Impact Report

An Analysis of a Novel Approach to Crop Climate-Resilience

Rice University iGEM

Human Practices

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Project Summary

Introduction

The overwhelming evidence pointing to the detrimental effects of climate change has garnered public and private support for research into the resulting impacts on various industries. Ventures into agricultural adaptations have become increasingly prevalent since the success of this industry directly correlates to physical and socioeconomic survival.

To comprehend how climate change affects agriculture, we need to look at the affected physical and biological processes. At the most basic level, climate change can be quantified by temperature and precipitation. In studies conducted on climate change, conclusions were drawn that the majority of impacts on agriculture are driven by trends in temperature rather than precipitation [1]. Using current climate data, models of future yield indicated that changes in temperature will be more important than changes in precipitation on the national and regional scales in response to climate change. [citation]

Temperature is critical because it plays a significant role in the regulation of vegetative metabolic processes that influence the production of biomass. According to Teixeira *et al*., most cropping areas will be exposed to record average air temperatures by 2080 [2]. Higher temperatures mean less frequent photosynthesis, reduced light interception due to faster phenological development, and increased incidence of drought.

While these long-term, upward trends in heat have been extensively studied, not enough research has considered high stress, short occurrence heat waves that have become more common with global warming [3]. These areas of crop heat stress overlap with major agricultural regions, including eastern China, northern United States, southwestern Russian Federation, and southern Canada. In fact, in 2010, Russian production areas experienced unexpected extreme temperatures that affected over 20% of the crops. This in turn led to wheat prices increasing by up to 50% in the international market. Without adaptive initiatives, there will be more extreme crop losses, specifically for those agricultural lands that exist at high latitudes, which will undoubtedly hurt the global economy. Therefore, while the importance of prioritizing agricultural adaptations is generally concerned with the survival of a nation's population, to truly understand the gravity of the situation we face, it is paramount to adopt a global perspective. We must incorporate the international market into our calculations because food security is an international venture; there is no advantage in treating each country in isolation. Therefore, it is critical that we utilize the same intensity of focus on both food-insecure and food-secure regions. Thus far, technological measures have not sufficed in reducing the disparity between developing and developed countries; this will prove to have negative implications on our future if not addressed soon.

Project Proposition

Synthetic biology is a major influencer in developing solutions to many global problems, including agricultural issues. Our team decided to make use of these innovative avenues to propose a novel solution

to confer climate resilience to crops. In particular, we are engineering the rhizosphere using the bacteria *Pseudomonas putida* to localize and form a biofilm around the roots of crops, sense ambient temperature changes beyond the optimal growth range, and counter appropriately with a heat stress response..

More specifically, we aim to design a synthetic circuit in *P. putida* that will confer greater stress resistance to *Arabidopsis thaliana*, a plant commonly used as a model organism that is also closely related to various species of edible crop plants. Because *P. putida* is naturally found in symbiotic relationships with many plants (including *A. thaliana*), it was an excellent choice for native metabolite exchanges. In terms of the mechanism, the enzymes used to mediate climate resilience only need to be expressed once the plant experience heat-induced stress. To achieve this, our circuit will be temperature-regulated by an RNA thermometer. Since *A. thaliana* grows best between 18 and 23°C, we optimized the thermometer such that at 30°C the circuit is functional.

Problem

Contextual Statistics

As mentioned previously, changes in global temperature and precipitation offer prime assessments of the degree of climate change. As temperatures rise, the capacity of the air to hold moisture increases, which makes rainfall thereafter more drastic and threatening. If there is insufficient water and nutrients and the temperature is outside of a crop's optimal range, then any historical yield increases can be reduced or reversed. Climate change has also brought rising CO_2 levels that actually reduce nutritional value by lowering the concentration of proteins and essential minerals in plants like wheat, rice, and soybeans. These sorts of issues will become much more commonplace as trends continue into the future. [4]

It's also important to note that even though agriculture represents a small portion of U.S. GDP, the U.S. is still the global leader in agricultural production and commodity exports. Looking at maize trends in particular, a temperature increase of 4 °C decreases U.S. production of maize by 50% [5]. This same temperature change makes it significantly more likely for the top 4 maize-producing nations - the U.S. Brazil, Argentina, and Ukraine - to suffer a simultaneous crop failure of 10% to 86%. However, not just maize would suffer the repercussions of climate change. The U.S. produces 41% of the world's corn and 38% of its soybeans, which are two of the four major sources of caloric energy [6]. One study combining the previous approaches of agronomic studies, simulation models, hedonic models, and panel of yields found that above 29 °C for corn, 30 °C for soybeans, and 32 °C for cotton any further temperature increase would dramatically decrease crop yields [7]. This trend was found consistent across factors such as time, location, crops, and sources of temperature and precipitation variation. Moreover, they predicted that holding current growing regions fixed, area-weighted average yields decrease 30-46% under the slowest warming conditions and 63-82% under the fastest warming conditions. This has major implications on the future of food security in both production and price.

