
17. How does the wattage of a light bulb affect energy use?
18. Which properties of different glue holds two boards together best?
19. Which properties of different popcorn pop fastest?
20. Which type of battery makes toys run longest?
21. Which type of diaper holds the most water?
22. What properties of different hand sanitizer disinfect hands better?
23. Are more expensive golf balls worth it?
24. Does using a cell phone (calls or texting) affect reaction time?
25. Do white candles burn at a different rate than colored candles?
26. Does the presence of detergent in water affect plant growth?
27. Do the same types of mold grow on all types of bread?
28. Does light affect the rate at which foods spoil?
29. How permanent are permanent markers? What solvents (e.g., water, alcohol, vinegar, and detergent solution) will remove the ink? Do different properties of different types of markers produce the same results?
30. Is laundry detergent as effective if you use less than the recommended amount? More?

Many more past projects are available for viewing: https://secure.youthscience.ca/virtualcwsf/

Appendix 2: Resources Used to Develop this Resource Package

https://www.sciencebuddies.org/science-fair-projects/science-fair/science-fair-project-display-boards

How Does Fertilizer and Hormone Affect Different Plant Root Growth?
By: Jocelyn Pon Silverspring School Saskatchewan, Saskatoon

Background

This experiment interested me because I enjoy doing experiments on plants. I was curious what treatments (liquid fertilizer and plant hormone) would grow the longest and healthiest roots with two plant types. This experiment is useful to gardeners because they want their gardening plants to thrive. Plant roots that are healthy and long help to create strong plants because they can reach further down in deeper layers of the soil to access more nutrients and moisture for the plant. This is especially useful in years of drought or low moisture conditions.

Purpose

The purpose of this experiment is to investigate which type of plant (rosemary and menthol) and which type of treatment (powdered hormone and liquid fertilizer) will grow the longest/healthiest roots. The method for investigating this experiment will be to measure the roots with a ruler at day 15 and day 30. This experiment is important because it will help me discover which plant and fertilizer will grow the longest/healthiest roots. After I have completed this experiment, I will be able to share which plant treatment is best for a rosemary and menthol plant if you would like to grow long and healthy roots.

Background Research

Plant hormones are plant growth regulators meaning that they control the plant growth through chemical communication. Plant hormones consists of signal molecules made in specific location. Plant hormones are involved in development of plant cells and cell differentiation.
Liquid Fertilizers are mixed with concentrate and water. Fertilizers are usually a blue or green dye. The liquid fertilizer acts as plant food (nutrients) for the plant.

Hypothesis

My hypothesis for this experiment is that the plant treated with powdered hormone and liquid fertilizer will most successfully grow the longest and healthiest roots. This will happen because the rooting hormone adjusts how the plant grows. Plant hormones are also plant growth regulators that help plants grow. Plant hormones can also get rid of any bacteria or fungi that may be introduced during the cutting process. The liquid fertilizer is plant food for the plant meaning that liquid fertilizer gives nutrients to the plant. The liquid fertilizer consists of mineral salts that the plants can absorb quickly. Liquid fertilizer allows the growing process to be faster. These two treatments (fertilizer, rooting hormone) will have an advantage of growing long and healthy roots for plants.

Variables

Independent Variables: Rosemary and menthol plants each with two plant treatments: hormone and fertilizer treatments.

Dependent Variable: The root growth measured by length (m).

Constant Variables:

- temperature/space for growing
- amount of plant treatments applied to each plant
- measurement days
- amount of light
- plants and types of treatments (each plant received each treatment)
- tools/materials

Controlled Variable: The plant grown in water alone.

Materials

- 0.355L transparent cups
- 0.000125L of fertilizer treatment for each cup
- 0.000125L of rooting hormone treatment for each cup
- 8 Menthol plant cuttings that are 0.05-0.06m
- 8 Rosemary plant cuttings that are 0.02-0.04m
- Jump star Grow light with fluorescent light
- Sturdy table
- Sticker labels
- Pen
- 0.15L of water for each cup
- Timer

Procedure

1) Trim 8 menthol plant cuttings about 0.05m-0.06m each.

2) Trim 8 rosemary plant cuttings about 0.03m-0.04m each.

3) Fill the 0.355L plastic cup up to 0.15L of water for each plastic cup.

