Abstract

Productive societies are founded on the abundance of agricultural produce. However, not all communities have access to reliable sources of nutrients, such as rice. A global food crisis exists: 854 million people are malnourished and 25 thousand people die yearly from inadequate diets. Addressing this problem involves the improvement of crop yield by enhancing traditional agricultural methods, which are currently limited by inefficient energy outputs. Our solution entails the induction of efficacious C4 photosynthetic characteristics into C3 photosynthesis-based plants through the mass-application and advancement of gene-editing techniques. While multi-gene editing tools have not been developed to an extent which our solution can be implemented on a cost-effective and widespread level, the untapped capabilities of the highly-accurate CRIPSR/Cas9 gene-editing tool along with recent headway in DNA replication techniques posits a future in which supercharged photosynthesis is more than a possibility, bringing to fruition the goal to feed millions of malnourished people.

Improving Photosynthesis: Multi-gene Editing Techniques to Induce C4 Characteristics into C3 Plants

Present Technology

The prospect of improving the efficiency of photosynthesis is by no means a new one. The current, evolving technique of doing so is by inducing characteristics of plant cells which use C4 photosynthesis into plant cells which use the C3 photosynthetic pathway.[1] By inducing C4 characteristics into C3 plants, scientists hope to solve the problem of inefficiency presented by Rubisco (RuBP), a photosynthetic enzyme involved in the C3 photosynthetic pathway. [1] Rubisco governs the rate at which leaves absorb carbon dioxide, and has a great impact on a plant's energy output.[2] However, Rubisco is inefficient, and loses up to 25% of carbon taken in by leaves. In addition, at temperatures above 30°C, the use of Rubisco in the C3 process leads to a 40% decrease in photosynthetic efficiency.[3] Since the C3 process is used in plants such as rice, which makes up 19% of daily diets worldwide, the process must be made more efficient as quickly as possible.[3]

In order to minimize this inefficiency, scientists recently have begun to use several techniques to reengineer Rubisco's structure. One technique is protein design, a technique in which scientists use computer-based structural modeling to optimize the efficiency of Rubisco. [4]However, this technique is not fully developed - one reason for this setback is the lack of understanding of protein dynamics. Before researchers can make large enough advancements in protein modeling, they must have reliable knowledge of the way which proteins interact, knowledge that researchers still lack today.

Another conventional method to improving photosynthesis is through the modification of plant genes. One technique currently used to do so is nuclear transformation, in which nucleus-encoded components of the photosynthetic process are re-engineered to optimize the photosynthetic pathway. However, this procedure has several limitations which inhibit its development in the future. Firstly, transgene silencing often inhibits the gene expression of genes inserted into a plant cell.[4] In addition, today's technologies lack the ability to efficiently transform large DNA segments (more than 10 genes at a time).

In order to resolve the problems of transgene slicing and multi-segment editing, a new system must be implemented to allow for efficient editing and improvement of the photosynthesis process. The proposed technique of using the CRISPR/Cas9 editing technique solves these problems and others.

History

Scientists and researchers have been pursuing the improvement of photosynthesis for centuries. The study of the process of photosynthesis began, in fact, in the 1600s when Jan Baptiste van Helmont observed that after 5 years of growth, a tree had gained 165 pounds while the soil under it had only lost 2 oz.[5] While early philosophers believed that plants gained all their nutrients from the soil, van Helmont reasoned that the plant had to have gained its weight by other means.[5] Soon after this discovery, biologists' perception of photosynthesis further matured. Scientists today understand that photosynthesis is the combination of two connected series of biochemical reactions - light reactions and dark reactions. The light reactions use light energy from chlorophyll to create high-energy molecules which are used in the dark reactions to synthesize carbohydrates, a form of chemical energy stored by plants.[6]

Based on scientists' research, farmers began to breed plants in order to optimize photosynthesis based on the physical characteristics of plants. In 1985, researchers found that by encouraging phenotypes which were compatible with changes in soil nutrition, water, and other environmental factors, they could increase crop yields and feed more people globally.[7] However, scientists began to find that the genetic potential of plants seemed to be reaching a ceiling in which even plants bred to be more productive could not improve further. Soon, scientists turned to improving the physical structure of plant cells to improve their output. In 1988, Johann Deisenhofer, Robert Huber, and Hartmut Michel were awarded the Nobel Prize in Chemistry for their ground-breaking research which determined the structure of a photosynthetic reaction center.[7] This development led other scientists around the globe to search for ways to modify this structure in other plants in order to yield the most optimal output.