Clearly, a focus on sustainable yield output is necessary to combat the negative effects of climate change on the agricultural industry. Because this market is globally interdependent and has far-reaching effects

that are not quarantined to drastic economic downfall, research into affordable methods of producing more crops under the changing growing conditions must be prioritized.

Future Implications

But what exactly does the future hold? How specifically will populations be affected by climate change. At the most rudimentary level, the effects of climate change can be summarized as a decrease in food production and a dramatic spike in prices. In 2050, a world population of 9.1 billion will require raising food production by 70% on average (from 2005/2007), although developing nations will need to double their production [8]. However, whereas 1.8 acres per capita are available for crop and pasture lands today, only 0.6 acres for crops and 1.1 acres for pasture lands will be available in 2050; at least 1.2 acres per capita is necessary to maintain current American dietary standards [9]. Food production must somehow increase on a decreased set of resources. Economically, this directly translates to a significant increase in prices. For every 1% increase in the demand of food, the price at the farm gate increases by 4.5%. With reference to current population growth and food consumption trends, the U.S. will cease to export food by 2025 as the domestic need will be of higher priority. Consequently, the U.S. will lose $40 billion annually, increasing the trade deficit. Internationally, this puts the lives of millions of people around the world at risk since the U.S. is the world's largest food exporter.

Thus, we need a solution to mitigate the effects of a smaller rural labor force, climate change, and unsustainable farming methods. We have to pour resources into the bioenergy and biotechnology industries to maximize efficiency and production. However, production increases alone will be insufficient to ensure food security; we need a holistic approach with poverty reduction strategies, safety nets, and a rural development program.

One of the first steps, though, is an attempt to increase production, especially since we are currently experiencing yield plateauing in rice and wheat in some of the most intensively farmed regions of the world. Farms need to adopt manufacturing identities and introduce technological changes in hardware, software, and liveware that extend beyond the field [10]. Such improvements in genetic editing, simulated natural mutations, and other biotechnologies will boost profits by cutting costs and increasing yields, which will ultimately benefit consumers in the form of lower prices.

As the problem of food security coupled with climate change becomes more prevalent, the biotechnology industry will become integral to the physical and economic survival of the world. We aim to play a part in the solution.

Project Goals

Synthetic Biology

Since agriculture is particularly affected by climate change, we want to design a product that makes plants more resistant to extreme temperatures. Genetically modified plants are an avenue that many researchers and farmers have already turned to in order to increase crop resilience, but engineering rhizobacteria can be a more modular avenue to conserve the wide array of crop species and cultivars that are needed to maintain biodiversity and culinary variety. Given the extensive characterization of plant-microbial interactions, engineered bacteria provide a promising route to achieving crop climate resilience. Altering th/e rhizobacteria, rather than the plant itself, will hopefully also avoid some of the negative connotations associated with genetically modified organisms and increase the accessibility of our product.

There have always been manual attempts to mitigate the effects of drought and other stressors on plants, such as irrigation and raised beds. However, they tend to be costly and not readily adaptable to changes in weather patterns. Another approach many researchers have used is transplanting bacteria evolved for extreme climates into the rhizosphere of another crop [11]. While this method has seen positive results on the growth of the plant, a synthetic biology-focused approach provides a cheaper, convenient, and optimizable solution [12].

