Utilizing RPE Rubisco to enhance carbon dioxide fixation in transgenic corn

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Excessive greenhouse gas emissions play a significant role in the global climate crisis. Carbon dioxide (CO_2) is the principal greenhouse gas emitted by human activities that contributes to the greenhouse effect, resulting in rising global temperatures. Lowering atmospheric CO_2 levels is critical to slowing the rate of global warming and preventing the catastrophic impact of climate change. A possible solution is to enhance plants' intrinsic ability to absorb CO_2 through photosynthesis. Several groups have introduced cyanobacterial CO_2-concentrating mechanisms (CCMs) into plant chloroplasts as a strategy to enhance crop photosynthesis and yield. However, the rate of photosynthetic carbon fixation in CCM-enhanced plants is still limited by the ribulose-1,5-bisphosphate carboxylase oxygenase (Rubisco) enzyme. Using recombinant DNA technology, we propose to create CCM-enhanced transgenic yellow dent corn (*Zea mays var. indentata*) that utilizes the RPE Rubisco protein, found in the endosymbiont of the deep-sea tubeworm *Riftia pachyptila*, which was determined to have a higher carboxylation efficiency and ready-to-deploy enzyme capacity that does not require chaperones. CCM-enhanced corn has the potential to concentrate CO_2, while RPE-Rubisco may increase corn's ability of CO_2 fixation. In effect, the CCM-RPE-Rubisco-enhanced corn can be tested for large-scale agricultural production as a product that contributes to decreasing atmospheric CO_2, alleviating global warming while producing food for general consumption.

Keywords RPE-Rubisco, carbon dioxide, Agrobacterium, transgenic plant, climate change

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Background

The greenhouse effect (Figure 1) describes the process by which solar infrared energy passes through the atmosphere and reaches the planet, where it warms the surface before being reradiated into the atmosphere. Greenhouse gases, like carbon dioxide, water vapor, nitrous oxide, and methane, form an atmospheric layer that acts as an insulator, reducing the amount of heat that escapes back into space and thus keeping the planet warm. This is especially essential at night so the Earth can maintain a safe and stable temperature suitable for life (Denchak, 2019).

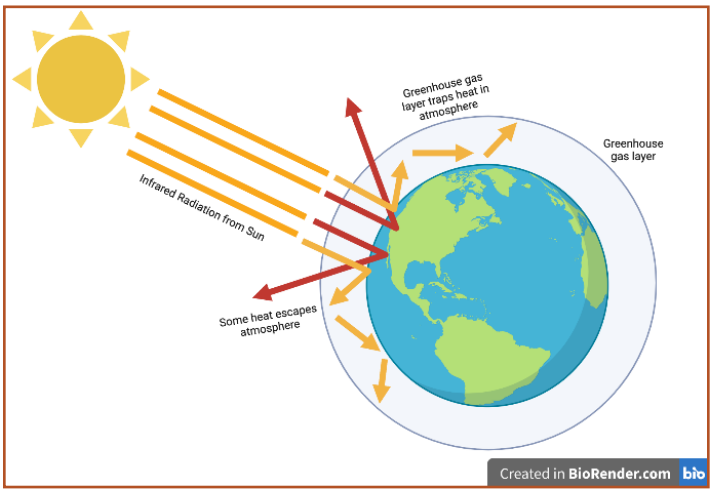

Figure 1: Greenhouse effect. *Ultraviolet radiation from the sun loses energy as it enters the Earth's atmosphere, releasing infrared radiation (heat). The earth's surface absorbs part of the radiation and reflects the rest. The layer of greenhouse gases stops most of the reflected heat from returning to space, thus insulating the planet and maintaining a stable temperature.*

Human activity has increased the greenhouse gases released into the atmosphere, thus disrupting the carefully created "balance" that keeps the planet from growing too hot or too cold to support life. In addition to CO_2, gases with higher Global Warming Potential (GWP) than CO_2 have also been added to the atmosphere (table 1). 1 ton of CO_2 is the base unit for GWP, meaning it has a GWP of 1. Nitrous oxide and methane, though found in smaller quantities, have GWPs of 265 and 28, respectively, and therefore are also threats due to their increased capacity to trap heat in the atmosphere (IPCC, 2014). Despite its lower GWP, the high atmospheric concentration (412.5 ppm) of CO_2 distinguishes it as a greater threat, as its atmospheric concentration creeps past 400 ppm in recent years. Other greenhouse gases, such as water vapor and fluorinated gases, are also emitted from varieties of manufacturing and industrial processes. As more and more countries industrialize, carbon dioxide (CO_2), emitted through the burning of fossil fuels, has become one of the biggest contributors to the greenhouse effect. Higher levels of atmospheric CO_2 not only result in a higher global average temperature but also causes several significant changes in the planet's climate. The changing weather patterns alter and devastate ecosystems worldwide. Warming trends force species to migrate to higher latitudes or higher elevations where temperatures are more conducive to their survival. Similarly, as sea levels rise, saltwater intrusions force key species to relocate, adapt, or die. The rapid rate of change makes adaptation nearly impossible, thus removing critical organisms from the food chain (McKie, 2013). In addition, flooding and droughts threaten the safety of both human settlements and natural habitats. The melting of glaciers results in an unreliable water supply to human settlements that rely on spring thaw (Denchak, 2019).