Scientists realized that by editing the genomic structure of plant cells, each step of the photosynthetic process could be improved by introducing favorable characteristics to these cells. The practice of gene editing began in 1973 when Herbert Boyer and Stanley Cohen created the first transgenic organism by inserting antibiotic resistance genes in the plasmids of an E.coli bacterium.[7] Soon after, in 1975, Frederick Sanger and his colleagues advanced the process of DNA synthesis by separating DNA nucleotides based on their location after they are subjected to an electrical current.[7] This technique, now known as the Sanger Coulson Technique, allowed for the automatic and efficient interpretation of DNA sequences.

Future Technology

Today, the improvement of photosynthesis is limited by researchers' inability to make multiple edits quickly and efficiently. In 15-20 years, however, the recently-developed

CRISPR/Cas9 system of editing (or similar techniques) could be applied to modifying the genes which result in the C3 photosynthetic pathway. In October of 2015, researchers at MIT used a novel bioinformatics-based approach to discover new proteins similar to Cas9, which allow for specific manipulation of genes.[8] These researchers developed a number of computational modeling systems which search genomic databases from NIH (National Institutes of Health).[9] As computational processing power and modeling systems improve, the researchers expect their algorithms to be able to search for similar proteins which allow for precise DNA modification. Even within a few months of searching, the researchers' algorithms helped them discover three new proteins similar to Cas9 - C2c1, C2c2, and C2c3.[9] If these results were found in just months, it is evident that 15 years from now, computational searching algorithms will improve to a point at which discovering similar mechanisms will be easy. As the field of precise DNA manipulation becomes saturated, researchers will be more able to apply techniques similar to Cas9 to editing plant cell DNA and improving the photosynthetic pathway.

Similar to their use in searching for proteins like Cas9, computers will be used in 15-20 years in order to predictively model the efficiency and effects of the CRISPR/Cas9 technique. As the field machine learning develops, it will be more and more involved in predictive modeling. In fact, researchers have begun to see modeling's potential to improve the output of CRISPR/Cas9. The company Azimuth is currently developing a machine learning-based predictive modelling software that will allow biologists to accurately identify the best guide RNA for CRISPR/Cas9.[10] As the technique improves, scientists will be more able to predict the effectiveness of the CRISPR/Cas9 technique and thus will be able to make very precise modifications to induce C4 characteristics into C3 plants.[10]

Even though basic C4 characteristics may be seen in a C3 plant, these plants still primarily rely on the C3 system. In order to convert their photosynthetic process completely, researchers must be able to re-engineer plant cells to make them produce cells in a specific arrangement.[11] In this arrangement, one set of cells captures carbon dioxide and the other concentrates the captured CO2.[11] At the moment, researchers lack knowledge of the genes which produce these sets of cells. However, years from now when photosynthetic research has developed further, scientists will be able to use both the tools of multi-gene editing and knowledge of the genes involved in producing C4-like characteristics.[11] Since dozens of genes are involved in the production of C4 structures, it will likely be several years before the scientific community is able to specifically point out which genes produce which component of the C4 plant cell structure.