4) Label your cups according to the type of plant and the treatment of the plant.

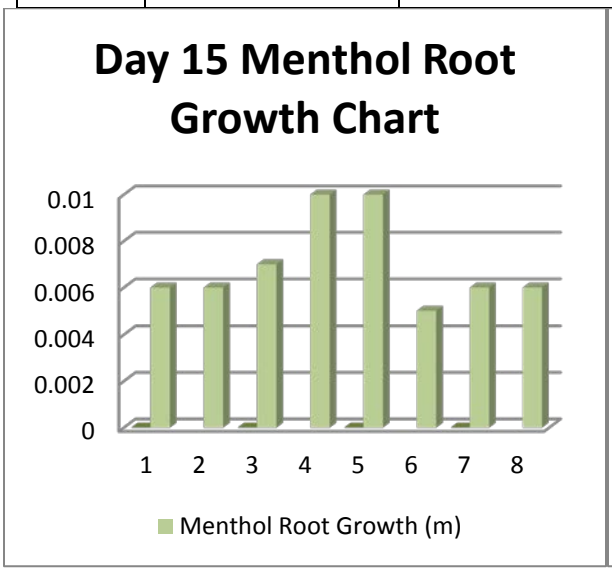
5) Using a pipette measure 0.0005L of liquid fertilizer separating the fertilizer equally into four of the plant cups

6) Using a ¼ teaspoon measure 0.0005L of rooting hormone and distribute it evenly for four of the plants

Results/Observations

Table 1: Day 15 Root Measurement of Rosemary and Menthol Plants

Day 15	Treatments	Rosemay Plants (m)	Menthol Plants (m)
Trial 1	Water	0.005	0.006
Trial 2		0.06	0.006
Trial 1	Fertilizer	0	0.007
Trial 2		0	0.01
Trial 1	Hormone	0	0.01
Trial 2		0	0.005
Trial 1	Hormone/ Fertilizer	0	0.006
Trial 2		0	0.006



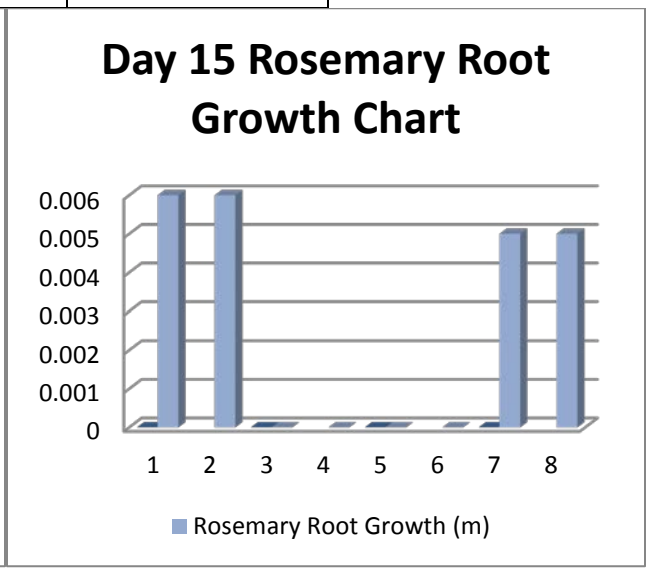
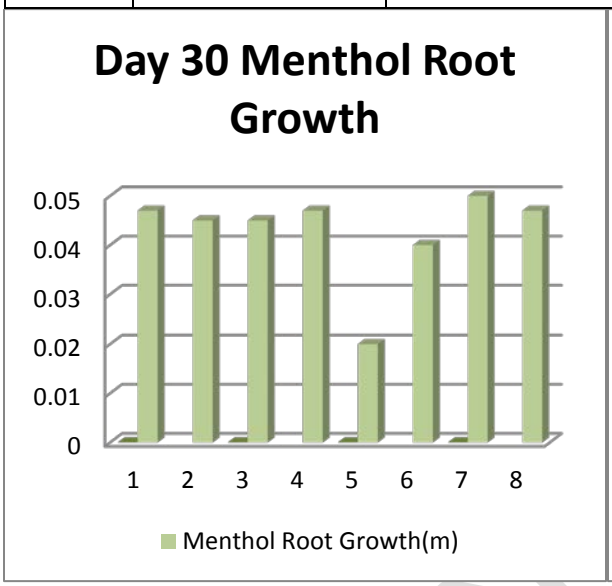
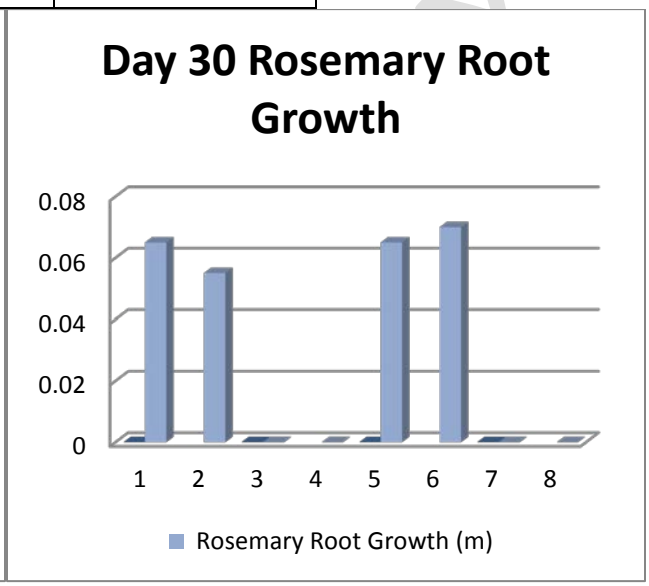


