A Novel, Genetically Modified Seed Design for Increased Biofuel Production and Poverty Alleviation in Indonesia

Ananya Dua

A Novel, Genetically Modified Seed Design for Increased Biofuel Production and Poverty Alleviation in Indonesia

Ananya Dua [1]

[1] **Monta Vista High School, Cupertino, California**

Abstract

Straddling the equator, Indonesia is a tropical country with a hot and humid climate and high temperatures in the 90s; its location and frequent rainfall make it conducive to the prolific growth of the tobacco plant. Indonesia is a major grower and exporter of tobacco and is home to the fifth-largest market for tobacco consumption in the world. Researchers estimate that Indonesia's population of 242 million consumes 182 billion cigarettes each year (Nichter, 2009). And, this consumption has increased rapidly over the years, largely due to the mechanization of the cigarette industry. Data reveals that 62% of men smoke in Indonesia (Nichter, 2009). Smoking is not common among women, but among men, it starts young and persists for the majority of their lifetime. Studies have shown that initiation begins early – over a quarter of urban and rural people start consuming tobacco at 10 years old (Zheng, 2011). This early exposure also leads to detrimental side effects, with tobacco-related mortality accounting for 10% of total deaths in Indonesia (Dasar, 2018).

In Indonesia, tobacco companies are financially powerful and successful because they are the largest source of government revenue after oil, timber, and gas (Reynolds, 2013). Their integration into the government makes it easier for them to promote and encourage their agenda. Revenues from cigarette excise taxes are considered critical to the functioning of the Indonesian government (Nichter, 2009).Unfortunately, in Indonesia, smoking cessation programs have yet to receive the required attention from the government as a preventative strategy for reducing the popularity of the smoking culture. Apart from cigarette taxes, government support for cessation programs still falls short due to their reliance on the tobacco industry to support the Indonesian economy and the livelihoods of farmers, who deem tobacco green gold and the primary source of their income (Bader, 2011).

Recently, due to the COVID-19 pandemic and conflicts with the government, tobacco farmers in Indonesia are facing increasing challenges. About 57.23 percent of tobacco farmers are now living in poverty, significantly higher than the national average of 9.66 percent in 2018 (Sahawedo, 2020). Roughly 40 percent of tobacco farmers are now reliant on governmental social protection programs (Sahawedo, 2020). Extreme weather and complicated excise taxes aggravated by the COVID-19 pandemic have made local farmers helpless and powerless. Tobacco farmers are at the bottom rung of the industry and have felt the pinch. Farmers are accusing the intermediate buyers of increasing prices and forcing them into accepting demands and are grappling with middlemen taking commission fees out of their payment. Farmers are suffering losses rather than making profits from the harvest, due to the large sum of money they have to shell out upfront for the harvesting of tobacco (Sahawedo, 2020). A separate study of 1350 smallholder tobacco farmers in Indonesia found that tobacco farming is not profitable for the farmer, that most farmers are poor, and that many suffer from green tobacco disease, which is a type of nicotine poisoning that occurs while handling tobacco plants.Workers are at especially

high risk for developing this illness when their clothing becomes saturated from tobacco (Astuti, 2020).

Aiming to eliminate smoking in Indonesia is a noble initiative. It would help prevent the numerous deaths each year and help the country move towards a healthier and more sustainable lifestyle. But, this motive will negatively affect the farmers and the economy. With the diminishing demand for tobacco, tobacco farmers would inevitably lose their main source of income. The government would also suffer a loss in revenue, as tobacco is one of their largest profiting industries.

Thus, I suggest an alternative use for tobacco that will allow farmers to continue supporting their lifestyles while also helping curb tobacco consumption in Indonesia – genetically engineered tobacco seeds grown for production of biofuel. The high sugar and oil contents in the genetically engineered seeds make them the optimal choice for producing biofuel; this would effectively ensure that companies would seek out these tobacco farmers to buy their plants, which would, in turn, provide them with a steady source of income to provide for their families, without having to directly sell tobacco and contribute to the nationwide crisis. Tobacco farmers who have been experiencing loss of profitability, resulting in moderate to severe repercussions to their livelihoods, would be the most open to this proposition. Other tobacco farmers who may not have experienced any personal loss due to the declining smoking industry will likely still want a safety net in the event of their sales dramatically decreasing within the next few years. On top of that, the product addresses the finite and rapidly depleting reserves of fossil fuels. In 2019 alone, 95 billion gallons of fuel were consumed by the aviation industry (E. Mazareanu, 2021). Fossil fuels are non-renewable resources; at the rate at which their reserves are being burnt, there would inevitably be a shortage shortly. Tobacco biofuel, on the other hand, is a renewable resource since tobacco plants can be continuously grown and harvested, thus ensuring that an aviation fuel shortage can be avoided.

