

## USING CROP TRANSCRIPTOME AND METABOLOME ENGINEERING INNOVATIONS TO SOLVE IRAN'S MAJOR FOOD AND ENERGY PROBLEMS

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*"With the name of God, we Agricultural scientist are proud to allow no politics among us, but feeding the world and preserving of this plentiful planet through Science, Technology and Innovative Ideas"*

Mariam Sticklen

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The title of this international conference reminds me of a quote from John F. Kennedy. He said:  
*"A man may die, nations may rise and fall, but an idea lives on. Ideas have endurance without death."*

John F. Kennedy

My name is Mariam Sticklen, and I am the Professor of Crop Improvement and Biofuels in the Department of Plant, Soil and Microbial Sciences at Michigan State University.

I am looking forward to opening the doors of my laboratories, department, college and university in collaborations between any of the universities in Iran and our scientific teams. The College of Agriculture and Natural Resources at MSU is proud to have trained many thousands of scientists around the globe on using their ingenuity in research to feeding and fueling the world through Science, Technology, and innovative Ideas.

I believe that with the powerful weapon of 'education', we can stand together shoulder-to-shoulder to come up with innovative ideas for changing the world of hunger and energy shortages. And indeed, I agree with an observation of my greatest hero, Nelson Mandela, who recently died on December 5<sup>th</sup> of 2013, when he said, and I quote:

*"Education is the most powerful weapon which you can use to change the world."*

Nelson Mandela

I also strongly agree with another hero of mine who compared knowledge with ideas or imaginations. That man is Albert Einstein who once said, and I quote:

*"The true sign of intelligence is not knowledge but imagination and ideas."*

-Albert Einstein Here at Michigan State University, we not only provide knowledge, but we train our team members how to come up with new and innovative ideas. My international team has so far received 14 U.S. and International Patents. I do invite graduate student candidates and sabbatical professors from Iran to join my team to help create more new ideas and innovations.

In order to come up with innovative ideas for a country, one has to first define the country's existing shortages. And of course, it is not my place to address the social or political matters of a society, rather to see how we scientists can help each other in solving the agricultural problems, mostly the food and fuel shortages around the globe.

Let me tell you with all honesty that unlike what most people around the globe might believe, Iran's major natural resources are not Iran's petroleum, nor its high energy radiation resources;

rather they are Iran's intelligent human resources. And I repeat: Iran's major natural resources are indeed, its' intelligent people. However, these blessed human resources need to be further trained and nourished for their maximum innovative idea capacities. The petroleum oil and high energy radiations are finite, but the capacity of a country's human intelligence is not!

As an agricultural country, Iran has been "self-sufficient" in food and feed production for the past three decades.

As per a recent report, Iran food security stands at 90%. As per another report, 1/3<sup>rd</sup> of Iran's land is suitable for farmland, and also the country enjoys a sizable agricultural sector. Iran's farmable lands produce 10% of Iran's Gross Domestic Product (GDP). However, a certain percentage of a few major crops (for example, wheat) have been imported to the country due to drought, diseases, and water shortage problems within the country.

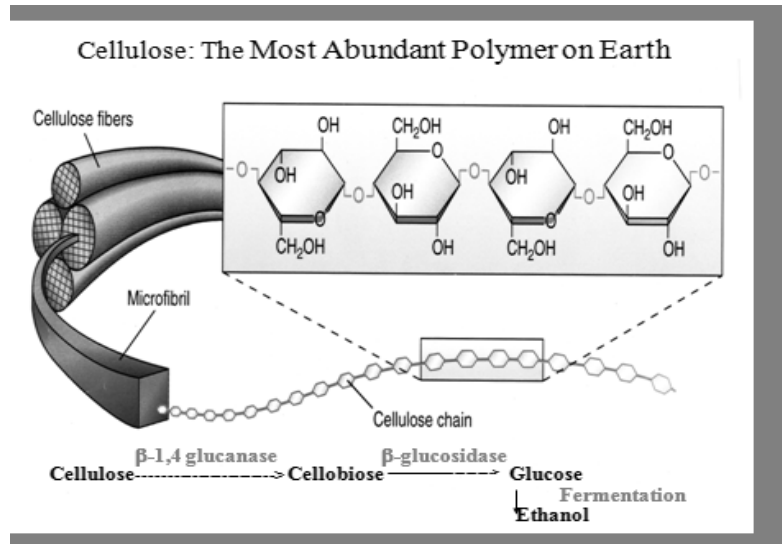
In this Keynote speech, I would like to address four major initiatives that Iran's might wish to consider for its future of Agriculture. These include:

1. Investing much more in the education of its young agricultural scientists by sending them abroad for training for 1-4 years with a firm condition that they will return to the country.
2. Iran needs to develop innovative technologies to produce bioenergy crops and to convert close a half billion tons of agricultural wastes (i.e. rice straw, wheat straw, and other agricultural wastes) and bioenergy crops into biodiesel, biobutanol, and natural or recombinant industrial co-products.

