Introduction

Throughout nature, carbohydrates and proteins are observed to have homogenous chirality; with carbohydrate configuration in a right-handed configuration and proteins existing naturally in a left-handed molecular configuration. While the reason for this exclusivity is still unknown and highly debated, it is hypothesized that our galaxy has a chiral spin and a magnetic orientation. In the case of proteins this has a direct correlation with formation and configuration of amino acids. The theoretical chiral spin and magnetic orientation of our galaxy results in cosmic particles polarizing starlight in a unidirectional circular fashion. Because the starlight only has one type of spin, it is hypothesized that this type of light degrades D-enantiomers (right-handed conformers) of amino acids more than it does L-enantiomers (left-handed conformers), resulting in a preference for L-form amino acid molecules in the Milky Way[1]. What if the same were true for carbohydrates? Is light the reason that D-oriented carbohydrates are preferred? If so, could we alter the orientation of carbohydrate molecules by manipulating exposure to different types of polarized light?

Exposing a fast-growing carbohydrate producing organism such as *Brassica napus* ssp. *Pabularia* to different forms of polarized light, organismal growth and development may show polarization effects. Over time, *B. napus* may show preference for a specific type of polarized light and, over many generations, undergo an evolutionary change that causes the organism to begin producing L-oriented enantiomer carbohydrates as a direct result of the type of polarized light to which it was exposed.

What is polarized light? Unfiltered light from the sun consists of light waves that vibrate in multiple planes, but light waves that vibrate in a single plane are said to polarized. Polarized light comes in a variety of forms--most commonly linear, circular and elliptical[2]. Circular and elliptical polarizations can further be distinguished as either right or left-handed depending on the direction in which the waves of light are rotating in that particular plane[3]. Transforming unpolarized light to polarized light is done through a process known as polarization, which involves a filter made of long chains of molecules aligned in a uniform direction, typically glass or transparent plastic. When light is transmitted through the filter, electromagnetic vibrations that are aligned in the same direction, or are parallel to the long-chained molecules, are absorbed by the filter. Vibrations that are not absorbed pass through the filter and are polarized since they only vibrate in the direction opposite of the plane of light waves which were absorbed by the long-chained molecules[2]. Subjecting *B. napus* to these different types of polarized light may show optimization of light absorption that the stomata of the plants absorb best, leading to a variety of results which could alter the configuration of carbohydrates produced through photosynthesis by the organism in study.

Why use *B. napus*? Photosynthetic organisms have been shown to prefer one handedness of circularly polarized light over another. Marine algae exhibit better growth when subjected to right handed polarized light than left-handed[4]. In a similar experiment, pea plants grew taller and faster under left handed polarized light[5]. *Brassica napus*, commonly known as kale, will be the model organism for this study on the effects of polarized light upon the configuration of monosaccharides and disaccharides. Hardy and resilient, this member of the cabbage family will provide quantifiable characteristics as a result of environmental manipulation[6].

How will carbohydrate configuration be analyzed? High Performance Liquid Chromatography (HPLC) is a chromatographic technique which uses small stationary phase particles and high pressure to drive the solvent through the column, separating components of the mobile phase. Columns are specific to the separation requirements of the mobile phase such as separation according to size, chirality, or polarity. The stationary phase is typically highly pure, spherical, microporous silica particles that are permeable to the solvent but can trap or slow particles of the mobile phase in order to allow particle separation[8]. Results of HPLC are quantified via computer programs, and a graphical representation of the data can be analyzed to determine components and the amount in which they were present. In this experiment, a reverse phase column will be used to separate the carbohydrate molecules by size and charge. Reverse phase refers to the use of a nonpolar stationary phase with a polar mobile phase. The molecules will elute according to relative polarity with the more polar molecules being eluted quicker.
Following purification by RP-HPLC, the isolated monosaccharides will be tested on the polarimeter for inversion of chirality. The polarimeter subjects a solution sample to a beam of plane polarized light. When this light passes through the sample, the chirality of the monosaccharide will refract the polarized light by a certain angle (i.e. optical rotation), either in the right (dextrorotatory) or the left (levorotatory) direction. This direction is not indicative of chirality, but this optical rotation is inverted for enantiomers[9]. If the *B. napus* grown in one particular type of polarized light caused some change to the chirality, the optical rotation would be a similar magnitude but in the opposite direction.

