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Feeding a Growing Population--Feasibility of Leghemoglobin as Visual Marker for Facilitating Plant Genetics Based Nutritional Improvement

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Notes:
Rebecca Caldbeck won the first place in the Physical and Engineering Sciences category. She also won the second place in the Biological Sciences category.
Dr. David Hildebrand was the faculty mentor.

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Abstract

As the population continues to expand, it is becoming increasingly relevant to find sustainable and affordable sources of high-nutritional value food, specifically protein and fatty acids.

One such crop of interest is Glycine max, commonly known as soybean. The leguminous plant is of high importance globally with its far-reaching economic applicability. Soybeans are a versatile crop with a wide-ranging habitat, making them a readily available food source. Besides their abundance, this crop is also one of the most economical sources of protein on the planet.

With their already blanketed global reach, the introduction of improved genetics to facilitate improved nutritional quality in the soybean crop would be an asset to humanity as a whole. The commonplace nature of soybeans would likely facilitate the acceptance of improved soybean products. Soybean seeds enriched in omega-3 fatty acids, as well as leghemoglobin, would provide an invaluable addition to human health worldwide.

The goal of the project is to increase levels of omega-3 fatty acid and nutritious protein content, through the employment of leghemoglobin as a visual marker for facilitating plant genetic improvement in soybeans. To date, leghemoglobin has not been tested for visual marker value, thus making the findings of this project novel.

If successful, through improving availability of high-nutritional value food from plants, the project could be environmentally beneficial in improving the current production of protein via reduction of necessary land area and water usage when compared to conventional meat production.

Soybean seeds enriched in omega-3 fatty acids, as well as leghemoglobin, would provide an added health benefit to consumers. In addition to soybeans specifically, having an effective visual marker to facilitate genetic improvement would be an asset to further development of more nutritional food products in general for a myriad of crop species. The project has the potential to have far-reaching impacts on the food, health, and agricultural industries.

Introduction

The ever-expanding global population has been a long developing issue. Global studies of natural resource bases illuminate concerning signs of degradation and unsustainable usage. The recent intensification of food production has led to the decline of several ecosystem services. Depletion of soil nutrients, freshwater, forests, and biodiversity, as well as desertification, and erosion, are all starkly vivid indicators of the issue. The productive capacity of land and genetic resources shall continue to wane at a distressing rate unless land usage becomes more sustainable and investments are made towards convalescence of the environment. Through raising public awareness of the issues, the implementation of sustainable practices has the potential to leave lasting impacts on the future of the agricultural industry. In recent times, the interest in alternative bio-based nutritional products is growing.

As the population continues to expand, it is becoming increasingly relevant to find sustainable and affordable sources of high-nutritional value food, specifically protein and fatty acids.

One such crop of interest is *Glycine max*, commonly known as soybean. The leguminous plant is of high importance globally and has far-reaching economic applicability for its numerous products on the global market (Sharma *et al.,* 2014). Worldwide production of soybeans in 2018 topped 346.02 million metric tons globally, with 119.52 million metric tons coming from the United States alone (USDA, 2018).

Soybeans are a versatile crop with a wide-ranging habitat globally, making them a readily available food source. Besides their abundance, this crop is also one of the most economical sources of protein on the planet.

With their already high global reach, the introduction of improved genetics to facilitate improved nutritional quality in the soybean crop would be an asset to humanity as a whole. The commonplace nature of soybeans would likely facilitate the acceptance of improved soybean products. Soybean seeds enriched in omega-3 fatty acids, as well as leghemoglobin, would provide an invaluable addition to human health worldwide.

Leghemoglobin is an abbreviated version of legume hemoglobin. Soybeans are leguminous plants, meaning they are capable of fixing atmospheric nitrogen in their roots. Leghemoglobin is a type of protein that is chemically bound to heme, a non-protein component commonly thought of as part of hemoglobin. Hemoglobin is a protein that carries heme, which is an iron containing molecule that is required for life. For animals, the heme molecule is primarily carried by hemoglobin and myoglobin. For soybeans, the heme molecule is carried by leghemoglobin. Leghemoglobin is exclusively found in the nodules resulting from symbiotic relationship between legume roots, soybeans in this instance, and Rhizobia bacteria (Wiborg *et al.,* 1982). Plant-based substitute giant, Impossible Foods, was the first company to commercialize the fact that this heme molecule is what gives meat its taste. The flavor of heme from soybean plants and the heme from animals is strikingly similar (Fellet, 2015).