Iran uses its petroleum oil as energy in Agriculture, but Agriculture should be a major source of energy by itself. Iran only has about 10% of the total global oil reserve. You might not know that the 2<sup>nd</sup> country on earth with largest oil reserve (1<sup>st</sup> being Saudi Arabia with 21% of the global oil reserve) is Canada. Although Canada has 15% of the global oil reserve (50% more than Iran), it has the 2<sup>nd</sup> largest biofuels program (first country being the U.S.).

As a consultant to the Canadian Government on their Biofuels program, I travel to Canada every other year to assist Canadian Government with its Network of Centers of Excellence in Biofuels Research, especially on converting of their crop biomass residues into biofuels and industrial chemicals.

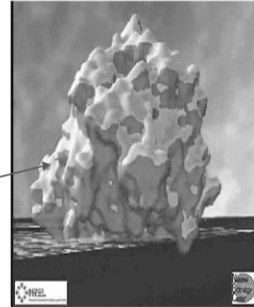
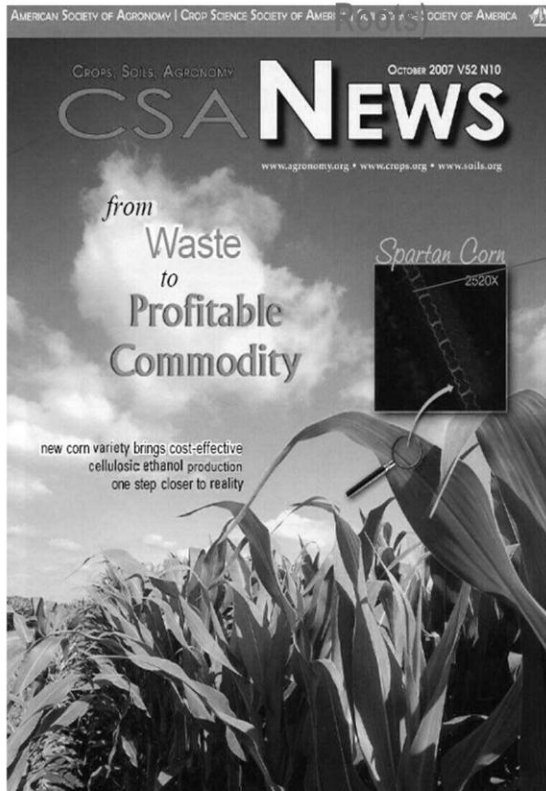
The crop's vegetative wastes are made of cellulose and cellulose is made of sugar (see the figure below).



A group of at least three enzymes convert cellulose into fermentable sugars for biofuels and industrial products. These enzymes include endo and exo-glucanases and the Beta-glucosidase. (see the lower portion of the figure above). Chemical engineers and bio-processors produce these three enzymes in *E. coli* microbes and grow them in bioreactors at high costs, and then extract and use these enzymes to convert crop vegetative biomass into fermentable sugars for production of alcohol fuels such as ethanol or butanol.

Our team has produced crops that self-produce all of these three microbial enzymes in their leaves and stems, and not in their seeds, flowers or roots. The gene for the first enzyme, endoglucanase (E1), was taken from a microbe that lives in Hot Springs in Colorado, and engineered in corn and rice genomes. The corn genotype produced was called “Spartan Corn”. So the corn vegetative wastes could be simply and cheaply converted into biofuels (see figure below reported by Crop, Soil and Agronomy News in 2007). The U.S. Patent #7,696,411 to Sticklen et al. entitled “Transgenic plants containing ligninase and cellulase which degrade lignin and cellulose to fermentable sugars” was issued on April 13, 2010 on this idea and its follow-up research. This patent has been since exclusively licensed to a biofuel company for its commercialization.

## Spartan Corn I Produces an Endo-cellulase within the cell Wall Areas (Apoplast) of Leaves & Stems (Not in Seeds, Pollens or

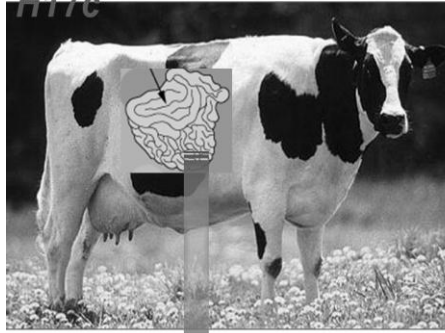


Thermostable E1  
(Gene from NREL)

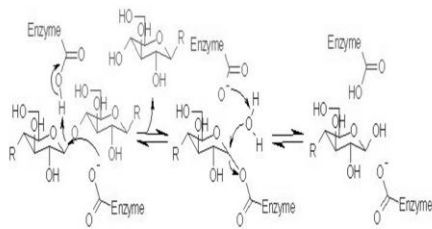
The gene for the 2<sup>nd</sup> enzyme, microbial exoglucanase was taken from another thermo-stable microbe and engineered in the corn genome by Sticklen's team.

