Advances in Crop Science and Technology

Sticklen, Adv Crop Sci Tech 2015, 3:3
http://dx.doi.org/10.4172/2329-8863.1000171

Short Communication Open Access

Advances in Crop Synthetic Biology: A Platform that Bridges the Expertise of Crop Molecular Biologists, Engineers, Software Developers and Mathematical Modelers

Mariam Sticklen*

Department of Plant, Soil and Microbial Sciences, Michigan State University, East Lansing, MI 48824, USA

There were enormous numbers of controversies among public around the applications of genetically modified (GM) crops in early 1990s, and no one could assume that over 90% of certain crops in the U.S. agricultural fields would be genetically modified after only a decade, in year 2000. Despite the fact that there are still organizations that oppose GM crops or at least wish that the GM crop products be appropriately and rightlylabeled, yet a new paradigm shift from GM crop technology has emerged, as:plant synthetic biology".

Plant synthetic biology has been recently effectively applied in embedding of completely artificial genetic code circuits into naturally existing plants used as framework of "chassis" [1].

The solid foundation of synthetic biology is rooted from the system biology i.e. a technology that quantitatively characterizes the molecules and the way that the networks of molecules behave [2].

While crop. genetic engineering deals with transfer of single genes or a combination of single genes into a crop genome, the synthetic biologyof cropsdeals with engineering-based principles plus mathematical modeling for designing, constructing and testing of a completely new crop genetic system for production of better food, fiber, biofuels and value-added products such as polymers and advanced biotech drugs. Therefore, synthetic biology requires bridging of the expertise of molecular biologists, engineers, software developers and mathematical modelers who can work together to understand how the natural crop genetic circuits work together, and predict, fabricate and incorporate its better options into the crops of interest.

In the conventional crop genetic engineering, the host plant already has an operational network for most of the genetic codes that naturally behave. In case of synthetic biology, the whole network is synthetic. Therefore the fundamental differences between the two are the "collection, application and modeling of the quantitative data involved with the technology, and the fact that crop synthetic biology approaches need engineering concepts and precise simple or complex mathematical modeling [3].

The complexity of the mathematical modeling in synthetic biology depends on the complexity of the coding circuits. For example,circuits that consist of molecules such as transcription factors that control the expression of other genes might require a more complex mathematical modeling than a circuit consisting of pathway enzymes without the involvement of transcription factors.

Microbial synthetic biology involves the synthesis and applications of a whole circuit in microbes. As compared to microbial synthetic biology, crop synthetic biology has the advantage of the fact that coding circuits already exists in crops, and therefore it is possible to mimic suchcircuits while developing a predicted advanced version of such circuits.

Initially, a team of scientists [4] designed and successfully engineered a plant genetic system with *artificial* oscillatory network as an artificial clock using mathematical modeling for gene expression rates and decay rates of the transcription and repressor proteins. The team used the green flourescence protein (GFP)color reportergeneto observe the gene expression and decay as the GFP glows in the dart upon its gene expression and stops glowing upon the decay of its gene expression. While GFP gene has been previously expressed in many plants via simple genetic engineering, the [4] experiment confirmed the possibility of engineering the complete genetic circuits including a time sensitive clock in plant cells.

Then, another group of scientists [5] successfully developed a well designed synthetic biology system of two communicating sets of cells, one group of cells as the "sender" cells and the other group as the "receiver" cells. By including the GFP gene in the system, they successfyly observed the intracellular communications that mimics eukaryotic organisms' cell to cell communications.

Via conventional genetic engineering, scientists have already produced accumulated oil in tobacco and *Arabidopsis* vegetative tissues either by overexpressing of the key enzyme that is associated with lipid biosynthesis pathway [6] or by blocking of a chemical reaction that causes lipid hydrolysis in plants [7]. It is expected that using an advanced synthetic biology, scientists should be able to develop a crop that contains a combination of artificial genetic coding circuits for lipid biosynthesis along with regulatory systems that can produce such oil only in the crop vegetative wastes. Studies of system biology of an oil crop lipid metabolic pathway [8] must consist of the rational design of each gene involved in the pathway, the pathway's genetic systems and the other systems of such oil crop for the purpose of producing oil. In more advances in understanding of lipid metabolic pathway, thisshould not be impossible because plants already contain the naturally existing operational biological gene circuits with varying kinetic behaviors for producing oil. Therefore, scientists can synthesizecircuits designed to over-express some of the key genes and transcription factors that are associated with lipid metabolic pathway.