Table 1:*Relative atmospheric abundance and GWP of greenhouse gasses*

Gas	Atmospheric abundance	GWP
Carbon dioxide	400 ppm	1
Methane	1800 ppb	28
Nitrous oxide	325 ppb	265

Furthermore, CO_2 reacts with water molecules (H_2O) to form carbonic acid, and the increased acidity makes aquatic environments uninhabitable to many species (Lindsey, 2021). CO_2 levels are higher than they have ever been in at least 800,000 years, at approximately 412.5 parts per million (ppm) (Lindsey, 2021). Even if the global temperature rises one degree (1ºC), there could be catastrophic effects. Thus, it is vital to reduce current atmospheric quantities of CO_2.

Currently, climate change is countered in a variety of ways. On the consumer level, making educated choices, such as using fluorescent light bulbs rather than regular light bulbs, takes small steps towards sustainability. On a larger scale, an industrial push towards utilizing renewable energy reduces fossil fuel combustion and CO_2 emissions. These methods, though capable of making change, are currently ineffective because the acts of consumers alone are unable to counteract the significant carbon footprint of companies, and there has been significant pushback in the US to proposals to switch to renewable energy.

On the other hand, a viable solution is capitalizing on plants' ability to consume CO_2. Plants already regulate the amount of CO_2 in the atmosphere through photosynthesis, the process by which plants and other organisms convert light energy, CO_2, and water into chemical energy for growth and reproduction. With a total of 7,000 billion tons of CO_2 in the atmosphere, plant photosynthesis decreases this amount by more than 100 billion tons annually (Baslam et al., 2020). Different elements of the photosynthetic cycle are potential targets for human alteration to improve photosynthetic traits, increase CO_2 consumption, and benefit agriculture.

Plants and algae absorb CO_2 and produce the oxygen that we use to breathe through their process of photosynthesis (figure 2) (Baslam et al., 2020). Photosynthesis begins in the thylakoids, flattened

sacs inside the chloroplast. In the thylakoids, the chlorophyll absorbs sunlight and transfers electrons to the photosystems, releasing ATP and NADPH (chemical energy), and splitting H_2O to release O_2 gas. ATP and NADPH then travel into the stroma (aqueous fluid within the chloroplast), where the Calvin cycle reactions take place. In the Calvin Cycle, CO_2 joins to RuBP (ribulose-1,5-bisphosphate, a 5-carbon molecule) with the help of an enzyme called Rubisco, converting atmospheric carbon into a usable carbon source, creating two molecules of 3-phosphoglyceric acid (3-PGA). The energy from NADPH and ATP then converts these 3-PGA molecules into a three-carbon sugar, G3P. Some of these G3P molecules go on to make glucose, and the rest go on to "regenerate" RuBP for the next cycle (Gunther, n.d.). This process can be made more efficient through the alternation of Rubisco and thus increase CO_2 consumption.