Table 2: Day 30 Root Measurement of Rosemary and Menthol Plants

Day 30	Treatments	Rosemary Plants(m)	Menthol Plants (m)
Trial 1	Water	0.065	0.047
Trial 2		0.055	0.045
Trial 1	Fertilizer	0	0.045
Trial 2		0	0.047
Trial 1	Hormone	0	0.02
Trial 2		0	0.04
Trial 1	Hormone/ Fertilizer	0.065	0.05
Trial 2		0.07	0.047





Conclusion

The purpose of my experiment was to test which treatments and plant cuttings would grow the healthiest and longest roots. The experiment results showed that the menthol with hormone and fertilizer grew the healthiest and longest roots. Therefore plants treated with hormone and fertilizer will grow the healthiest but also the longest. The menthol with hormone and fertilizer grew 0.05m ± 0.02 followed by the second trial with 0.047 ± 0.002. Plants treated with hormone and fertilizer grow the longest and the healthiest because rooting hormone are plant growth regulators. Plant hormone are chemicals that encourage and stimulate root development. New cells form calluses at the plant cut causing the roots to grow. In rooting hormones there are three groups that support the root growth of plants including auxins, gibberellins and cytokinins. Auxins and gibberellins support the root development of the cutting plant whereas cytokinins assist the cutting plant by development of cell division. Liquid fertilizer is necessary for a cutting plant because fertilizer provides nutrients.

Acknowledgment

I would like to thank Rick Block from Agriculture in the Classroom (Saskatchewan) for helping me with my science project and providing me with the experiment supplies. I would also like to thank Ms. Knoblauch (Silverspring teacher) for her guidance and preparation for the Science Fair.

References

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Targeting the Overexpression of MAD1 with SGOL1 and PP2A Inhibition
Deeksha Kundapur