Breakthroughs

Although genome editing has revolutionized in the past few years, through innovations such as TALEN and ZNF, a more accurate and efficient genome editing tool must be invented in order to edit the genomes of plants at a more expansive level. Thus the introduction of the CRISPR-CAS9 tool prompts a new era of gene editing, as it allows for a more precise and scalable method of replacing the targeted DNA sequence with the desired one. [12]

Gene Editing and Insertion

Originally designed to understand certain bacterial genes through the introduction of artificial virus vectors, the CRISPR-CAS9 system has transcended the barriers of merely affecting the genes of a few germ cell lines to completely altering the genetic sequence of living somatic cells.

The CRIPSR-CAS9 system was first implemented by utilizing a "guide" RNA to locate the targeted DNA sequence, which then would form the CAS9 nuclease, an enzyme designed to cut the targeted DNA strands.[12] This process would allow scientists to observe the effects of the targeted gene by comparing the effects of the mutated and therefore derelict gene on the organism.[12] However, these methods were largely irreproducible and prone to error, as the section in which the DNA strands were cut and repaired contained mutations that scientists could not control. A breakthrough is needed to enhance the CRISPR-CAS9 technique in such a way that when the targeted DNA is cut, a new strand of DNA would be introduced in the system as the template with which the cell can use to replace the previously cut DNA perfectly and without error, thus allowing the cell to duplicate with transgenic properties.[13] This improved process is very precise; the targeted DNA strands are replaced with exactly the desired DNA sequence, allowing scientists to not only understand the full functions of that gene, but also create new proteins expressed from the genes and by extension, new phenotypic characteristics for that organism that would carry through cellular reproduction.[13] However, it is a formidable challenge to enable the DNA polymerase to copy the non-native DNA with high fidelity. In essence, researchers need to modify the properties and behaviors of the DNA polymerase to fit the experiment they are conducting, which in this case would be gene editing. One way scientists can circumvent this problem is by introducing enzymes which are tailored to proofread specific mismatches of the DNA polymerase. By optimizing the enzymes of the DNA polymerase to increase the effective processivity, scientists can decrease the time needed to copy the DNA and can increase accuracy. Thus, genome editing would be far more accurate and could yield results with lower error rates. If breakthroughs can be made to improve the accuracy of the DNA polymerase which duplicates the induced genomes, this new gene-editing technology can be

readily applied to the genes responsible for the encoding of the enzyme Rubisco in plants, allowing the conversion of C3 photosynthetic properties to C4.

Multigene Editing

Whereas previous attempts at gene editing could only target the genetic sequence of one gene, the CRIPSR-CAS9 system is able to target multiple genes at once, prompting its applications to entire cell lines. However, to change the millions, if not billions, of cells involved in the mutation of a particular characteristic is still a challenge; a breakthrough in this area is needed in order for this technology to be applied directly to plant cell lines. The ability to apply CRISPR-CAS9 to entire plant cell lines overcomes the current struggle of scientists to get plants to completely switch over from their usual form of photosynthesis to the induced C4 photosynthesis.[14] Researchers need to engineer the plants to produce specialized cells in a precise arrangement: one set of cells to capture carbon dioxide surrounding another set of cells that concentrate it, which is the distinctive wreath anatomy found in the leaves of C4 plants. However, scientists still don't know all the genes involved in producing these cells and suspect that they could number in the dozens. Through the CRISPR-CAS9 system, more research can be done to precisely identify which genes affect the photosynthetic properties of the plants and then scientists can alter the genes to best fit the genetic templates of the plants performing C4 photosynthesis.

Design Process

Throughout our design process, we encountered various potential solutions to the global hunger problem through the improvement of photosynthesis. Three potential features we thought of but rejected are carbon uptake, breeding techniques, and protein design.

Carbon Uptake

One major factor in the efficiency of photosynthesis is the speed at which carbon dioxide is transferred from the site of intake in the leaves to the chloroplast where photosynthesis takes place. One method for improving this process could be to model the carbon dioxide channels and bicarbonate transporters of algae and various photosynthetic bacteria.[15] These structures are not present, however, in the chloroplast membranes of terrestrial plants. By creating similar structures in the cells of plants who undergo C3 photosynthesis, the speed of carbon dioxide transport could be increased.[16] This improvement would lead to less opening and closing of the stomata and thus less use of water. However, we choose not to develop on this idea because as opposed to our proposed idea of induced C4 photosynthesis, there is no concrete evidence of the benefits of doing so. The complexities involved in developing such structures in plant cells are too great to be overcome within the next 15-20 years. Gene editing, however, is developing rapidly and growing in popularity as more and more advanced techniques are created.