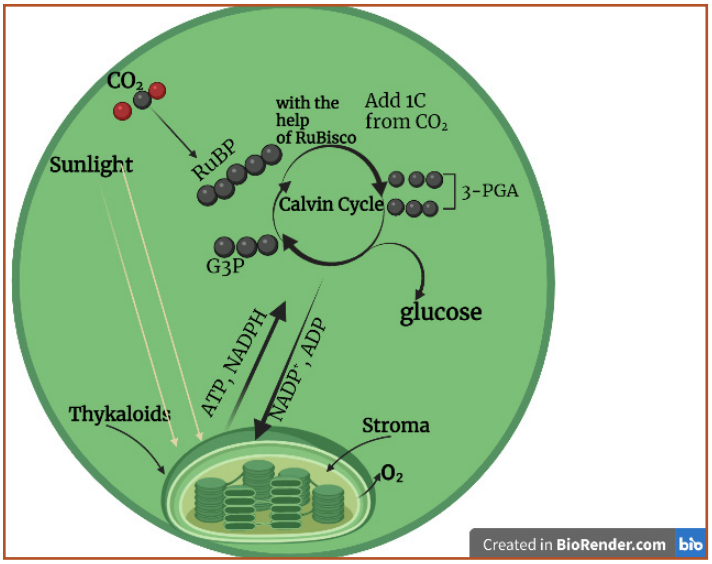

Figure 2: Photosynthesis at the macro level. *In photosynthesis, light reactions first occur in the thylakoids of the plant cell. Sunlight, through light reactions, releases oxygen gas and produces ATP and NADPH. After exiting the thylakoid membrane, ATP and NADPH enter the stroma (inner space of the chloroplasts) and join with carbon dioxide gas from the environment. These molecules then go through the Calvin cycle, producing sugars in the form of glucose.*

As previously described, the Rubisco enzyme is a key part of photosynthesis. Rubisco is an enzyme found in most photoautotrophs allowing them to fix carbon dioxide in the Calvin-Benson-Bassham (CBB) cycle. However, Form I Rubisco enzymes from a variety of plants, algae, and cyanobacteria all have low rates of carboxylation, with significantly lower efficiency than most enzymes (Zhang et al., 2021). Additionally, high levels of atmospheric O_2 in the environments of many photoautotrophs facilitate the photorespiration process, which consumes energy and releases CO_2 (Cai et al., 2014). Therefore, plants alone are incapable of reducing atmospheric CO_2 levels at a rate high enough to counter climate change. Thus, countering deforestation alone is not enough to offset climate change. Though plants store high quantities of carbon, it is not reasonable to plant enough trees to completely offset atmospheric CO_2. Thus, further action is needed.

Previous attempts to improve the efficiency of Form I Rubisco have had limited success. Instead, a higher carbon-fixation efficiency can be found in the Form II Rubisco enzyme from bacteria that live in endosymbiosis with the deep-sea tubeworm *Riftia pachyptila*, known as RPE Rubisco. The higher carboxylation efficiency of this enzyme allows the bacteria to provide *R. pachyptila*, which lives near CO_2-rich hydrothermal vents, with enough energy to support its extremely fast growth rate (Zhang et al., 2021). Thus, if common plants such as corn employ RPE Rubisco, the rate of carbon fixation by plants can be greatly improved.

We propose to engineer transgenic corn plants that incorporate the Rubisco gene from the tube worm through agrobacterium-mediated transformation, the most common method of engineering plant genomes. The transgenic corn plants will have increased capacity for CO_2 consumption—approximately 3 times more in vivo and 19 times more in vitro (Zhang et al., 2021). Fields of this RPE Rubisco-enhanced crop can be planted to replace current corn crops. Additionally, they can be planted around factories, cities, or other large CO_2 producers to offset emissions. The food grown can be fed to humans or livestock and provides significant agricultural benefits. If successful in corn, similar enhancements could be applied to plants more suited for growth in urban settings (parks, gardens, etc.) or perhaps even house plants for filtering CO_2. Overall, we hope to engineer an efficient crop to offset CO_2 emissions with limited need for resources and resistance to drought.

Systems level

In normal plants, the Rubisco enzyme is a limiting factor in CO_2 consumption. While most enzymes can work on thousands of molecules per second, an individual Form I Rubisco enzyme processes only three CO_2 molecules per second (Goodsell, 2000). If RPE Rubisco was used instead, we could increase the molecules of CO_2 processed per second, thus increasing the overall amount of CO_2 adsorbed. To increase the efficiency of fixation, we propose to genetically engineer yellow dent corn by inserting the RPE Rubisco gene via Agrobacterium-mediated transformation (figure 3).