However, unlike the single transgene genetic engineering technology, the scientists working on plant synthetic biology for producing a novel oil crop must first logically design the networks, measure the stability of the networks' steady-state, model their behaviors, and finally assemble the system in form of genetic circuits

---

***Corresponding author:** Mariam Sticklen, Department of Plant, Soil and Microbial Sciences, Michigan State University, East Lansing, MI 48824, USA, Tel: 1 517-355- 0271; E-mail: stickle1@msu.edu

Received April 15, 2015; **Accepted** June 13, 2015; **Published** June 20, 2015

Citation: Sticklen M (2015) Advances in Crop Synthetic Biology: A Platform that Bridges the Expertise of Crop Molecular Biologists, Engineers, Software Developers and Mathematical Modelers. Adv Crop Sci Tech 3: 171. doi:10.4172/2329-8863.1000171

with standardized appropriate parts, and with predictable and reliable expected functions [9].

Because the synthesis of most metabolic pathways such as the one for oil biosynthesis or for plant cell wall biosynthesis circuits are so large, scientists working on such synthetic biology also need to use the routine engineering techniques called"decoupling and abstraction"i.e. breaking of the whole circuits into smaller modules or pieces, and testing of each pieceprior to their assembly [2].

Like in engineering, the prefabricated parts such as DNA pieces must be combined following the engineering rules of "assembly standard" including speed, versatility, laboratory autonomy and full combinational potential where the parts can become interchangeable. Despite the above rules, the need for mathematical modeling of each piece of the circuits or each prefabricated part is important in order to assure the rationality of the designed genetic circuits [2].

Also, in an ideal 21st century affordable manufacturing scenario, the pre-fabricated parts of any circuits should also be potentially used in different synthetic biology hosts such as different crops.

A very powerful DNA assembly system called "Golden Braid" has been used in plant synthetic biology research. This DNA assembly system contains DNA building modules (parts) to be used in different crop synthetic biology approaches, a system that can be optimized for many uses. The Golden Braid DNA assembly modules arealready commercially sold in form of a "tool kit" for their uses in plant synthetic biology research [1].

The authors team used the techniques of RNAi genetic engineering and produced a corn crop that its cell walls contains about 8% less lignin, and therefore not only its stover could be converted into fermentable sugars with less needs for pretreatment processes, but also the energy saved by plants by producing less lignin was shifted to producing more cellulose resulting in more fermentable sugars [10,11] Using synthetic biology of corn crop, it might be possible to improve the systen by first logically designing the circuits network, measuring the stability of its steady-state, modelling their behaviors, and finally assembling the system in form of genetic circuits with standardized appropriate parts, and with predictable and reliable expected functions. Therefore the crop synthetic biology is much more complicated, but also more precise with better predictable results.

The author's team also produced a corn crop that its stover expresses all three microbial cellulase enzymes (endoglucanse, exoglucanase and beta-glucosidase) needed to convert the plant cellulose into fermentable sugars. This transgenic corn cropself-produces all of these three cellulases in its stover (not in flowers, seeds or roots) and such stover could be converted into fermentable sugars without the needs for any externally applied microbial cellulases [12]. If the system was to be synthetically made using the technologies associated with synthetic biology, it should be possible to logically design circuits networks of crop cell walls consisting of cellulase genes, measure the stability of their steady-state, model their behaviors, and assemble the system in form of genetic circuits with standardized appropriate parts, and apply the system for production of a novel corn crop with predictable and reliable expected functions of self producing cellulases.

It might be also possible to combine both of the above examples to produce a bio energy crop with ideal cell walls that have high cellulose and hemicelluloses molecules; but with stronger but yet more bioprocessable lignin contents.

Plant synthetic biology can also include multiple genetic regulatory system designs [13], such as switches or promoters to turn on and off the genes as they are required [2].

Synthetic biology of *E. coli*-producing butanol by Michelle Wang's team is a great example of the potential of the powerful synthetic biology [14]. It is also encouraging to see the most recent advances in comprehensive DNA assembly framework developed for plant synthetic biology [1].

In using the plant synthetic biology, it is also possible to predict the directed evolution and use such predictions to design novelcrops similar to those selected in vivo for the best traits [15], or produce novel cereal crops that can fix nitrogen, fix carbon more efficiently with a much more effective photosynthesis, produce and store more sugars, have water use efficiency, disease and pest resistance and more nutritious seeds and vegetative tissues.

Despite all of its prmisses that potentially can advance the foold and energy security and produce low-coss industrial products, there are multiple challenges associated with the application of the crop synthetic biology. The challenges are; (1) unavailability of most naturally existing biological genetic pathway systems and the ambiguity of the certain genes involved such as those in cell wall synthesis pathway [16] or plant fatty acid biosynthesis and assembly pathways for oil production [17], (2) limited and impeding ability to rationally designthelanguage of the plants genetic system [18,19] and its gene circuits for the finest predictable direct evolution, (3) the unpredictable evolution of the genes' regulatory sequences, and (4) the acceptance of synthetically produced crops by the public.