Our project aims to perfect the thermosensor capabilities of RNA thermometers within bacteria. Many RNA thermometers that have been well characterized are optimized for 37°C or higher [13]. However, this temperature is well above the typical growth conditions plants experience. For example, rice grows best between 24° and 30°C and wheat grows best between 19°C and 25°C, indicating a greater need for thermosensors that can alter gene expression at lower, specific temperatures [14]

We aim to design a synthetic circuit in *P. putida* that will confer greater stress resistance to *A. thaliana*, a model plant species. We chose *P. putida* because it is naturally found in many rhizospheres and has a symbiotic relationship with plants, including *A. thaliana* [15]; this means metabolite exchange is native between *A. thaliana* roots and *P. putida*. The enzymes used to mediate climate resilience only need to be expressed once the plant begins to feel heat-induced stress. To achieve this, our circuit will be temperature induced by an RNA thermometer. Since *A. thaliana* grows best between 18 and 23 °C, we decided to optimize our thermometer for 30°C. At around 30°C, our thermometer will begin to melt, which will allow for it to be transcribed and induce expression of the downstream genes. We are using a fluorescent protein as a means of detecting and quantifying expression.

The genes we are expressing have all been previously shown to help plants cope with environmental stresses. IAA works to expand lateral root growth, ACC deaminase reduces ethylene, a compound shown to hinder plant growth [16], and trehalose synthase helps mitigate the effects of drought in plants [17].

By using the plant model organism *A. thaliana*, we will be able to see how flowering plants like rice, maize, wheat, and soybean (which comprise 2/3 of human caloric intake globally [18]), will grow with our temperature induced system of plant growth promoting enzymes.

Application

Our project is part of a larger conversation about plant survival amidst climate change. We hope that our temperature-dependent system will aid in the development of a commercially-available product that farmers in warming regions, which includes much of the world, can treat their crops with to mitigate the effects of drought and heat on crop yield. Ideally, the result of our research would be a fertilizer-like product that can be applied once but act for several growing seasons, thus reducing the need for constant upkeep. This would be especially important in areas with scarce resources.

Modeling

Existing RNA thermometers are generally suited for 37°C and do not undergo conformational changes between 25°C and 30°C. However, as most plants die by this upper operating temperature, we found it necessary to design new thermometers to experience conformational change between 25°C and 30°C. Using a genetic algorithm to address the challenge of designing thermometers due to the complexity of RNA folding, we discovered our optimal candidates.

Essentially, RNA thermometers regulate translation based on temperature such that there is a higher probability of base pairs forming a stem loop structure at lower temperatures. At higher temperatures, the thermometers "melt," which means there is less base-pairing. We created the stem-loop structure by creating a variable region, which is the altered sequence of the complement of the ribosome binding site (RBS). These mutations ensured that the RBS would be locked into the thermometer's structure so that it would be inaccessible to the ribosome at lower temperatures; they also allowed us to optimize the targeted melting temperature.

We pulled from several sources to ultimately design a program unique in its speed and automated nature, which can create and test libraries of RNA thermometers in a custom temperature range without extensive technical expertise. This was done via percentage base pair optimization and secondary structure change optimization algorithms. We utilized a Python 3 library called Distributed Evolutionary Algorithms in Python (DEAP) to access components to build the genetic algorithm, Scalable Concurrent Operations in Python (SCOOP) to evaluate potential candidates, and NuPack to evaluate base pairing and secondary structure.

Education and Outreach

Synthetic biology is a growing field that can open up a world of possibilities. However, like any new technology, there are unknowns associated with it and it can be a source of concern. Talking to local farmers, we found that many had misconceptions about what a GMO entails causing them to have concerns with our project from the get go. Even though most seemed supportive in our project and

interested in the problem we were trying to tackle, in the end the negative connotation of GMOs made them hesitant to accept our project. While we did try to talk to stakeholders in person, the argument to be open to GMOs is a long conversation which is in part why we decided to write this report. We are also fortunate to be able to talk to students at various education levels and various backgrounds in biology. Our presentations ranged from middle schoolers to visiting undergraduates and the goal was to provide insight about synthetic biology and use our project as an example of its possibilities. As a team, we've seen through the years that it is hard to fully delve into iGEM without an understanding of synthetic biology. Our college course was intended to act as a better transition for students coming into iGEM or students simply interested in learning more about synthetic biology.

Economic Impacts

The following TOWS Matrix highlights various factors and strategies associated with ultimately increasing the positive economic impact of our project. On the left margin, internal factors of strengths and weaknesses are noted; on the top margin, external opportunities and threats are identified. Within the matrix, we have identified strategies to increase our positive economic impact. The SO Maxi-Maxi Strategy identifies strategies that build on strengths to maximize opportunities; the ST Maxi-Mini Strategy pinpoints strategies that tailor strengths to minimize threats. The WO Mini-Maxi strategy characterizes strategies that minimize weaknesses by utilizing opportunities; the WT Mini-Mini Strategy names strategies that avoid both weaknesses and threats.