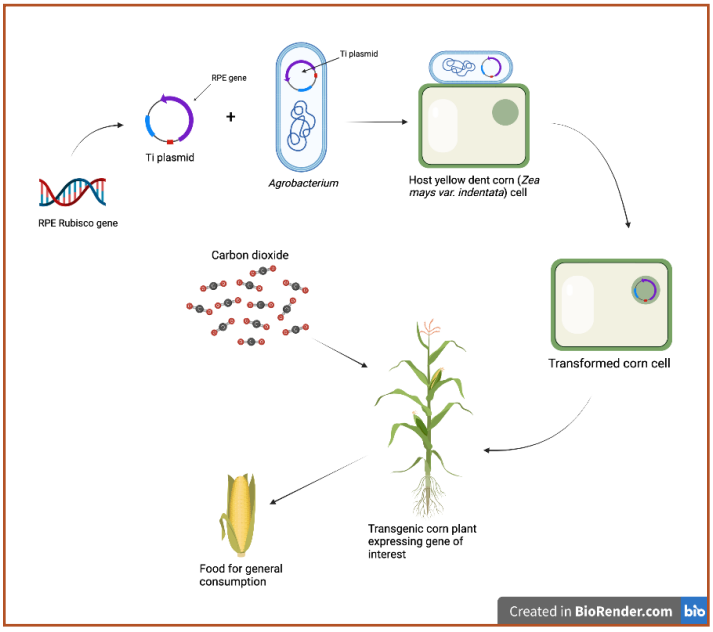

Figure 3: Overall system. *A Ti plasmid containing the RPE Rubisco gene will be expressed in CCMs-enhanced corn cells via Agrobacterium-mediated transformation, leading to corn plants with enhanced CO_2 consumption that also create consumable products.*

RPE Rubisco has been found to be vastly more efficient than other forms of Rubisco. It is 19 times more efficient in vitro and 3.6 times more efficient in vivo than 7002 Rubisco, another high-functioning expression of the enzyme (Zhang et al., 2021). Applying this increase in efficiency to real-world farms, we reason that our genetically modified corn would increase CO_2 consumption by around 3.6 times. We will need to perform experiments with expressing the enzyme in corn to assure a 3.6 times increase in efficiency, as it may not perform as well outside of its native context.

Further, it is important to note that implementing this gene may not translate directly to 3.6 times consumption, as farming and transportation equipment currently used in corn agriculture also emit CO_2. Nevertheless, these agricultural processes are already used, meaning that this modified corn would be more efficient in carbon consumption than currently grown yellow dent corn.

One such community to which our corn could be applied is Michigan, a state which emits 162 million metric tons of CO_2 each year (*Rankings: Total Carbon Dioxide Emissions,* n.d.). According to the Michigan State University, a typical Michigan corn field absorbs 16.33 metric tons of CO_2 per 4 thousand square meters each year (Thelen, 2007). Michigan corn fields typically grow field corn (*Zea mays*), of which yellow dent corn is a derivative (*Field Corn*, n.d.). However, if they made the switch to our genetically modified corn, and CO_2 capturing increased to the predicted 3.6 times, each field would capture 58.79 metric tons of CO_2 per 4 thousand square meters each year.

Applying this on a larger scale, the United States grows an average of 350 billion square meters of corn each year (United States Department of Agriculture, 2022). This translates to 15 billion bushels nationwide each year. On average, one acre of corn in the US absorbs 7.26 metric tons of CO_2 yearly (Bennett & Cornett, 2018). Thus, as it currently stands, the corn annually grown in the U.S. captures 620 million metric tons of CO_2. At the same time, it takes 371 grams per square kilometer of CO_2 to grow corn in the US due to fossil fuels in farming equipment, transport, and even soil fertilizers (Stecker, 2013). Thus, the nation's corn currently has a net CO_2 capture rate of 492 million metric tons per year.

Instead, if our modified corn is used, which is 3.6 times more efficient, the amount of CO_2 captured would increase to 2.2 billion metric tons a year. When again factoring in the amount of CO_2 produced in the farming process, the nation's corn would have a net CO_2 capture rate of 2 thousand metric tons per year. Since the nation emits around 36 billion tons of CO_2 each year, we would need around 16 times the current land used for corn to make the nation completely carbon neutral, around 5.5 billion square kilometers (IEA, 2022). As the nation already devotes 3.6 billion square kilometers to crops but once used 3.8 billion square kilometers in 2000, this is not unimaginable but may require partnering with other nations to utilize more fertile land area (Shahbandeh, 2022).

RPE Rubisco has the potential to significantly increase the ability of plants to sequester CO_2. However, while Rubisco catalyzes the CO_2 fixation involved in the plant's growth, it also catalyzes an energy wasteful photorespiration process that consumes oxygen. Therefore, for our design to work, our corn must be enhanced by CO_2-concentrating mechanisms (CCMs) to ensure that carboxylation is favored over oxygenation. During oxygenic photosynthesis, light energy transfers water taken up by plant roots to carbon dioxide to produce carbohydrates and oxygen. Several groups have successfully demonstrated the introduction of cyanobacterial CCMs into the chloroplasts of rice and tobacco (Iñiguez et al., 2021). There is precedent for porting microbial enzymes and machinery focused on carbon fixation into other organisms.