While more fundamental research is needed to reduce most of the above challenges, the author expects major public concerns on the applications of synthetic biology. However, such concerns would lessen as soon as the benefits of synthetic biology become more obvious to the public. Public needs to become educated by social scientists and by the media on the most humanitarian benefits of the system biology, such as the one developed for the creation of the award winning anti-malarial drug produced through synthetic microbes [20]. Such drug can save millions of human lives in the developing nation where malaria is the most cause death, especially in children.

References

1. Sarrrion-Perdigones M, Vazquez-Vilar J, Palaci B, Castelijns J, Forment, et al. (2013) GoldenBraid 2.0: A comprehensive DNA assembly framework for plant synthetic biology. Plant Physiol 162: 1618-1631.
2. Bowen TA, Zdunek JK, Medford JI (2008) Cultivating plant synthetic biology from system biology. New Phytologist 179: 583-587.
3. Drublin DA (2007) Designing biological systems. Genes & Development 21: 242-254.
4. Elowitz MB, Leibler S (2000) A synthetic oscillary network of transcriptional regulators. Nature 403: 335-338.
5. Basu S, Gerchman Y, Collins CH, Arnold FH , Weiss R (2005) A systemic multi cellular system for programmed pattern formation. Nature 434: 1130-1134.
6. Andrianov V, Borisjuk N, Pogrebnyak N, Brinker A, Dixon J, et al. (2010) Tobacco as a production platform for biofuel: Overexpression of Arabidopsis DGAT and LEC2 genes increases accumulation and shifts the composition of lipids in green biomass. Plant Biotechnol. J 8: 277-287.
7. Slocombe SP, Cornah J, Pinfield-Wells H, Soady K, Zhang Q, et al. (2009) Oil accumulation in leaves directed by modification of fatty acid breakdown and lipid synthesis pathways. Plant Biotechnol J 7: 694-703.
8. Sticklen M (2013) An Advance in Crop Science and Technology: Potentials for Producing the High-Value High-Calorie Triacyglycerols Commodity in Crop Vegetative Wastes. Advances in Crop Sci. and Technology e104.

9. Andriantoandro E, Basu S, Kraig DK, Weiss R (2006) Synthetic biology: New engineering rules for an emerging discipline. Mol Sys Biol 2: 0028.

10. Park S-H, Mei C, Pauly RM. Garlock BE, Dale R. Sabzikar H (2012) Down-regulation of Maize Cinnamoyl-CoA Reductase via RNAi Technology Causes Brown Midrib and Improves AFEX™-Pretreated Conversion into Fermentable Sugars for Biofuels. Crop Sci. 52: 2687-2701.

11. Park S-H, Ong RG, Mei C, Sticklen M (2014) LigninDown-regulation of Zea mays via dsRNAi and klason lignin analysis. 89.

12. Park S-H, Ransom C, Mei C**,** Sabzikar R, Qi C, et al. (2011) In the quest of alternatives to microbial cellulase mix production: Corn stover-produced heterologous multi-cellulases readily deconstruct lignocellulosic biomass to fermentable sugars. J Chem Techno Biotechnol.

13. Gardner TS, Cantor CR, Collins JJ (2000) Construction of a genetic toggle switch in Escherichia coli. Nature 403: 339-342.

14. Wen M, Bond-Watts BB, Chang CY (2013) Production of advanced biofuels in engineered *E. coli.* Current Opinion in Chemical Biol 17: 472-479.

15. Yokobayashi Y, Weiss R, Arnold FH (2002) Directed evolution of a genetic circuit. Proc Natl Acad Sci, USA. 99: 16587-16591.

16. Sticklen M (2007) Feedstock genetic engineering for biofules. Crop Sci 47: 2238-2248.

17. Vanhercke T, ElTahchy A, Liu Q, X-Rong Zhou, Shrestha P, et al. (2014) Metabolic engineering of biomass for high energy density: oilseed-like triacylglycerol yields from plant leaves. Plant Biotechnol J 12: 231-239.

18. Pedersen M, Phillips A (2009) Towards programming languages for genetic engineering of living cells. J Royal Society of Interface.

19. Umesh P, Naveen F, Rao MA, Nair S (2010) Programming languages for synthetic biology. Syst Synth Biol 4: 265-269.

20. Priscilla EM, Purnickand R, Weiss (2009) The second wave of synthetic biology: from modules to systems. Nature Reviews Mol Cell Biol 10: 410-422.

Citation: Sticklen M (2015) Advances in Crop Synthetic Biology: A Platform that Bridges the Expertise of Crop Molecular Biologists, Engineers, Software Developers and Mathematical Modelers. Adv Crop Sci Tech 3: 171. doi:10.4172/2329-8863.1000171