While the following matrix is not overtly economic, each tactic will manifest itself in an economically favorable fashion, specifically through increased collaboration with agricultural workers and research industries to more effectively design and apply our technological solutions. With better application, we can increase crop production and support a global economy in the face of climate change. While we recognize our competition with other biotechnology ventures specific to agriculture and also understand that, as a whole, the bioengineering market suffers at the hand of the ongoing GMO debate, we have hope that prioritizing education of our genetically modified rhizobacteria and *not* the actual crop will encourage support and dispel misconceptions from conscious consumers and research groups. In the end, our project can only serve to benefit the local, state, national, and international economic markets by sustaining and improving agricultural production on a mechanistic level with the specific rhizobacteria circuits and on an employment level by maintaining the job security of our agricultural workers and opening the job market to bioengineers and food safety/security fields.

TOWS Matrix		
	External Opportunities (O) - Companies like Pivot Bio and Ginkgo Bioworks - Farmers expressed interest in applying crop yield solutions in response to heat stressors	External Threats (T) - GMO/non-GMO debate and effects of a more conscious consumer - Possibility of *P.putida* being outcompeted in the wild - Other biotechnologies available for crop yield, performance, etc.
Internal Strengths (S) - Root interactions rather than genetic modification of the plant - RNA thermometers closer to optimal growing temperatures of crops - Plant-growth promoting rhizobacteria is cross-compatible between different plant models - Cheaper, noninvasive, convenient, and optimizable solutions	SO: Maxi-Maxi Strategy - Reduce the need for synthetic and environmentally unsound practices to confer climate resilience by working with large bioengineering companies to deliver our method in a refined and cost-effective manner - Make novel RNA thermometers commercially available to positively impact the biotechnology industry - Expand collaboration with local and regional farmers to identify needs, test out proprietary solutions, and refine technologies for mass production on a national and international scale	ST: Maxi-Mini Strategy - Provide education, infographics, and media coverage on the idea of modifying rhizobacteria for a symbiotic relationship with an unmodified plant; could eventually weaken the GMO market and strengthen the non-GMO and research industries to increase agricultural production and sustain the economy - Increase research into other bacteria that have positive interactions with plants to strengthen the market; use a similar framework to this project - Competing with other biotechnological solutions will likely weaken our market; however, increased research and application of the project on smaller local and regional scales can help build a successful reputation

Internal Weaknesses (W)	WO: Mini-Maxi Strategy	WT: Mini-Mini Strategy
- *P. putida* itself is not generalizable to all crops - Biocontainment issues with proposed fertilizer-like vector	- Utilize the expertise of Pivot Bio and Ginkgo Bioworks that have worked with various organisms to further enhance the productivity of *P. putida* and other viable rhizobacteria - Collaborate with a biocontainment agency to identify weak points in our design and application; reduce the cost impact of environmental mishaps	- Use other root bacteria like *Azospirillum brasilense* (positive root interactions with ~ 35 botanical families) and *Bacillus amyloliquefaciens* (anti-fungal properties to reduce competition); increasing the breadth of research will allow for greater production of a wide variety of crops essential to the global food market - Biosecurity training ideas: create a time-sensitive kill switch to reduce the effects of water runoff and the costs of addressing biocontainment issues

Environmental Impacts

Biotechnology for crop climate resilience is not a new venture. One comprehensive report detailed several companies that are already involved in the task of "improv[ing] agricultural precision and risk mitigation" [19]:

> Sistema Biobolsa: This company aims to bring sustainable agricultural practices to farmers worldwide and produces innovative biodigester technology to address issues of waste management, fertilizer overuse, increased costs of fossil fuels, and negative health effects of using wood fuel. The hybrid reactor-biodigester they manufacture converts manure of animals into biogas energy resources and a natural fertilizer.
>
> DryGro: This agricultural technology company focuses on growing crops on already unproductive and arid land. An added benefit is that the feed ingredients developed uses much less water than traditional agriculture.
>
> CARE Honduras: This project combines efforts of CARE International and Mexichem Honduras to create a simple rainwater harvesting method using a geomembrane bag; this is great for storage

and distribution and is even compatible with micro-irrigation efforts. This geomembrane bag is especially innovative as it protects water from contamination and is quite cost effective.