The current climate crisis is another important issue that the product tackles. In 2019, 915 million tons of carbon dioxide were emitted from air travel, which represents around 2% of total emissions worldwide (Air Transport Action Group, 2020). Although it doesn’t seem like an exorbitant percentage, the airline industry is one of the fastest-growing industries in the last 20 years, averaging a 5% annual increase in flights (Jocelyn Timperley, 2021). Air travel is primarily dependent on fossil fuels, so to get to the root of the problem, we have to find sustainable alternatives that are much less harmful to the environment, such as tobacco biofuel; it could reduce around 75% of carbon dioxide emitted from air travel (Aisling Irwin, 2016). Hence, these genetically modified seeds—that eventually produce tobacco biofuel—would contribute to efforts aiming to contain the ongoing climate crisis.

The sharp decrease in demand for air travel over the past few years due to the COVID-19 pandemic, coupled with rising jet fuel prices have sent most airline companies into a state of financial decline. The US jet fuel prices hit a 13-month high, doubling to almost $750 per metric tonne over the year, according to data by S&P Global Platts. The rising oil prices would have normally passed onto the consumers through increased ticket prices, however, due to the uncertain times of COVID-19, airlines are already running below the normal capacity. In a time when companies are providing discounts to encourage air travel, increasing ticket fares would only cripple the already struggling industry. To aggravate matters, most airline companies stopped hedging their future fuel requirements during the crisis in the oil market last year, leaving them more exposed than ever. To quantify the situation, RyanAir has lost around $300 million during the pandemic period while Delta Airlines has had only one profitable quarter this year. The airline industry is suffering largely from fuel prices and would need a substantial alternative fuel resource to survive.

The recent string of climate change policies and the rising awareness amongst people about the devastating effects of greenhouse gasses have caused a chain reaction, driving people to prefer cleaner ways of energy. To comply with the public demand for increased reliance on renewable or cleaner ways of energy, many fuel companies are expected to cut down on their fossil fuel extraction programs. This renewed climate deterioration awareness also means that more people would prefer cleaner, environmentally friendly modes of transport rather than those with pernicious impacts. This has already been since in the automobile industry as more people switch towards electric cars or hydrogen fuel cell cars over traditional petrol cars. A similar change is expected in the airline industry, with the United Nations Framework Convention on Climate Change (UNFCCC) recent Kyoto Protocol, most countries have to reduce their emissions and mitigate CO2 release. This program was renewed at the 'Durban Platform for Enhanced Action' and has seen many countries like Norway, Sweden, and Finland set out policies to significantly reduce their emissions by 2030 via a system of quota demanding SAF(sustainable aviation fuel) to be around 30%. These policies would mount greater pressure on the aviation industry as it lacks a sustainable, clean source of fuel for lengthy and high-weight travels.

Taking into account all factors: pandemic, changing attitude towards the use of fossil fuels, increased awareness about drastic environmental effects, sharply growing air travel demand, and fastly depleting reserves of fossil fuels, the aviation industry needs a sustainable, functioning, substantial, and socially acceptable fuel source and the product addresses that. Grown from a farm through sustainable farming methods (rejecting pesticides and insecticides), it has public validation. Increased sugar and oil concentrations mean it has greater energy per kJ, allowing it to sustain prolonged, heavy flights. With minimal expenditure on genetically engineering the product and stable cost of services, large fluctuations in the price of the product would not exist, resolving the high oil price chaos. Its hybrid properties of withstanding droughts and higher yield mean it can be grown almost anywhere, during any time of the year, and in magnanimous quantities, therefore there is no risk of depletion.

Besides the numerous concerns of the airline industry that the service addresses, it also heals many of the agricultural industry’s ails. The service produces tobacco with minimal nicotine levels as the room is made for sugar and oil content. This allows the crop to be grown without fears of the crippling green disease, which takes 1.6 million lives each year. Lower nicotine levels also mean that the crop is less susceptible to being attacked by animals.