Background: Cancer is the leading cause of death within Canada, accounting for 30% of mortalities [1]. While there is promise in treating cancer through improved drug-radiotherapy combinations, the non-specific mechanisms of current anti-cancer therapy causes damage to normal tissue [2]. Chromosomal instability (CIN) is a hallmark of cancer that is brought on by the overexpression of checkpoint proteins [3]. The spindle assembly checkpoint (SAC) prevents aneuploidy and CIN by arresting cell cycle progression until centromere and microtubule tension is sufficient enough for cell division [4]. The concept of synthetic dosage lethality (SDL) provides a new approach to identifying therapeutic targets with the exploitation of essential gene interactions unique to SAC protein overexpressing tumour cells. A SDL interaction occurs when the overexpression of one gene is lethal only in the presence of a second, nonlethal mutation [5]. Protein phosphatase (PP2A) physically interacts with the protein Shugoshin-like 1 (SGOL1) within the SAC to ensure proper chromosome cleavage.
Purpose: Due to biological and functional relevance, the possible SDL interaction of the SGOL1 and PP2A proteins with MAD1 overexpression was tested quantitatively and through imaging.
Materials & Methods: ***Cell culture*****:** The MAD1 inducible DLD1 human colorectal cancer cell line was cultured at 37°C and 5% CO_2 in RPMI 1640 medium, supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin at 10,000 units/mL. HEK 293T cells, used for lentiviral production, were similarly cultured, but in DMEM medium. To induce overexpression of MAD1, tetracycline was added to the culture medium at a final concentration of 500 ng/mL.
Resazurin Cell Viability Assay: Cells were seeded in 96-well plates and treated with cantharidin at various concentrations once 30% confluency was reached. At time of cantharidin addition, half the plate was treated with 500 ng/mL of tetracycline to induce MAD1 overexpression and the other half was given an equivalent dilution of 70% ethanol. At 96 hours post treatment, 10% resazurin was added to each well and incubated at 37°C and 5% CO_2 for one hour. Fluorescence at 544 nm excitation and 590 nm emission wavelengths and absorbance at 570 nm were read every hour for three or four hours using the SpectraMax M5 plate reader.
Immunofluorescence Imaging: Chambered coverglass was coated with fibronectin and incubated during cell preparation. Cell culture was trypsinized and counted, then split into chambered coverglass wells. Cells were fixed using methanol stored at -20°C and incubated with Triton X-100. After being blocked in 1% normal goat serum/PBS, antibodies NEDD1 and Tubulin were added and cells were incubated in dark. DAPI solution was used to stain cells and fix them for the confocal imaging. Confocal images were isolated for red, green, and blue stains and analyzed.
Lentivirus Hairpin Preparation. Three million HEK 293T cells were seeded to thirteen 10 cm plates and incubated for 24 hours at 37°C and 5% CO_2 to reach 70% confluency. The cells were transfected with 5400 ng of the lentiviral packaging plasmid psPAX2, 600 ng of the envelope plasmid pMD2.G and 6.0 µg of each SGOL1 and luciferase control hairpin using X-tremeGENE 9 DNA Transfection Reagent according to the manufacturer's instructions. Eighteen hours posttransfection the media of each plate was changed for viral harvest media containing 6.4 g of bovine serum albumin instead of fetal bovine serum. The next day, the media was collected containing the lentivirus and the media was replaced, to be collected the following day and combined with the first viral harvest. Each hairpin lentivirus was aliquoted into 2 mL stocks and stored at -80°C.

Western Blot Analysis: DLD1 cell pellets were collected and lysed with RIPA Lysis Buffer and 1X Halt Protease Inhibitor Cocktail and 1X Halt Phosphatase Inhibitor were added and incubated on ice for 30 minutes. The protein concentration of each lysate was measured using the BCA protein assay kit. Following the addition of 1X sodium dodecyl sulfate reducing buffer and 4X loading dye, the samples were boiled and 30 µg of the cell lysate was subjected to 4-12% SDS polyacrylamide gel electrophoresis. The electrophoresed proteins were then transferred to a membrane and the proteins of interest were detected using primary rabbit polyclonal antishugoshin and anti-GAPDH. Goat anti-rabbit secondary antibodies conjugated with horseradish peroxidase were used to be detected by Low ECL Western Blotting Substrate.

100K Race Proliferation Assay of SGOL1: Lentiviral transduction of DLD1 cells was performed by seeding with 8 µg/mL polybrene, the addition of lentivirus, and selection the following day with 2 µg/mL puromycin.The lentivirus of shLuc, sh1, sh8, sh9, and sh12 were transduced into MAD1 inducible DLD1 cells, selected and grown between 70 to 80% confluency. Each knockdown cell line was split to 100, 000 cells in two different wells of a 6 well plate for an uninduced set with ethanol added and an induced set with tetracycline added.

The cells were counted when a well reached approximately 80% confluency.

Results: ***Evaluation of PP2A SDL Interaction using Chemical Genetics:*** Using chemical genetics to model the SDL effect observed in the screen between the depletion of PP2A in MAD1 overexpressing cells, the anticancer activity of the PP2A inhibitor cantharidin was utilized. Overall, inhibition of PP2A showed a SDL interaction with MAD1 overexpression in cancer cells, as selective killing of cancer cells was taking place. Results were most optimal with a concentration of cantharidin between 2 µM and 4 µM (Figure 1). Thus, the chemical genetic experiments suggest that the inhibition of PP2A appears to exhibit SDL interaction with MAD1 overexpressing cancer cells through the use of cantharidin.