Cyanobacterial CCMs enable relatively rapid CO_2 fixation by elevating intracellular inorganic carbon as bicarbonate, then concentrating it as CO_2 around the Rubisco enzyme in specialized protein micro-compartments called carboxysomes. A simplified

carboxysome from *Cyanobium* has already been created within the tobacco chloroplasts. The endogenous Rubisco large subunit gene was replaced with cyanobacterial Form-1A Rubisco large and small subunit genes, along with genes for two key α-carboxysome structural proteins. This minimal gene set produces carboxysomes, which encapsulates the introduced Rubisco and enables autotrophic growth at elevated CO_2 consumption. Photosynthetic and yield improvements have already been observed when manipulating Rubisco's degree of activation along with advanced CCMs in crops. Further engineering Rubisco carboxylation capacity continues to be a promising target for improving photosynthesis and yield (Long et al., 2018).

Device level

We chose yellow dent corn as our chassis because it is the most common variety of corn grown in the United States. This corn is widely used in agriculture and can be used for human and animal consumption (*Yellow Dent Corn*, 2015). Thus, it would be easiest to implement on a large scale, as farmers already understand how to grow it and the public is comfortable eating it. On a genetic level, yellow dent corn is the most commonly genetically modified corn (Schaufler et al., 2018). Thus, there is precedent and abundant information on how to best edit it.

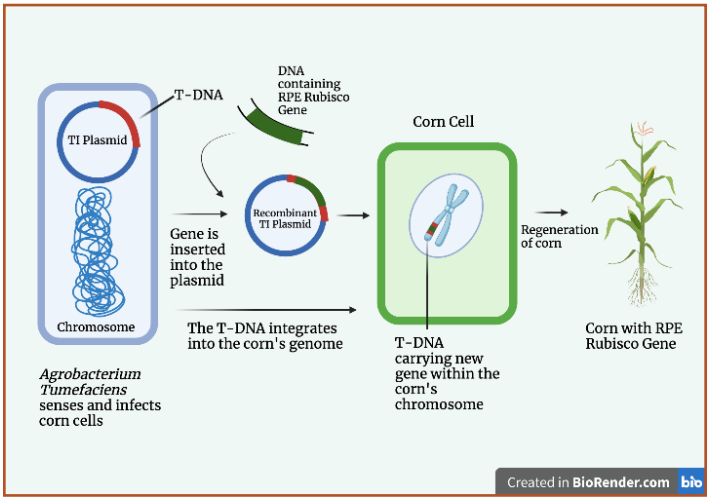

Figure 4: Agrobacterium-mediated transformation of corn cells. *Agrobacterium Tumefaciens (containing a chromosome and TI Plasmid with T-DNA) senses and infects corn cells. DNA containing the RPE Rubisco gene is inserted into the plasmid and the recombinant T-DNA integrates into the corn's genome. In the corn cell, we have T-DNA carrying the new RPE Rubisco gene within the corn's chromosome. With the regeneration of corn, we have corn with the new RPE Rubisco gene.*

We specifically chose *Agrobacterium tumefaciens* because this bacterium is an important tool in plant biotechnology due to its natural ability to transfer DNA into the genomes of host plants. Genetic manipulations of *A. tumefaciens* have yielded considerable advances in increasing transformational efficiency in several plant species and cultivars. Modulating the expression of various mediators of *A. tumefaciens* virulence can lead to more successful plant transformation; thus, applying synthetic biology to enable targeted engineering of the bacterium may enable new opportunities for advancing plant biotechnology.

We plan to apply *Agrobacterium*-mediated genetic transformation using binary vectors (figure 4). This is a widely used method for generating transgenic plants. This system utilizes the ability of the bacteria *Agrobacterium tumefaciens* to insert foreign DNA into the corn genome. The Agrobacterium binary vector system is derived from natural tumor-inducing (Ti) plasmids. Agrobacterium transfers a region of the Ti-plasmid known as the transfer DNA (T-DNA) into corn, where it is integrated into the host genome. The T-DNA is delineated by flanking 25 bp T-DNA border repeat sequences in direct orientation with one another. Typically, in the binary vector system, all tumor-associated intervening T-DNA sequences are removed, leaving the T-DNA border repeats, which flank and direct host integration of the user's sequence of interest (Hwang et al., 2017).

The complete binary vector system consists of two parts. The first is the T-DNA binary vector that contains two T-DNA repeats bracketing the DNA sequence, which will be inserted into the corn host. The RPE Rubisco is cloned into this portion of the binary vector. The second plasmid, referred to as the *vir* helper plasmid, encodes components necessary for integration of the region flanked by the T-DNA repeats into the genome of plant cells. Prior to transforming the target plant cells, these two plasmids are brought together in *A. tumefaciens* by co-transformation, co-electroporation, or conjugation. When the binary vector and the *vir* helper plasmid are both present in the same Agrobacterium cell, proteins encoded by the *vir* helper plasmid act in trans on the T-DNA border repeat elements to mediate processing, secretion, and host genome integration of the sequence between the left and right border repeat elements (Kroemer, n.d.).