The A more innovative idea came to me when I was flying to present an invited speech at one of the five U.S. National energy labs. The idea was; cattle eats silage for food and energy. What is the ultimate gene that converts the cattle's silage into energy, and where does this gene come from? The answer was, well there are microbes in cattle's 2<sup>nd</sup> stomach that convert the grass or silage into energy. The gene my team transferred from the microbe that lives in the cattle's gut into the corn genome and rice genome was the same gene that converts the silage into energy in its 2<sup>nd</sup> stomach (see figure below).

*Butyrivibrio fibrisolvens*  
H17c



Hydrolysis of disaccharides into glucose



Mechanistic details of beta-glucosidase activity



A yet far newer and more energy efficient technology that is now in progress in our laboratories at Michigan State University is production of pure oil (whether vegetable oil or biodiesel oil) in wheat straw, rice straw and other crop vegetable wastes through oil metabolic engineering.

Oil seeds produce oil, but crop vegetative biomasses (that are abundant and cheap) do not produce sufficient oil. The idea is to produce oil in crop leaves and stems, just as canola and soybean seeds produce oil. Figure below shows the oil biosynthesis pathway of oil in oil crop seeds.

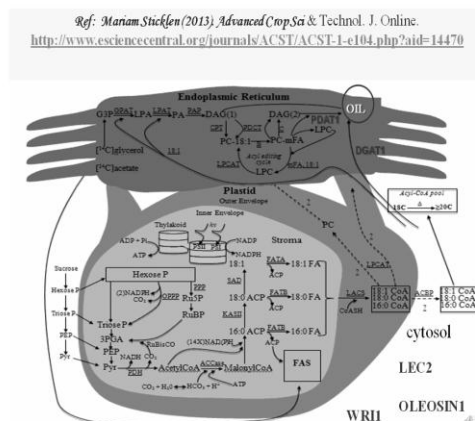
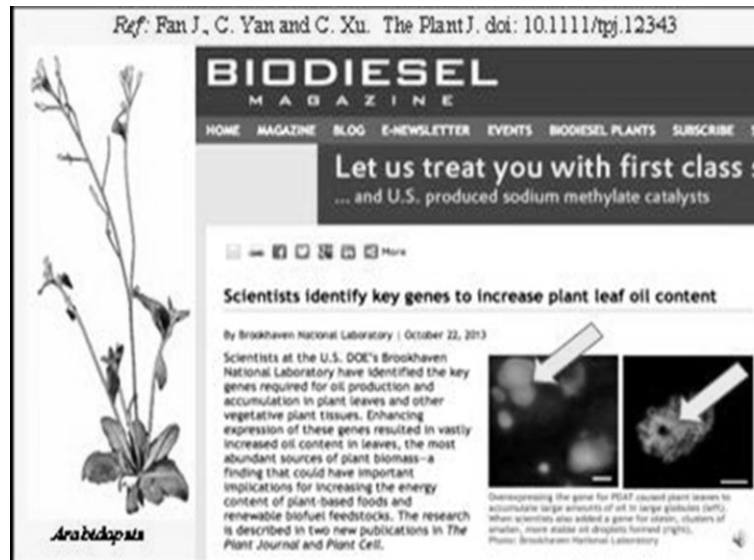


Fig. 1. A simplified version of oil (TAG) biosynthesis pathway (from: Sticklen, 2013). The enzymes that are shown in red color are either in low quantity or absent in crop vegetative tissues.

In that figure, there are four enzymes (shown in red). These enzymes are produced in oil crop seeds, but not enough in crop leaves or stems. By overexpressing the genes of these four enzymes that are not produced enough in crop vegetative leaves and stems, my team is now working on a new discovery to produce about 20% oil in wheat and rice straw.

The basic research of the work was just reported in this journal by one of the former MSU PhD students (see the news below).



Therefore, Iran can indeed produce ethanol and butanol, and also biodiesel oil in its crop vegetative wastes such as wheat straw and rice straw. In fact, some countries such as Egypt still burn their rice straws to waste, resulting in environmental and health problems including asthma. Rice straw could be used for production of biofuels and industrial bio-based chemicals.

3. The 3<sup>rd</sup> initiative in Agriculture that Iran might wish to consider is staying ahead of the climate change research.