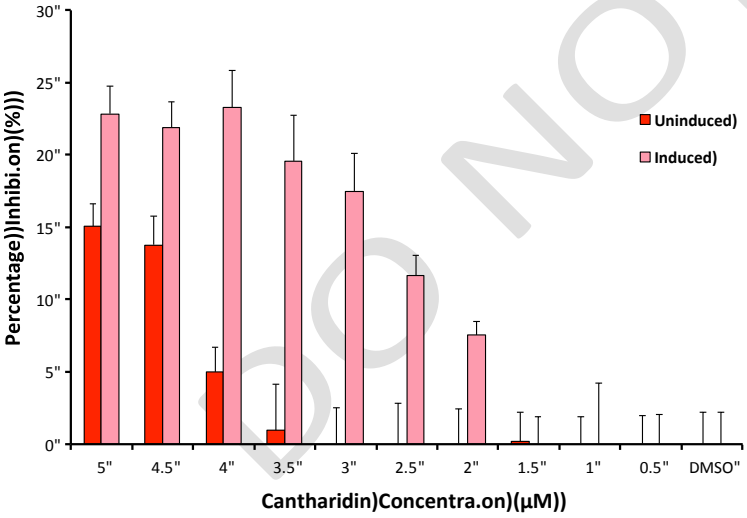

Figure 1. Cantharidin inhibits the viability of MAD1 overexpressing tumour cells. DLD1-MAD1 cells were split to 5000 cells per well of a 96-well plate and cantharidin was added at the indicated concentrations two days later. At time of cantharidin addition, half the plate was treated with 500 µg/mL of tetracycline to induce MAD1 overexpression and the other half was given an equivalent dilution of 70% ethanol.

Immunofluorescence Imaging of Cantharidin-Treated Cells: Immunofluorescence imaging exhibited formation of micronuclei within cantharidin-treated cells (Figure 2). It can be hypothesized that the results are actually due to the PP2A inhibition leading to aneuploidy within daughter cells. Furthermore, the reason this interaction leads to death as opposed to further tumor metastasis is likely

because the CIN caused by MAD1 overexpression in conjunction with aneuploidy from PP2A inhibition leaves defective repair mechanisms for the cells.

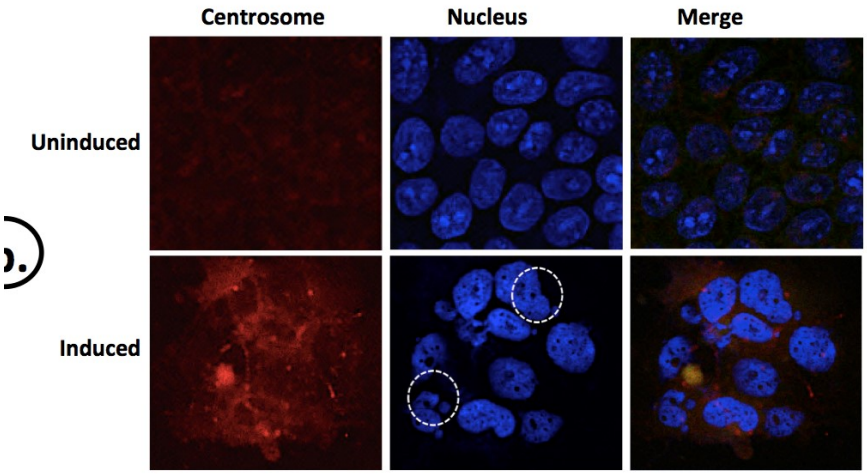

Figure 2. Cantharidin treatment immunofluorescence imaging. DLD1MAD1 cells were treated with 3 µM cantharidin for 48 hours and imaged using the confocal microscope. The results display formation of micronuclei.