To make sure that we infect the plant with the desired soil bacterium, we propose to use an *A. tumefaciens* strain, AGL-1, as it allows for Agrobacterium-mediated genetic transformation of maize. We will insert a T-DNA binary vector that has a kanamycin gene, allowing for the Agrobacterium to be resistant to kanamycin. This antibiotic can be used as a selective marker to kill or prevent the growth of bacteria that do not have resistance. Knowing that our strain will have this resistance, we can use the antibiotic to eliminate the

unwanted *A. tumefaciens* strains, leaving us with our preferred strain, AGL-1 (Gold Biotechnology, n.d.).

Parts level

We created a T-DNA binary vector by modifying an already known *Agrobacterium* vector to enhance the expression of carbon dioxide fixation (figure 5). Corn are monocots, possessing one cotyledon: an embryonic leaf. ZmUbi is known to be a strong promoter and drives high-level gene expression in monocots, which increases the rate of transcription (Zou, 2014). By modifying this *Agrobacterium*, more of the RPE Rubisco gene will be expressed. We will likewise add Kana, a selectable marker that allows plants to be resistant to kanamycin (table 2). This ensures that the plant's bacteria are not killed or inhibited from growing.

Additionally, we will utilize EGFP. EGFP is a stabilizer that provides green fluorescence, brightness, pH resistance, and environmental protection. Using DNA recombinant technology, we can combine the EGFP gene into the RPE Rubisco gene and insert it into the yellow dent plant cells (Zou, 2014). If the cell produces green fluorescence, we know that the plant cell will express the target gene. This will be used primarily in proof of principle experiments and may be removed from the final product in order to increase market acceptability.

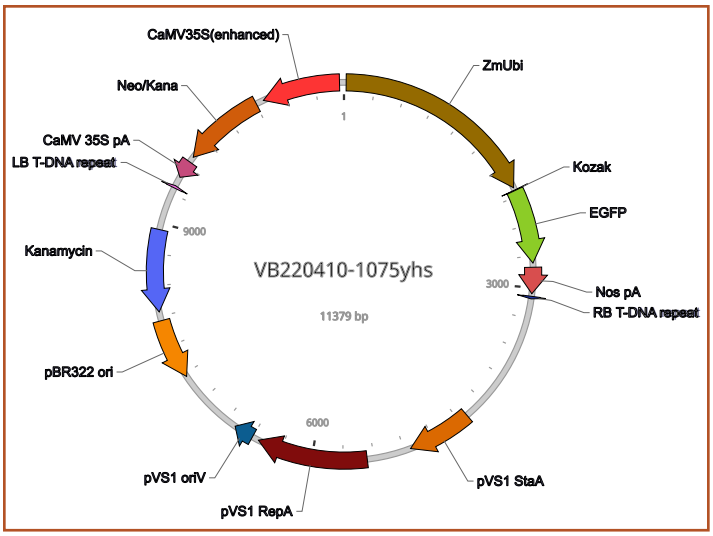

Figure 5: Vector. *Modified T-DNA binary vector containing ZmUbi, EGFP, and Neo/Kana*

Safety

Although we are not at the stage where we are directly handling RPE Rubisco, when we begin working with RPE Rubisco and the corn cells, we will follow lab safety guidelines and wear gloves, goggles, and all necessary PPE (personal protective equipment) to protect ourselves and minimize contamination to our experiment. The specific bacteria we are using is *A. tumefaciens* which has Plant Biosafety Level 1 (BSL-1P). These microbes present minimal potential hazard to laboratorians and the environment. Organisms with BSL-1P are also not known to consistently cause disease in healthy adults.

The transgenic corn does not pose any biosafety hazards to the general public because we are only changing the speed at which the corn utilizes carbon dioxide to create glucose—the primary makeup of the corn itself is unchanged. Before growing our corn in an outdoor environment and feeding it to livestock, we will first grow it in a lab under controlled conditions and monitor it to see if it will release any unexpected chemicals into the air or soil. Furthermore, we can test the safety of the corn crop by feeding it to a controlled population of lab mice or cows, allowing us to monitor the effect that ingesting the corn has. This allows us to check for any adverse effects of eating the corn before feeding it to a large population of cows.