DAI Maker Lab Hardware: This initiative utilizes the vision and needs of end users to create innovative hardware solutions for places with little to no resources. Such efforts include a water pressure telemetry for small utilities under the USAID-funded IUWASH Plus project.

ECOM Agroindustrial: This sustainable supply chain company has developed Sustainable Management Services (SMS) that offer processes for farmers to adopt climate technologies to simultaneously increase production and conserve the environment. Termed precision agriculture, SMS involves techniques for farmers to input the right amounts of each resource into their crops at specific times to maximize yields.

CIMMYT: Christian Thierfelder is working on multi-level intercropping systems through which the assortment of crops, tillage, and no-burn policies allow for gains in soil organic matter, increased yield, etc. Essentially, this allows for less inorganic fertilizer and more food production.

Greenseeker Handheld Technology: To cope with ineffective nitrogen fertilizer practices, CIMMYT developed the GreenSeeker technology, which basically utilizes Normalized Difference Vegetation Index (NDVI) sensors to determine exactly what amount of nitrogen would be appropriate in the second application of the fertilization cycle (when farmers typically overuse). This technology helped avoid more than 14,000 tons of CO_2 emissions while also increasing profits for farmers.

These technologies barely scratch the surface of the innovations that have become available over the years to address issues of climate change and food security. But where does our project fit in? In many of the above technologies, the goal is to adapt farming practices to cope with issues of climate change. However, our project goes directly to the plant itself to modify its response to temperature stressors that generally mark climate change. What use are the above technologies if the crop itself dies at certain temperatures that are slowly becoming the norm as global temperatures rise? Our project challenges these issues by exploiting the natural heat-shock response and symbiotic relationships with rhizobacteria that various crops possess to release particular enzymes and encourage survival.

Our main concern, though, is biosecurity in regards to the containment of our genetically modified bacteria. After attending a biosecurity workshop, we realized that our bacteria risked contaminating runoff and other water supplies after significant precipitation. To mitigate this vulnerability, we combined our genetic circuit with the Tol plasmid to create an effective, geographically-based kill switch. Including these constructs with our own plasmids will address the biosecurity concerns raised about how we can stop our bacteria from propagating into unintended environments.