In a period of increasing land pressure, formerly agricultural lands make room for housing projects and commercial avenues. Fewer cultivable land means decreased yield for the farmer and smaller profits. However, the hybrid vigor of the product allows us to produce double the yield in half the land area, while requiring fewer resources. Fewer fertilizers, less water, and no pesticides are all of these features that significantly cut down the farmer’s expenses, boosting profits, and making tobacco a financially viable crop again. Additionally, the product's resistance to diseases like Blue Mold and Black Root Rot means that epidemics would no longer be a threat to the farmer and they would not have to experience devastating financial losses to them.

Ultimately, many global issues need to be dealt with, and the seed design has targeted these three causes to serve humanity: providing a source of income for tobacco farmers, jet fuel shortage, and climate change.

I plan to produce seeds that are a hybrid of two genetic modification methods. One of these methods works to increase oil production and resilience by suppressing the FAD2 gene, which is responsible for reducing the overall oil content. The other depends on overexpression of DGAT-1 and LEC-2 genes that increase the production and regulation of TAG (Triacylglycerol) and the rate of photosynthesis. For this process, I will be employing varieties used in the production of Begerac's ITB-6184 strain due to its optimum sugar to nicotine ratio.Though these are two gene manipulation methods already developed by scientists, the process of hybridizing genetically-engineered seeds (as opposed to naturally optimum seeds) has not yet been done. Recent research has established that the combination of genetic engineering and hybridization leads to copious benefits, including greater plant yield, resistance to disease and drought, and a healthier overall supply, and mine will be one of the first products to implement this knowledge into the development of the seed.

In Nicotania Tobacum, the FAD-2 gene codes for the key enzyme that is responsible for the biosynthesis of polyunsaturated fats in non-photosynthetic tissues like roots and developing seeds. FAD-2 gene in the endoplasmic reticulum utilizes phospholipids as substrates with NADH, and NADH-cytochrome as electron donors. FAD-2 gene is divided into further four types: FAD2-1, FAD2-2, FAD2-3, and FAD2-4; all variations are dependent on the site of their function and the pattern of gene expression during transcription. My project focuses on the FAD2-2 variety. Expressed at a low level from vegetative to maturation state during seed development, FAD2-2 is the major gene responsible for the synthesis of linoleic acid. Coding for fatty acid desaturase enzyme, FAD2-2 is responsible for desaturating oleic acid to linoleic acid at the mesocarp and the maturing seeds, covering the greatest function area of all the four varieties. The FAD2-2 gene expression decreases as the temperatures go from 15oC to 35 oC, while its expression increases with the level of light intensity and in case of wounding or a pathogen attack.

After using genomic flanking the T-DNA was cloned by plasmid rescue to isolate cDNA and genomic clones of FAD2, a cDNA containing the entire genomic sequence of FAD2 was expressed in FAD2 mutant plants. The Gel Blot analyses of FAD2 mRNA levels showed that the gene is expressed throughout the plant and transcription levels are in excess than needed for oleate desaturation. Therefore, inhibiting the FAD-2 gene to a certain level would not harm the plant and it will maintain its useful functions through oleate desaturation like plant development and tolerance against salt and cold.

In the second base strain, the two other Arabidopsis genes, DGAT and LEC-2, will be used. DGAT gene consists of two varieties, DGAT1 and DGAT2. Both DGAT genes are responsible for coding acyl-CoA: diacylglycerol acyltransferase (DGAT) enzymes. These enzymes are involved in the biosynthesis of Triacylglycerols (TAGs). Triacylglycerols are major storage molecules of metabolic energy in living organisms. Triacylglycerols (triglycerides) (TGs), a major type of neutral lipid, are a heterogeneous group of molecules with a glycerol backbone and three FAs attached by ester bonds. TAGs serve many vital functions in living organisms like storage molecules for energy utilization and synthesis of membrane lipids. Heightened LEC-2 levels which are attained through the gene's expression catalyze seed development and enhance the levels of oil storage. Increasing the DGAT enzyme concentration would not only speed up the rate of this reaction but also increase the overall TAGs production, leading to greater oil content.

The two strains are crossed together for a given number of generations, isolating the required characteristics, similar to what is done during selective breeding of livestock. The desired characteristics in the resulting hybrid offspring are higher oil and sugar content, greater plant height, and lower nicotine levels. The hybrid vigor possessed by these offspring leads to greater plant height, plant number, overall yield, and area, and the ability to withstand tough environmental conditions and survive droughts. These seeds also have increased disease protection, especially against Blue Mold and Black Root Rot.

