Hong Kong Student Science Project Competition 2023

Template of Extended Abstract (Investigation Design Proposal)
(Word Limit: 1,600 words, Pages: 3 pages only)

Team Number: SDBC243

Project Title: Tackling Excessive Use of Nitrogen Fertilizers by Nitrogen-fixing Engineering

Project Type: Investigation Design Proposal

***To our best knowledge, there are / ~~are no~~ * similar works in the field*; (if there are,) related research links are as below:**

https://www.sciencedaily.com/releases/2020/01/200115093433.htm

The enhancement our project proposed / the difference with related research are:

We have come up with an idea to prove the hypothesis made in the research, which talks about how nitrogen-fixing genes and bacteria could help grow crops faster without the use of nitrogenous chemical fertilizers. Since the research news does not include any detailed explanation on how to engineer a normal crop into being capable of fixing nitrogen, we delved deeper into this topic by thinking of a method that is used to transfer and duplicate different genes and insert them into a plant colonizing bacteria. As the aim of our project correlates with a vast number of different social problems and sustainable development goals, we wish our method to be practical and straightforward.

Please delete if not applicable. The competition values the originality of works. Students must do enough literature research to ensure that their works are unique and list relevant reference materials before starting research or invention.

I. Background

In this project, we aim to modify an agricultural crop so that it can fix nitrogen by introducing certain important genes such as flavonoids in legumes into the plant genome. This project is based on one principle, which is that nitrogen fixation by legumes is a partnership between a bacterium and a plant.

Legume crops, such as chickpeas and lentils, require significantly less fertilizer than other crops, because they've developed a symbiotic relationship with bacteria that grow within their root tissues. These bacteria convert nitrogen gas to ammonia through a process called biological nitrogen fixation. (Nitrogen-fixing genes could help grow more food using fewer resources, 2020).Biological nitrogen fixation is the process that is mediated in nature only by N-fixing rhizobia bacteria (*Rhizobiaceae*, α-*Proteobacteria*) (Sørensen and Sessitsch, 2007). Other plants benefit from N-fixing bacteria when the bacteria live in close association with the other plant. In legumes, the bacteria live in nodules on the roots. Within these nodules, nitrogen fixation is done by the bacteria, nitrogen is absorbed by the plant. (Flynn, Idowu, 2015)

We want to extract the gene of interest from legumes into other plants such that the genetically-modified plant can adopt a similar system like legumes to conduct the nitrogen fixation. GM crops are usually regarded as dangerous as people may not know much about it. In this project, we indicate the process of how we modify the plants and inform people that there is no safety concern.

II. Objective(s)

Our target is to modify the genes in potatoes, which normally cannot fix nitrogen from the atmosphere, such that they can do so after the modification by attracting rhizobia, a nitrogen-fixing bacteria commonly found in legumes. This can reduce the excessive use of nitrogenous fertilizers and alleviate many issues commonly found in society, such as water shortages, water pollution and more.

III. Hypothesis

It is expected to genetically-modify potatoes that have no ability to fix nitrogen to be able to release flavonoid and attract bacteria to fix nitrogen for them, developing a similar system as leguminous plants. To prove this hypothesis, we will reproduce the genetically-modified potatoes in the greenhouse and compare the genetically modified plants with normal plants and that with additional chemical fertilizers. We measure the amount of flavonoid released around the roots of different potatoes and the time for potatoes to grow for a same height to check if the genetically modified plants can adapt to flavonoids and the nitrogen-fixing bacteria (rhizobia) attracted, plus, to ensure the genetically modified plants have similar abilities as plants sprayed with chemical fertilizers to result in a higher crop yield.

IV. Methodology

After extracting the CHSA gene from legumes green peas, we will carry out the experiment as follows.
Firstly, locate the CHSA gene from the green peas by PCR. Then isolate the CHSA gene from green peas, the CHSA gene must be isolated from others on the chromosome. To accomplish this part, we use Type II restriction enzymes. Type II restriction enzymes cut the gene of interest and plasmid to produce sticky ends at fixed position with respect to their recognition, then we cut the desired gene out precisely from one DNA strip and splice it into the plasmid.

After that, insert this CHSA gene into plasmid, combine the fragments with DNA ligase, which is an enzyme that can catalyze the joining (ligation) of two large molecules by forming a new chemical bond, to link them to make a recombinant plasmid containing the gene. It seal the gaps between the sticky ends.

Firstly, use a micropipette to mix the plasmid and *E.coli*, which is the host for cloning CHSA gene, together. Then, insert a buffer into the solution. Centrifuge the solution for a while and incubate the mixture in ice. Then place the mixture in water at 42°C for 45 seconds and then place back in ice. The quick rise in the temperature is a heat shock. The plasmid will be able to mix into the bacteria after this process. Use a micropipette to transfer the mixture of plasmid with the CHSA gene and *E.coli* and spread evenly on an agar plate. Incubate the plate at 37°C for a few days. *E.coli* will be able to repeatedly divide and multiply. Resulting in a mass production of proteins coded by CHSA genes.