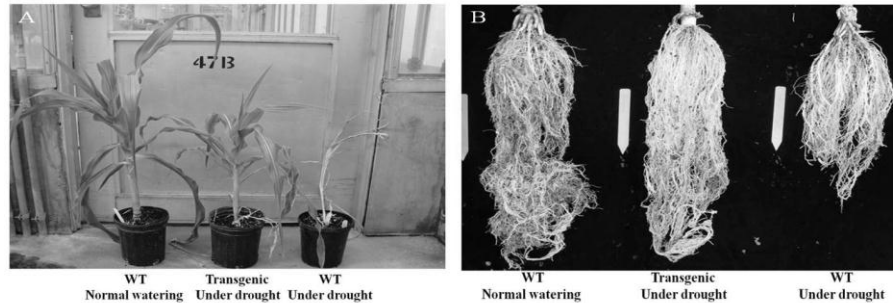
As much as some are pessimistic about the existence of the global climate change, the evidence of such changes are now obvious, not only to the scientists in the area, but among ordinary people who observe year after year of more drought and severe soil salinity resulting in reducing of agricultural yields. An example of Iran's climate change can be observed in Khozestan where temperatures are predicted to increase by 3.7°C, resulting in an increase in more water needs of about 2.00 mm/year. Furthermore, soil water evaporation due to higher temperatures will result in more soil salinity.

The crop to be most affected by climate change in Iran will be wheat with an increase water requirement. Therefore, effective Government policies are needed to be developed by authorities in Iran on developing crop varieties that are draught and salinity tolerant, plus needs for water usage management across the country, most specifically in the of Iran.

Iran needs to stay ahead of the global climate change games. To stay ahead of the climate change challenges, my international team of scientists have developed oat, rice and corn plants that are tolerant to drought and soil salinity. We transferred a combination of three different genes into different crop genomes, one gene being a transcription factor (master switch) for drought and salt tolerance taken from the crop of sub-Saharan Africa, sorghum. The team sat down and decided

to take the sorghum drought and salt tolerance master switch, and transfer it to other crop genomes.

Here are a few examples:



Looking at the left picture of the above slide, the dead plant was watered with 200 mM salt water and the plant on the further left is also the wild-type control plant which was watered with no-salt water. The plant in the middle is transgenic and was watered with 200 mM salt water.

On the right picture, you are looking at the roots of these plants, the root you see on the furthest right and furthest left were from control plants, but the root in most right was watered with 200 mM salt water, so it is so small. The root in the middle is from a transgenic plant that was also watered with 200 mM salt water, but it grew well just as it was no salt in its irrigation water.

Looking at another slide (see below), the transgenic and control oat plants were not watered for three weeks. The plant on the right died, and the plant on the left grew well and produced abundant seeds because it is transgenic.

A similar work was done on corn (see the slide of a corn field with Prof. Sticklen in the field)

4. Finally the 4<sup>th</sup> initiative that Iran might wish to consider is to invest in Crop Synthetic Biology research.

Although only a decade old and mostly applied to creating synthetic microbes, crop synthetic biology will soon become the future of the world's agriculture. It includes embedding circuits of completely artificial genetic codes into naturally existing crops.

Unlike crop genetic engineering that deals with transfer of single genes or a combination of single genes into a crop genome, crop synthetic biology deals with engineering-based principles and mathematical modeling to design, construct and test completely new 'synthetic crops' that can serve the world as more nutritious food, stronger fiber, higher calorie biofuels and value-added products such as better polymers and best and cheaper biotech drugs.

Some of you among the audience are experts in crop genetic engineering. In crop genetic engineering, the host crop already has an operational network of most of the genetic codes that naturally behave, and you will add a few extra genes to the crop's genome. In case of synthetic biology, the whole network is synthetic. The crop synthetic biology needs engineering concepts and precise mathematical modeling.

Similar to what I addressed in staying ahead of the climate change, Iran also needs to stay ahead of the Synthetic Biology research by training of its Agricultural molecular biologists, mathematical modelers and software engineers to collaborate in order to learn how the natural crop genetic circuits work together, and predict, fabricate and incorporate its better options for developing synthetic crops to be used for multiple purposes.

As I close my speech, I wish all of the Agricultural scientists in Iran the best in the development of their new and innovative ideas. And once again, I encourage your government to invest in higher education that leads into new ideas, greater imaginations and innovations.

Please do not hesitate to contact me at Michigan State University (Email: [stickle1@msu.edu](mailto:stickle1@msu.edu)).

In conclusions, I wish to thank Professors Vahdati and Eghbal for their kind Invitation to speak to you today.

God Bless Iran and God bless Iran's Agricultural Scientists