Another important safety issue is the potential for our crop to spread as an invasive species. To combat this, we propose to grow our crop in isolated fields. Further, since we chose corn as a host, our crop needs to be replanted yearly. Thus, any stray crops would die, allowing local ecosystems to maintain balance. If there is a fear of insects spreading the crop in a particular ecosystem, we could opt to grow the corn in greenhouses. At the same time, the process of planting and maintaining the crop could create more jobs to benefit surrounding communities.

Discussion

By inserting the RPE Rubisco present in the symbiote of deep sea tube worms into plants, we will be able to increase the carbon dioxide intake of plants by several times, thereby removing more CO_2 from the atmosphere and more efficiently converting it into usable energy for the plant. Corn would be a good vessel for the RPE Rubisco because of how commonly it is grown in the United States, maximizing the carbon dioxide intake. Yellow dent corn is the main type of corn that goes to feed our livestock; especially when nearly half of our corn grown in the United States goes to feed animals, it would be extremely beneficial to the environment to take CO_2 from the atmosphere and allow us to potentially yield more crop while using less land (United States Department of Agriculture, 2015). One of the main causes of deforestation is the need for more farmland. As the

Table 2: *T-DNA Binary Vector Components*

Name	Position	Size (bp)	Type	Description	Application Notes
ZmUbi	22-2014	1993	Promoter	Maize ubiquitin promoter	Strong constitutive promoter, drives high-level gene expression in monocots.
Kozak	2039-2044	6	Miscellaneous	Kozak translation initiation sequence	Facilitates translation initiation of ATG start codon downstream of the Kozak sequence.
EGFP	2045-2764	720	ORF	Enhanced green fluorescent protein; codon optimized based on a variant of wild type GFP from the jellyfish Aequorea victoria	Commonly used green fluorescent protein; ranked high in brightness, photostability and pH stability among all fluorescent proteins.
Nos pA	2805-3058	254	PolyA_signal	Nopaline synthase polyadenylation signal	Allows transcription termination and polyadenylation of mRNA transcribed by RNA polymerase II.
RB T-DNA repeat	3084-3108	25	Miscellaneous	Right border repeat of T-DNA	Upon recognized by Ti plasmid in Agrobacterium, it transfers the region between T-DNA border repeats to plant cells.
pVS1 StaA	4409-5038	630	ORF	Stability protein from pVS1 plasmid	Essential for plasmid segregational stability in Agrobacterium.
pVS1 RepA	5467-6540	1074	ORF	Replication protein from pVS1 plasmid	Permits replication of low-copy plasmid in Agrobacterium.
pVS1 oriV	6606-6800	195	Replication_ origin	Origin of replication from pVS1 plasmid	Permits replication of low-copy plasmid in Agrobacterium.
pBR322 ori	Complement (7470-8058)	589	Replication_ origin	pBR322 origin of replication	Facilitates plasmid replication in E. coli; regulates low-copy plasmid number when Rop protein is present (15-20) and medium-copy plasmid number when Rop is absent (100-300).
Kanamycin	Complement (8145-8939)	795	ORF	Aminoglycoside 3'-phosphotransferase gene.	Allows E. coli and Agrobacterium to be resistant to kanamycin.
LB T-DNA repeat	9364-9388	25	Miscellaneous	Left border repeat of T-DNA	Upon recognition by Ti plasmid in Agrobacterium, it transfers the region between T-DNA border repeats to plant cells.
CaMV 35S pA	Complement (9479-9653)	175	PolyA_signal	Cauliflower mosaic virus 35S polyadenylation signal	Allows transcription termination and polyadenylation of mRNA transcribed by RNA polymerase II.
Kana	Complement (9710-10507)	798	ORF	Neomycin phosphotransferase II gene	Allows plants to be resistant to G418 or Kanamycin.
CaMV35S (enhanced)	Complement (10583-11336)	754	Promoter	A chimeric promoter with the doubled CaMV 35S enhancer element	Strong promoter.

human population continues to increase, we will need more food to sufficiently feed everyone, and if we can successfully utilize RPE Rubisco in corn plants, it can help address that issue.

Rubisco is the most abundant enzyme on the planet, and by altering such an important enzyme, we risk impacting other aspects of the plant (Bar-On & Milo, 2019). RPE Rubisco in a corn plant should make the plant more efficient at taking in CO_2 and, therefore, more efficient at creating glucose for itself. RPE Rubisco was found to increase glycolate production 14-fold (Yang et al., 2021). However, at this stage of development, it is difficult to tell if it would create a more nutritious or different type of corn or if it will just cause the corn plant to grow quicker. In theory, this practice could be copied on a global scale to decrease CO_2 levels and combat world hunger. Other possible uses could be space exploration or to greatly counteract CO_2 emissions in urban center green roof farms.