Soybean nodules are comprised of four primary speces of leghemoglobin, LBA, LBC1, LBC2, and LBC3, as well as a number of minor components that are likely just modifications of the primary species (Wiborg *et al.,* 1982).

The goal of the project is to increase levels of omega-3 fatty acid and nutritious protein content, through the employment of leghemoglobin as a visual marker for facilitating plant genetic improvement in soybeans.

If successful, the project could be environmentally beneficial in improving the current production of protein, as production of leghemoglobin-based protein is already utilizing 95% less land, 74% less water, and producing 87% less greenhouse gases than conventional meat production, according to Impossible Burger.

Besides the environmental benefit, soybean seeds enriched in omega-3 fatty acids, as well as leghemoglobin, would provide an added health benefit to consumers. In addition to soybeans specifically, having an effective visual marker to facilitate genetic improvement would be an asset to further development of more nutritional food products in general for a variety of crops. The project has the potential to have far-reaching impacts on the food, health, and agricultural industries.

Methods

RNA was extracted from the root nodules of *Glycine max*. Pink to red undertones are indicative of a healthy nodule, so special care was taken to ensure only healthy and moderately mature nodules were selected for sample collection. RNA was extracted from the samples collected and was used to synthesize cDNA. The cDNA was amplified via PCR and validated via agarose gel analysis. Overnight cultures were prepared and then miniprep was performed to purify. A restriction digestion analysis was performed and verified with an agarose gel extraction and analysis. A-tailing of blunt-end PCR product fragments occurred and was followed by a ligation using the pGEM-T Easy Vector. The ligation results were transformed into E.coli using pGEM-T Easy Vector and then plated.

Results

**COVID-19 impacted research timeline and has delayed data collection. Result compilation is forthcoming with ongoing research.

Discussion

The value of leghemoglobin as a visual marker for facilitating plant genetic improvement in soybeans was analyzed in hopes of determining as effective marker to improve nutritional value of the crop, specifically via an increase in levels of omega-3 fatty acid and nutritious protein content. The visual marker could also potentially be utilized as an asset in the further development of more nutritional food products for a myriad of crop species. Genetic markers of value must display high heritability with limited environmental impacts on phenotypes, in order to be assayed without need for as much replication after patterns of inheritance have been configured (Bretting and Widrlechner, 1995).

The genetic marker of interest should be statistically independent and unliked physically from other markers, thus permitting it to be straightforward to observe throughout plant development, have limited to no impact on overall growth and health, and be sufficient for rapid and economically feasible analysis when utilized on research and development scale (Bretting and Widrlechner, 1995).

Marker-trait association must be carefully analyzed in order to ensure maximum practical breeding value. The potential leghemoglobin visual marker should be analyzed for compatibility with the traits impacting omega-3 content in soybeans. Closely related markers and traits typically result in higher efficiency and observed rate of success. Gene or association mapping, or linkage or recombination analysis could be utilized to facilitate understanding of the marker-trait association present (Jiang, 2012) The planted crop would have DNA extracted from tissue samples and analyzed via running PCR on the marker of interest, in this case leghemoglobin. The amplified PCR product would be scored and separated potentially via techniques such as PAGE. The individuals seen to be carrying the desired marker alleles would be selected and generations would be repeated, with continuous advancement of elite lines with the improved trait of increased omega-3 present (Jiang, 2012).

***More discussion forthcoming with ongoing research, delayed due to COVID-19.

Conclusion

**No substantial conclusion can be made as of yet, as COVID-19 impacted research timeline and has delayed data collection. Conclusion is forthcoming pending ongoing research.

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