Project Limitations

Challenges in Application

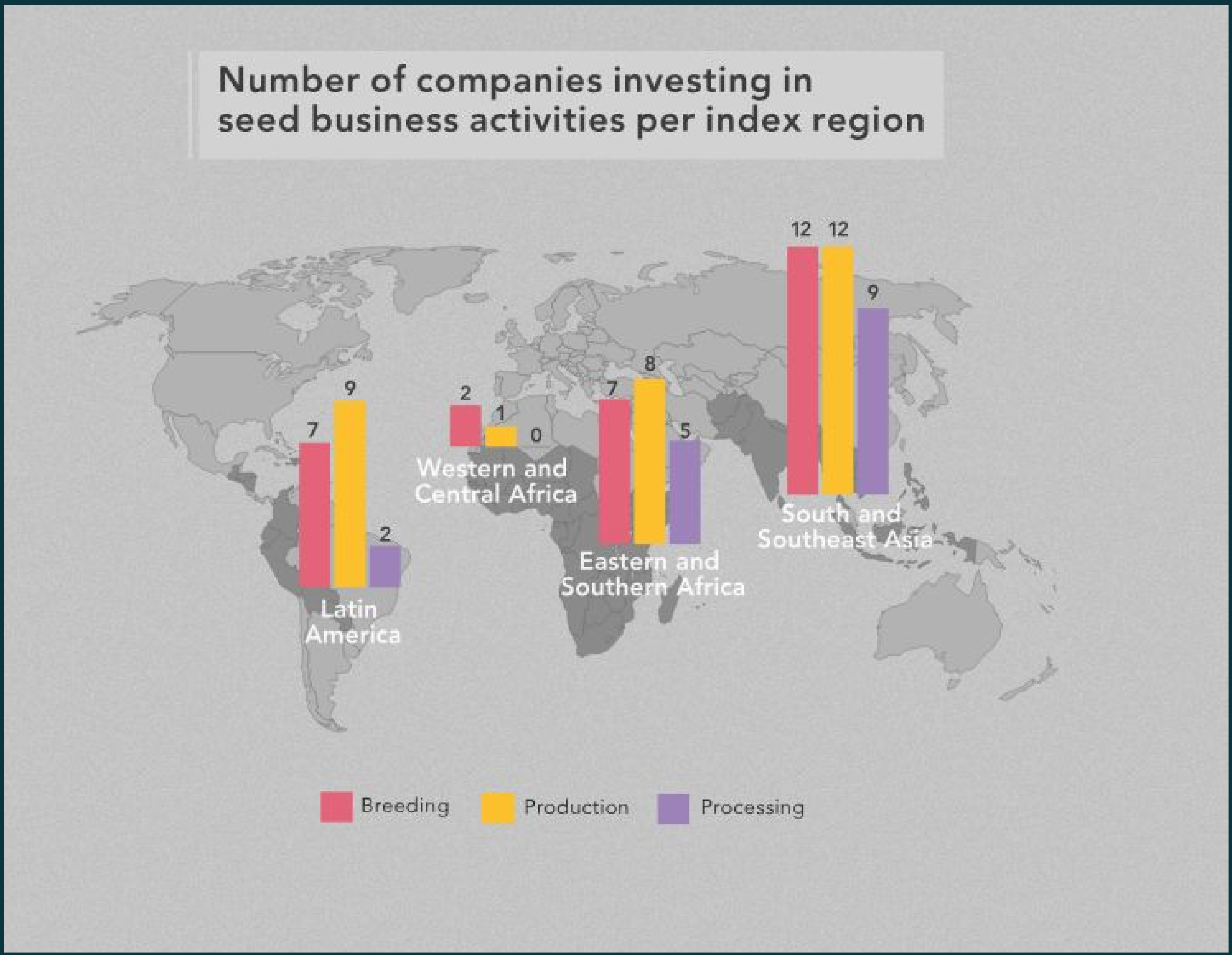
Our solution is comprised of a genetically modified root bacteria so we anticipate challenges in application on multiple fronts ranging from safety implications to farmers opinions to overall feasibility of our project. During our initial project brainstorming, we took into consideration the general fear surrounding the intersection of genetically modified organisms and food. While we know that safely and carefully genetically engineering the plant itself could also have positive effects, the rhizosphere was a more distant environment that people were comfortable with. The main concern, after talking to farmers, seemed to be the concept of genetically engineered material somehow being in the food we eat. Our engineered rhizobacteria can safely interact with the roots without the above ground plant being "disturbed". A safety concern that was brought up was what would have to vegetables and crops that we do eat the root for such as carrots and potatoes. The scenario that the farmers were concerned about, like one Cellar Farms farmer, was whether "the bacteria [will] somehow go inside the vegetable because it associates with it." However, as Espinosa-Urgel, Kolter, and Ramos clearly show, the establishment of the *P. putida* is around the corn roots [20]. Rhizobacteria are still hard to separate from plant roots, not because they are physically inside of the roots but rather their sticky interactions allow for a stronghold. However a strong wash with water should loosen their connection and farmers need not worry about the bacteria somehow making its way onto our dinner plate. However this did bring up a different concern with our project -- water that has the potential of removing our bacteria.

As a way to ensure that we were considering the safety and security implications, we attended a biosecurity training hosted by Rice graduate students. We were able to address safety concerns associated with our project in a systematic way and we realized that containment was the biggest concern with our project. Talking to farmers and considering our project in a biosecurity aspect, we came to the same conclusion that our bacteria would have to be applied carefully and in a limited area. We considered various methods of trying to control our bacteria populations and decided that a kill switch was the best option. The danger of bacteria replicating without control is a scenario that is imperative to avoid. We understand that containment issues would complicate the dispersal of our product. Farmers will have to be careful in application as we do not want our product to become an aid to weeds or other unwanted crops.

Once we are past these hurdles, our project seems to have great potential as a significantly non invasive method to help crops become more climate resilient. The farmers and experts we talked to gave positive feedback on our project inspiration because our end result is a big goal but an important one. While our product may at times be a hassle, it is a new option for farmers in a world of increasing temperatures and our non invasiveness has a nice appeal.