Another potential uncertainty is taking into account cellular respiration in plants in general. During plant respiration, rather than taking in CO_2 from the atmosphere, the plant takes in O_2 and releases CO_2 into the atmosphere (Gillespie, 2018). We are certain that RPE Rubisco would accelerate the rate at which CO_2 is converted into glucose, but it is difficult to determine if the plant would release more CO_2 at night due to the RPE Rubisco.

Additionally, the EGFP stabilizer that we use to provide green fluorescence is optional and can be used for proof of principle experiments. When the high capacity of the modified corn to remove CO_2 is proven, the fluorescence gene will be removed, so the corn will maintain its quality, marketability, and safety.

We predict the public would be concerned about the safety of transgenic corn. Genetically modified organisms (GMOs) are safe for human and animal consumption. The FDA states that genetic material found in GMOs does not transfer to consumers, and there is no difference between organic and GMO foods on consumer health (U.S. Food and Drug Administration [FDA], 2022). GMOs have no evidence of harmful side effects(Norris, 2015). Already, *Bacillus thuringiensis* (Bt) corn, which is engineered to decrease pests, is prominent in the US to feed both humans and livestock. 92% of corn planted in the United States in 2018 was genetically modified. Further, over 95% of meat and dairy animals eat GMO crops (FDA, 2022).

Nevertheless, Pew Research data reveals that 51% of the American public believes GMOs are worse for human health than organic foods. Further, 88% of the public believes GMOs result in health problems (Funk, 2020). Their data additionally suggested that approval for GMOs amongst the public was trending downwards, with more and more growing wary of adverse effects. To combat this, we hope to educate the public about the benefits, specifically by relating the need for our crop to the growing issue of climate change. Further studies on our specific crop's safety would help to assure the public that it is fit for consumption. If our efforts to sway the public about the safety of our crop fail, our crop can be used as feed specifically for livestock. This would fill another purpose while increasing public support.

Next steps

To continue, we will conduct an experiment as proof of principle. In a container, we will insert RPE Rubisco into yellow dent corn and leave a second container unmodified as a control group. In the same stable conditions, we expose these two groups to sunlight. Each day, we will monitor CO_2 consumption in parts per million by using a CO_2 meter to test and track CO_2 levels in each container. Moving forward, we will monitor CO_2 consumption, growth quality, and nutritional value first in a contained greenhouse and later in a contained field.

Another future step we hope to take is to decrease the amount of water required to grow our crop. This would make the corn cheaper and easier to grow and thus increase the likelihood that farmers would switch to our product. This can be achieved through further genetic motivation or through selective breeding. There are several genes that can be introduced to corn to improve water efficiency and increase drought resistance (McFadden et al., 2019). One example is the GmDREB1 gene. Transgenic wheat carrying this gene from soybean showed drought tolerance under a ubiquitin promoter (Shiqing et al., 2005). Another example is the HaHB4 gene. Transgenic wheat transformed with a mutated transcription factor (HaHB4) had increased water use efficiency and yield (Fernanda et al., 2019). These are two potential examples we may target to further improve the corn with enhanced CO_2 consumption. If we choose to only grow seeds from plants that require less water, over time, we will produce a plant that requires very little water to carry out necessary life processes while having no impact to yield.

Furthermore, making corn more nutritious will allow people to stay healthier while still enjoying food. Corn is used in many things, including food products, beverages, alcohol, and fuel. Products made with corn rather than food with processed flour can increase or maintain gut

health and help lower the chances of getting diseases such as heart disease and type two diabetes. Eating carbohydrates in corn, such as fiber, also lead to staying full for longer periods and between meals. Recently, scientists have found that inserting a bacterial gene causing corn to produce methionine, a key amino acid that corn lacks, can enhance the nutritional value of corn without affecting plant growth. Methionine is needed for growth and tissue repair, improving the tone and flexibility of skin and hair (Rutgers University, 2017). Implementing this with our other experiments and future steps could create more nutritious CO_2-consuming transgenic corn requiring less water without affecting the plant's growth.

Author contributions

All authors worked on general research, writing the abstract, and general editing. A.C., A.S., M.V., M.Z., and S.R. worked on background information. A.C. created the main diagram and formatted citations. A.C. and S.R. to detail how the Ti plasmid containing the RPE Rubisco gene will be expressed. S.R. and A.S. wrote about lab, community, and environmental safety and future development. S.R. and M.Z. wrote about the next steps for the project. Y.K. and G.L. worked on describing Agrobacterium and the specifics of the T-DNA binary vector. M.Z. and M.V. created figures and captions.

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