Objective: In nature, carbohydrates exist almost exclusively in the right-handed configuration. The reason for why carbohydrates tend to be in this right-handed configuration is unknown. However, polarized light may play a role in affecting the configuration of macromolecules such as carbohydrates. In this project, *B. napus* plants are grown entirely in either left or right handed polarized light. Then, the carbohydrates from the plant materials will be isolated and analyzed with a polarimeter to determine if the polarized light affected the carbohydrate configuration.

Methods and Materials

Constructing Polarized Environments
In order to prevent the introduction of confounding variables, all plants will be grown in identical conditions, with the only variation being the type of polarized light to which they are exposed. All plants will reside in a completely opaque environment that is in contact with continuous light only through the polarized windows, each of which will have the same dimensions. This will ensure that each plant in the varying conditions will be exposed to the same amount of light. The opaque environments will have a top window, which will allow light in through a polarized sheet. Polarizers used are American Polarizers Circular, both right and left-handed, and Linear; all three of which have equivalent transmittance values, ensuring the light that reaches the plants is of the same intensity.

Plant environments for this experiment were made with cardboard boxes of the same size, creating a cubic space of about 18”x18”x18”. Four identical environments were made for the four different types of light being test: linear, right-handed circular, and left-handed circular, and a control made with transparent plastic wrap. The interior of the boxes was sprayed with reflective spray paint in order to prompt further light absorption by the plants. The outside of the boxes was sprayed with black spray paint. A top window of each box was cut out about the size of an 8.5”x11” sheet of paper. From the inside

of the box, the polarizer was taped into this window opening, being sure no tape overlapped and would absorb any light that would come through the filter. On one side of each box a flap was cut into the box that would be large enough to dust the inside and remove excess moisture from the walls or filters if necessary. This would also serve as a gate to remove and replant later generations of the plant, and to water daily. Two small holes were made on bottom corners of one face of the box to allow air flow into the container. All boxes were then placed beneath a large nursery lamp that would give each the same amount of continuous light. Boxes were rotated every three days in a clockwise direction to ensure light distribution was equal.

B. napus Growing Conditions and Maintenance

Prepare four six-welled planting plates with Dr. Earth Pot of Gold Premium organic all-purpose potting soil. Fill potting soil to the top. Plant two kale seeds per well in the center of each well. Water 3mL per well everyday using a pipet through the side flap of the polarized boxed environments. After germination, prepare Miracle-Gro solution for indoor plants and water plant subjects once per week 3 mL per well of the growth solution. Everyday temperature, humidity, amount of water given, and observations should be recorded.

Germination is expected within the first 3-4 days, followed by a rapid increase in plant height toward the light source. These first leaflets are cotyledons, or seed leaves, and quickly become replaced with true leaves, which will resemble the typical short and bunchy kale form[10].

Homogenizing leaves and Isolating Carbohydrates

Once grown and harvested, *B. napus* stems, roots, and leaves were separated and frozen at -80 C overnight. Extraction of sugars from frozen samples began with grinding in 95% ethanol with mortar and pestle for 2-3 minutes. The resulting homogenate was vacuum filtered (LabTech VP18R Plus) through FisherBrand ashless filter paper and washed with 95% ethanol three times. The ethanolic extract adjusted to 50 mL/g of fresh weight (FW).[11]

High Performance Liquid Chromatography (HPLC)

Once the carbohydrates have been extracted from the homogenate and the polysaccharide sugars have been hydrolyzed and isolated, HPLC (Agilent Technologies 1120 Compact LC) is performed in order to separate and analyze the different sugar molecules. HPLC will be conducted at 20-25 degrees C. Insert the HPLC ZORBAX Eclipse Plus C18 Analytical 4.6x150mm 5-micron column (Agilent Technologies), which will contain a nonpolar stationary phase, into the HPLC. Create small sample solutions and known standard solutions by dissolving the plant isolate and known standards in 95% ethanol. Create a mobile phase of 75% acetonitrile and 25% water. If the elution is too quick, reduce the water in the mobile phase[12]. Run the ethanolic extract samples through the column with the mobile phase at a flow rate of 1.0 mL/min[13]. Use UV-Vis to conduct sample detection, setting a focus wavelength at 270 nm. Compare the results of the sugar samples with those of the known standards to determine identity of the plant isolate samples.

Polarimetry

After the carbohydrates are separated by HPLC, run the sugar samples in a polarimeter (Anton Paar MCP 200) in order to determine if any configuration changes have occurred. Add 95% ethanol to the sugar samples up to 2 mL and inject into the polarimeter. Record specific rotation.

Statistical Analysis

Perform a T-test and one-tailed ANOVA ($p<0.05$) after determining the alpha level of the experiment in order to verify if our results were due to chance or the dependent variable.