Effect on Industry

Our project is essentially trying to find a way to make crops more climate resilient, and overall we are tackling this in a pretty innovative method. There are some startups also looking into utilizing rhizobacteria but overall this is a new avenue so there is still a lot to be developed here and there is a lot of open area to experiment with. But trying to get crops to be more climate resilient is still a very large problem and there are currently other methods in place. The main way that farmers make their crops more climate resilient at the moment is through having climate resilient seeds. Some examples of products that are used include products by Pioneer: they offer seeds that are pest resistant, seeds that can maximize growth with limited land, and even seeds that can grow with minimal water (https://www.pioneer.com/us/products/corn/traits-technologies.html). While these are great advancements and help farmers, they are still far from a perfect solution. First of all, these seeds are often times significantly more expensive, making them unattainable for small farmers. Also these seeds are limited in the types of crops that they apply to as it does not apply to lentils. Additionally, the technological progress of these seeds is very skewed as seen by the figure below [21].



Our solution provides an alternative that account for many of the current shortcomings. As we are dealing with a root bacteria, it can associate with more crops -- broadening its scope from the get go. While cost is not an aspect that we can be competitive in, our solution is a much more of a one size fits all solution so that lessens the cost of having to get different types of seeds that are drought resistant. However, we do admit that our product may have the similar accessibility issues because of the cost aspect. While technically bacteria could regenerate itself and our initial supply of bacteria could become a permanent fix, we had to ensure that our bacteria had some sort of a control to stop that from happening. Our bacteria would have to be reapplied so as to ensure that the bacteria are only used from crops and cannot go towards other weeds and should also limit its possibility of escaping from the fields. So the repeated application will build up cost but should not heavily burden existing labor as it is something that can easily be incorporated into the normal soil tilling procedures or be applied alongside fertilizer or other soil substituents. And most importantly our solution does not make the plant itself a GMO, making our genetically engineered rhizobacteria and attractive alternative. The non-GMO factor can help to attract a larger audience and also lessen worries that the seeds have been manipulated too extremely. As we learned from talking to local farmers, the appeal of having organic crops can soothe personal questions and also is oftentimes a cost loss they are willing to take on. Considering all the advantages brought on by our project, we think that it could potentially have a positive and feasible impact in industry.

Patenting and Licensing

Our project has essentially two parts, the RNA thermometer aspect and the enzyme production aspect. RNA thermometers act as thermosensors, and we have designed RNA thermometers that will be temperature sensitive at lower temperatures that naturally found thermometers. Most well characterized thermometers that currently exist function around 37°C, which is around human body temperature. 37°C is also the optimal temperature for E.coli, so it makes sense that the well characterized thermometers coincide with a dependable model organism. Overall, the nature of RNA thermometers does tend to steer it towards higher temperatures because RNA structure depends on temperature. At higher temperatures, the RNA thermometer starts to unfold and no longer blocks translation. However, 37°C is still quite a high temperature, and by 37°C plants would have already died. So we decided to create our thermometers that were better suited for lower temperatures. As this is where the novelty of our project comes into play, we also consulted a local patent lawyer Dr. Melissa Schwaller. Dr. Schwaller was kind enough to meet with us in person to further talk about our project. The software that we are using to create our RNA thermometers has modifications made by our team, but the code is based off of the already existing NuPack. However, we are using their software to create novel RNA thermometers. After further discussion with Dr. Schwalle, we came to the conclusion that our RNA sequences were indeed novel and of our own creation. If we were to try to follow an entrepreneur path, our RNA sequences would be the way to go because they are innovative advances. However, the topic of science innovation brought about the question of are our RNA sequences more appropriate to be patented as novel sequences or do they belong in publishing and scientific papers? Surprisingly the two options -- patenting or publishing -- are not automatically mutually exclusive, though the cost of the process can quickly make it mutually exclusive. Hypothetically if time and money were not constraints, the path of attempting both patenting and publishing would be the best for something like our RNA thermometers. Novel thermometers that can unfold at lower temperatures would be scientifically significant as it would expand our understanding of

RNA thermometers. However, as our own use of RNA thermometers as a thermosensor that has an integral role in increasing crop yields shows, RNA thermometers can also be applicable in biological products. So, our RNA thermometers technically can be patented and licensed.

As for the second part of our project, the enzymes that we are producing are naturally produced. We are simply upregulating the pathway so that the plant can better utilize its resources before the onset of hotter weather which would kill the plant. Consequently, this aspect of our project is not as much patentable as our novel RNA thermometers.

GMOs

One of the most common issues in developing technologies to assist in the food security crisis is the creation and use of genetically-modified organisms (GMOs). The dynamic between support of GMOs and their counterpart, non-GMOs, is interesting, but its relation to meeting global needs is quite important to comprehend. Can these two concepts live in harmony with one another? How can they work together to meet needs? And what further research still needs to be done to satisfy patrons of all perspectives?