The modified plasmid, inserted with nitrogen-fixing genes, can then be inserted into plant-colonizing bacteria, which will be able to produce fixed nitrogen after the experiment. The plant-colonizing bacteria, genetically modified for the production of nitrogen, will then be inserted into potatoes for experiment. Those bacteria will live with the potatoes.

After copying the gene of modified potatoes, we allow the potato to multiply asexually. Then the potatoes are mass produced for us to check and compare the nitrate production rate and growth rate.

V. Expected Results and Impact of research

Modified potatoes will fix nitrogen and produce nitrate when they are inserted with CHSA genes. With its help, the potatoes will be able to attract nitrogen-fixing bacteria into the plant itself by releasing flavonoids. The plant's efficiency of nitrate production will be increased greatly. They will be self-sufficient with the nitrogen that they fix from the atmosphere to produce amino acids and proteins to grow. When this is the case, it results in a lower usage of nitrogenous fertilizer to ensure a high crop yield. Therefore, the pollution caused by the nitrogen fertilizer will be diminished and less algal blooms will appear in rivers, lakes or sea. We expect genetically modified plants to be able to grow faster. which can be tested by measuring the height of the plants respectively, and produce flavonoids to attract rhizobia, which can be tested by the dilution method. We collect 100g of soil near the roots of the plant for at most 20 cm from the respective plants and 10 cm in depth. Then we dilute the material we collected which is to be examined with water in powers of 10, and inoculate equal volumes of the diluted material into liquid media. If growth occurs from the inoculation of 1cc. of a 1:100 dilution and not from a 1:1000 dilution, the number of organisms present in the original material is said to be between 100 and 1000 per cubic centimeter. The reciprocal of the highest dilution which shows growth represents the most probable number of organisms present.

As we aim to modify plants to reduce the excessive use of chemical fertilizers, less chemicals and ammonia will be released into the ocean. Some of the pertinent major real-world problems include water shortages, water pollution and diseases originated from toxic seafood. The 2030 Agenda for Sustainable Development, adopted by all United Nations Member States, provides a unanimous scheme for peace and prosperity for people and the planet. At its core are 17 Sustainable Development Goals , which are an urgent call for action by all countries in a global partnership. In which we aim to tackle zero hunger, clean water and sanitization and life below water.

VI. If your team will compete the Sustainable Development Award, please indicate the specific sustainable development goal the project is related to, and provide justification for competing for this award. *(Word limit: 300 words)*

This project aims to tackle three sustainable development goals, which are zero hunger, life below water and clean water and sanitization. By mass producing the modified plants, we wish to reduce the excessive use of nitrogenous fertilizers in farmlands so less ammonia will be released into the ocean. This can inhibit the overgrowth of blue-green algae and reduce water pollution, such as eutrophication to limit the burden on the marine ecosystem. Below are the three aspects of sustainable development:

Economically, the cost of fertilizing crops can be lowered in the long term as modified plants no longer rely on absorbing nitrogen from chemical fertilizers, instead, they can fix nitrogen for their own usage. Moreover, the economy in coastal cities will be further developed as more types of non-poisoned fish can be caught instead of suffocating in oxygen-depleted waters or dying to the toxins in cyanobacterial algal blooms.

Environmentally, when less fertilizer is used, less ammonia gets released into the ocean, reducing eutrophication and cyanobacterial blooms. This lowers the chances of the algae blocking the sunlight and avoids the inhibition of photosynthesis, therefore, plants underwater can flourish. The ocean will become a more favorable environment for aquatic organisms to inhabit due to a more stable supply of oxygen. In the long run, it may also prevent fish species from becoming endangered.

Socially, lowering the frequency of algal blooms and its toxins in the ocean can improve the quality of the sea, which benefits our daily life greatly. Firstly, fishes such as tuna and sardine become safer for humans to consume due to the lowered chances of getting diseases like cholera due to ingesting toxic seafood. Secondly, as seawater is one of the main water sources, improving the quality of the seawater deals with water shortage problems in specific areas.

VII. If your team will compete the Social Innovation Award, please list the target group or social issue the project focuses on, and provide justification for competing for this award.
(Word limit: 300 words)

VIII. Conclusion

This project brings us to the insights of the nature of leguminous plants and allows us to think outside the box and come up with a method to solve the problems of water pollution and food shortage. This project aims to reduce the use of fertilizers by injecting CHSA genes extracted from leguminous plants (green peas) into crops (potatoes) so they can unlock the ability to develop a similar system like legumes to form nodules naturally by bacteria, producing flavonoids and attract rhizobia to fix nitrogen from the atmosphere. This method can not only reduce the use of fertilizers, but also enrich the soil quality nearby as nitrogen will be fixed in the roots of the plants. Since once this project succeeds, this will truly benefit us and solve some current problems such as hunger and water pollution in the following decades.

IX. Reference list

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