Results and Discussion

Three trials of *B. napus* were grown in the three specified polarized conditions (linear, left-handed circular, and right-handed circular) and one control (nonpolarized). After 4-5 weeks, the plants were harvested and separated into leaves, stems, and roots (Figure 1). Three representative leaves, stems, and roots were measured from each test group and the average was graphed (Figure 2). There was statistical difference between the left-handed and right-handed circularly polarized light samples.

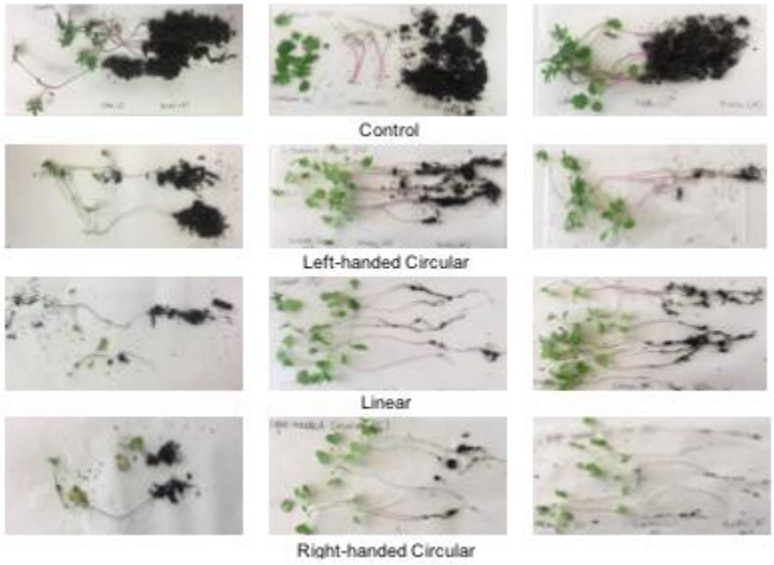

Figure 1. Trials 1-3 samples after harvesting. The leaves, stems, and roots were then separated and measured.

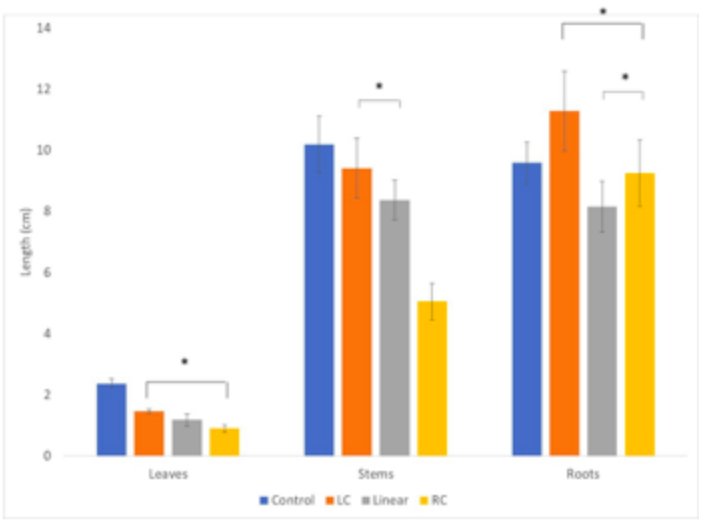

Figure 2. Comparison of the sample measurements among the variable conditions. A T-test was performed on the data and the asterix (*) represents $p<0.05$ to show statistical difference between the samples.

Conclusion

Overall, the *B. napus* exhibited a preference for the left-handed circularly polarized light. These findings are consistent with previous research performing a similar experiment with pea plants and their preference for different handedness of polarized light. Currently, there is no explanation for why dicots such as kale and pea plants have a preference for left-handed polarized light.

Quantitative analysis of the carbohydrate extracts is yet to be performed due to instrument malfunction. However, it is unlikely that any configuration change will be observed. If a configuration change occurred in the carbohydrates, the plant's stereospecific enzymes would be unable to process the L configured sugars. As all the plants grew, although to different amounts, there must not have been a configuration alteration. In order for the possibility of a plant produced L-carbohydrate, multiple generations must be grown entirely within the polarized environment for a mutation to potentially occur. Even if such a mutation were to occur and produce L-glucose via the Calvin Cycle, many more mutations must occur within the enzymes for the plant to use these L-monosaccharides in its own metabolic processes. Through the growth *B. napus* to various types of polarized light, it was found that the *B. napus* prefers the left-handed circularly polarized light of the three variables. However, using polarized light to induce plants to form left handed configured carbohydrates is highly unlikely.

References

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