Since its introduction in the mid-1990s, the market of genetically modified (GM) crops has increased significantly, adding 213 million tonnes and 405 million tons to the global production of soybeans and maize, respectively. The economic gains are just as plentiful - the net benefit was $18.2 billion in 2016 and $186.1 billion since 1996; 65% derived from increasing yield and production and 35% from cost savings [22]. Use is not relegated to developed countries, though. In fact, 67 of the 195 countries of the world have adopted biotech crops: 5 industrial nations grow GM crops, 43 countries import biotech crops, and 19 developing nations account for 53% of the global GM acreage [23]. Farmers are adopting such technologies because of the economic benefit and industries are supporting these ventures because of the nutritional value, crop yield, and diversity of market. The future offers much in the realm of GM crops. Paul S. Teng, ISAAA board chair, had this to say about the value of such biotechnology:

> "Biotech crops offer enormous benefits to the environment, health of humans and animals, and contributions to the improvement of socioeconomic conditions of farmers and the public. The recent production of next generation biotech crops — including apples and potatoes that are not likely to spoil or become damaged, anthocyanin-enriched super sweet pineapple, increased ear biomass and high amylose content maize, and soybeans with modified oil content, combined with the commercialization approval for an insect resistant sugarcane — provides more diverse offerings to consumers and food producers." [24].

There are downsides, though, but these negatives must be viewed within the context of the GMO and non-GMO market as a whole. GM crops are characterized by their two main commercial purposes: tolerance to glyphosate and glufosinate herbicides, as well as resistance to various insect pesticides. Graham Brookes and Peter Barfoot developed an aggregate analysis into the environmental impacts of GM crops globally [25]. They bring to the forefront some of the arguments that proponents of the anti-GM movement have voiced and present data that addresses such concerns. For instance, the aforementioned tolerance to herbicides has indeed brought on the problem of herbicide-resistant weeds

that has led to increases in the amount of active ingredient used in herbicides, which has deteriorated the environmental impact quotient (EIQ) of GM crops. However, what people fail to mention is that even conventional crop methods have seen an increase in the amount of active ingredient used in herbicide over the years. In fact, as a whole, GM cops still show an improvement when compared to the conventional alternative. Furthermore, they note that the herbicides used conventionally in the mid-1990s had significant resistance, which provided reason for GM technology to be made resistant to glyphosate as weed control.

ISAAA has provided an infographic that perfect summarizes the contributions that GM crops claim in food security and positive environmental impact [26]:

All in all, GM technology remains an area largely debated for its use, effectiveness, and safety, but what cannot be denied is how it has mitigated food security concerns by increasing crop production and exponentially contributing to the global economy. Clearly, more research will need to be done into the genetic modification of these crops and their effects on the ecosystem and human systems. Even within our project, we need to estimate consumer and farmer responses to using genetically modified bacteria to confer desired characteristics to crops. Are the crops we modify using these bacteria actually categorized as GMOs? It seems like this would not be the case as the crops themselves are not being modified; their responses are being influenced by bacteria surrounding them that are genetically modified to operate in various conditions. This is a gray area that would understably create cause for concern. Nevertheless, the opportunities are endless for this market and offer critical possibilities to solving the global food security problem; thus, this field warrants additional research and resources.

Conclusion

Scientific evidence points to future detrimental effects of climate change, particularly on the agricultural industry and food security. As global temperatures increase, crop yield will eventually decrease due to the optimal growth ranges of various crops being exceeded. Thus, we proposed a synthetic biology solution that utilizes bacteria that has established native metabolite exchange with specific plants. These bacteria will express certain enzymes when the plant experiences a heat stressor, which is signalled via RNA thermometers custom-designed for the plant's optimal growing range. While this project has several possible positive impacts associated with it in regards to improving crop production and contributing to the overall economy, setbacks include biosecurity in ensuring bacteria containment measures are met, as well as the elephant in the room of GMOs vs. non-GMOs. Although our project aims to avoid genetic modification of the crops themselves by just modifying bacteria that they have a symbiotic relationship with, education of farmers and consumers, as well as research into the effects of these modified relationships, will need to be effectuated to maximize the potential of such endeavors. Regardless, biotechnology is an up-and-coming factor that will play a significant role in protecting and improving our future, and we aim to take full advantage of it.

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