Use of Advanced Breeding Techniques

In order to achieve C4 photosynthesis in crops such as wheat and rice, the plants' genetic makeups need to change. As described earlier, altered crops need to be reengineered in order to produce specialized cells which follow the arrangement of cells involved in C4 photosynthesis. To achieve this change, researchers must analyze the plants' current DNA and make modifications in genes to produce the desired effect.[17] One way to achieve changes in genes is to breed plants together in a certain way to select the desired traits. However, this technique does not work when more than one or two gene changes are necessary. In the case of inducing C4 photosynthesis in C3 plants, dozens of changes must take place and traditional breeding methods

are not sufficient. For this reason, we chose gene editing rather than plant breeding. As compared to breeding, editing is more precise and allows for a larger number of changes.

Protein Design

There are many proteins involved in the process of photosynthesis which govern the processes of carbon dioxide absorption to ATP outputs. In order to improve photosynthesis as a whole, several of these proteins could be optimized or redesigned to further speed up the process.[18] However, we chose not to develop on this idea because of the current limitations of computational protein modeling. Although there could be a breakthrough in this field in the near future, the field still lacks a thorough understanding of the proteins involved in photosynthesis and lacks success in previous attempts to improve protein efficiency. Thus, we chose the technique of genome editing because of the probable future improvements in the technology.

Consequences

Positives: Crop Yield

The primary and most sought after advantage of induced C4 photosynthesis is its potential to revolutionize the global agriculture and food production industries, and make a dent in the problem of world hunger. As recorded in the past century, rice production in Asian countries accounts for 19% to 76% of total food energy (FAO).[19] Unfortunately, as the world's population grows, the demand for rice will continue to increase rather than stagnate. In fact, scientists state that by 2050, the global population will require a rice production rate of 1.3 billion tons per year. At its current rate, rice farming would only yield 915 million tons per year, creating a shortfall of 394 million tons of rice. In addition, MIT Technology Review states that scientists currently

estimate that over 33% of rice-producing regions have plateaued in production, failing to account for an increasing global population and increasing demand for rice.[19] This problem is not just global - it affects Americans too. As stated by the United Nations Food and Agriculture Organization, global rice yield growth has been decreasing and was lower in the past two decades than it was from 1961-1990. As the world population grows, this stagnation will affect others throughout the world, not just in America.

In order to avoid this shortfall, the means and output of rice production must be improved. Induced C4 photosynthesis is one solution for this problem. C4 photosynthesis, when compared with C3 photosynthesis, requires less water to produce the same output. According to MIT Technology Review, the implementation of C4 photosynthesis for rice production in China could feed 39 people per hectare as opposed the current rate of 26 people.[20] This 50% increase in number of people fed could be applied to rice exports and thus to others around the world, increasing the world's carrying capacity, and decreasing the world hunger problem.

Negatives: GMOs + Cost

Induced C4 photosynthesis entails further dependence on GMOs or unnatural food production. Although there is no concrete evidence of the harmful evidence of genetically modified organisms, many do not support an increased dependence on modified food. In addition, because of the procedures needed to modify crops, the cost of food production would increase, even if the output increases. This may be problematic to people in 3rd world countries where low-cost food is the only option in everyday life.

C4 plants also need more light than C3 plants to offset C4's reduced ATP efficiency. Adding C4 photosynthesis to plants such as rice and wheat means that in order to grow these

plants, more light is needed than traditionally would be needed. These plants tend to grow best in bright, dry, and warm areas and thus would suffer in other climates.

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