Currently, the global aviation fuel market is a rapidly-growing industry that is anticipated to generate $238.5 billion by 2026. With a shortage of worldwide aviation fuel, many companies are looking for alternative methods to produce this fuel, one of the most famous methods including tobacco. The optimally designed seeds are perfect for the purpose due to their increased sugar and oil levels, a significant advantage that improves the quality and selling price of aviation fuel.

In conclusion, this unique, never-before-done combination of hybridization and genetic engineering methods on the seeds ensures that this product will be successful in protecting the livelihoods of tobacco farmers while mitigating the aviation fuel shortage in the country and bolstering the economy.

References:

1. “Air Transport Action Group (ATAG).” International Airport Review, https://www.internationalairportreview.com/organisations/air-transport-action-group-atag/
2. Astuti, Putu Ayu Swandewi. “Why Is Tobacco Control Progress in Indonesia Stalled? - A Qualitative Analysis of Interviews with Tobacco Control Experts - BMC Public Health.” BioMed Central, BioMed Central, 19 Apr. 2020, https://bmcpublichealth.biomedcentral.com/articles/10.1186/s12889-020-08640-6.
3. Bader, Pearl. “Effects of Tobacco Taxation and Pricing on Smoking Behavior in High-Risk Populations: A Knowledge Synthesis.” International Journal of Environmental Research and Public Health, Molecular Diversity Preservation International (MDPI), Nov. 2011, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3228562/.
4. Dar, Aejaz A., et al. “The FAD2 Gene in Plants: Occurrence, Regulation, and Role.” *Frontiers*, Frontiers, 1 Jan. 1AD, https://www.frontiersin.org/articles/10.3389/fpls.2017.01789/full.
5. Irwin, Aisling, et al. “Tobacco-Powered Aeroplane to Be Tested 'within Months'.” Horizon Magazine, 25 Jan. 2016, https://ec.europa.eu/research-and-innovation/en/horizon-magazine/tobacco-powered-aeroplane-be-tested-within-months.
6. Mazareanu, E., et al. “The Impact of the Coronavirus Pandemic on Supply Chains and Their Sustainability: A Text Mining Approach.” Frontiers, Frontiers, 1 Jan. 1AD, https://www.frontiersin.org/articles/10.3389/frsus.2021.631182/full.
7. Nichter, Mimi. “Reading Culture from Tobacco Advertisements in Indonesia.” Tobacco Control, BMJ Publishing Group Ltd, 1 Apr. 2009,
8. Reynolds, Catharine. Global Assessment of Deforestation Related to Tobacco Farming. https://tobaccocontrol.bmj.com/content/8/1/13.
9. Riset Kesehatan Dasar (Riskesdas). Lembaga Penerbit Badan Penelitian dan Pengembangan Kesehatan (LPB), Ministry of Health, Indonesia. 2018.
10. Sahadewa, Gumilang Aryo. “In-and-out of Tobacco Farming: Shifting Behavior of Tobacco Farmers in Indonesia.” MDPI, Multidisciplinary Digital Publishing Institute, 15 Dec. 2020, https://www.mdpi.com/1660-4601/17/24/9416.
11. Timperley, Jocelyn. “Are We Too Late to Stop Climate Change?” BBC Science Focus Magazine, BBC Science Focus Magazine, 31 Mar. 2022, https://www.sciencefocus.com/planet-earth/can-we-stop-climate-change/.
12. Tian, Yinshuai, et al. “Design of High-Oleic Tobacco (Nicotiana Tabacum L.) Seed Oil by CRISPR-Cas9-Mediated Knockout of NTFAD2-2.” *BMC Plant Biology*, BioMed Central, 25 May 2020, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7249356/.
13. Zhang, Jiantao, et al. “Arabidopsis Fatty Acid Desaturase FAD2 Is Required for Salt Tolerance during Seed Germination and Early Seedling Growth.” *PLOS ONE*, Public Library of Science, https://journals.plos.org/plosone/article?id=10.1371%2Fjournal.pone.0030355.
14. Zheng R, Marquez PV, Ahsan A, Hu X, Wang Y. Cigarette affordability in Indonesia: 2002-2017. World Bank. 2018 4 Global Adult Tobacco Survey, Indonesia 2011.