Western Blot Assessment of Small Hairpin Knockdown Levels of SGOL1: Each shRNA packaged into lentiviral particles were separately transduced into DLD1 cells to produce stable SGOL1 knockdown cell lines upon selection for further analysis. In addition, a small hairpin targeting the luciferase gene (shLuc) was transduced to act as a negative control for SGOL1 knockdown. Western blot analysis of the knockdown lines indicated small hairpin one (sh1) and sh9 achieved virtually complete knockdown of SGOL1; sh11 and sh12 close to complete knockdown; and sh8 knockdown being the next best (Figure 3A).

100K Race Proliferation Assay of SGOL1 as a Potential Validation Hit: All hairpin knockdowns show the SDL trend of an increased degree of lethality in the MAD1 overexpressing cells (Figure 3B). The strongest SDL interactions were achieved by sh8 and sh9 as these hairpins show the greatest proliferation arrest in the MAD1 overexpressing cells and the lowest in the control cells.

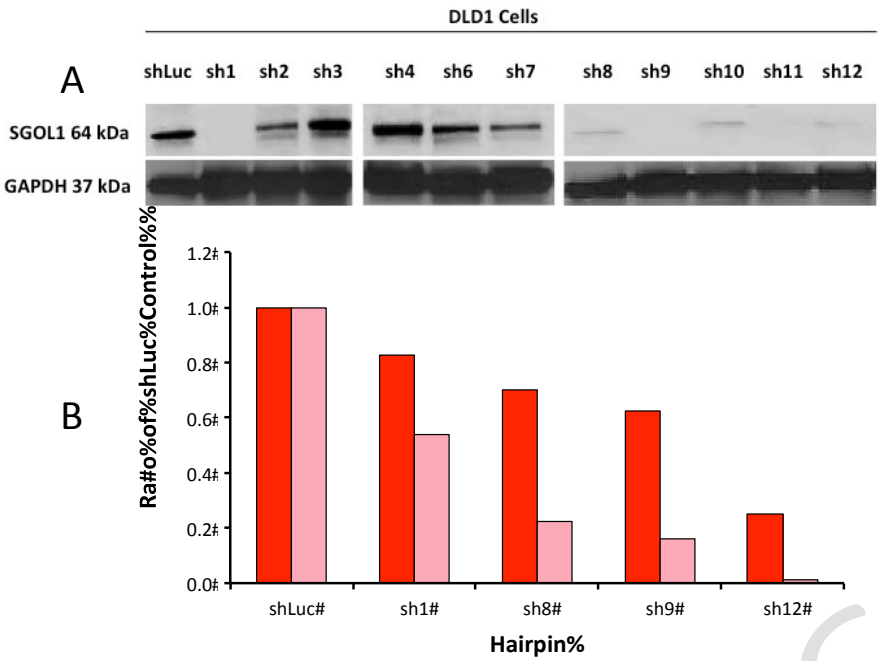

Figure 3. DLD1 cell proliferation effect by SGOL1 targeting hairpin knockdown.

A) Western blot analysis of the level of SGOL1knockdown caused by each hairpin.

B) Cell counts presented as a ratio of the shLuc control from the 100K race proliferation assay to assess the SDL effect in DLD1-MAD1 overexpressed cells through lentiviral hairpin knockdown, selection and split into control and MAD1 overexpressing populations.

Conclusions: Two novel SDL interactions were identified with SGOL1 and PP2A inhibition in conjunction with MAD1 overexpression. The economical and functional relevance of the study is extensively significant. Essentially, two novel anticancer therapeutic targets have been classified. As the field of SDL research is relatively new, this work contributes to pioneering the field with two successful interactions. Through cell viability assays and immunofluorescence imaging, the results of PP2A inhibition are indicative of an SDL interaction in a quantifiable way. In the future, this inhibitory effect can be translated into other cell lines, broadening the scope of this project's application. Similarly, the relation between SGOL1 inhibition and MAD1 overexpression exhibits extremely convincing potential as a SDL interaction and thus a anticancer therapeutic target. In the future, work towards developing peptide-based inhibitors will be done in order to translate this project into a possibly clinical stage. Overall, this work is significant in contributing to combination therapy and a future treatment for cancer. **Acknowledgments:** This project was guided by Shaina Templeton; it would not have been possible without her support and mentorship. Thanks to Dr. Franco Vizeacoumar for supervision, guidance, and the facilities to conduct this research at the University of Saskatchewan Health Sciences Building. And thanks to Frederick Vizeacoumar for his computational expertise.

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