# Synthetic Biology and its Applications in Agriculture

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## Abstract

Modern biotechnological tools are helpful for more refined improvement of agronomically important traits. Synthetic biology is a broader term applicable to genetic engineering approaches that construct either new biological elements or redesign existing biological systems to build new or improved functions/trait modification. This technology enables the introduction of multiple genes in a single transgenic event, either synthetically generated or derived from a foreign organism. Thus, it can address multiple challenges including improving photosynthetic ability, nutrient acquisition, tolerance to heat and drought stress, fungal diseases, insect pests, removal of antinutritional factors, improvement of quality of produce in a range of important crops which are required to maintain and improve yields and production efficiency in the era of accelerated incidence of biotic and abiotic stresses posed by climate change.

## Introduction

Synthetic biology is the engineering of biology *i.e.*, 'the synthesis of complex, biologically based (or inspired) systems, which display functions that do not exist in nature'. It encompasses approaches that design and construct new biological elements (e.g., enzymes, genetic circuits, cells) or redesign existing biological systems to build new and improved functions for useful purposes. It also includes creation of non-natural building blocks to replicate natural functions or develop novel functions (Design and construction of new biological parts, devices and systems). This engineering perspective may be applied at all levels of biological organization, from the molecular level to entire organisms. Synthetic Biology or 'SynBio' upgrades the potential of genetic engineering, enabling more rapid development of transgenic material with more complex modifications, which is favorable for the development of elite crop cultivars. Application of synthetic biology in agriculture shows:

1. The potential of transforming metabolic pathways, genetic circuits and plant architectures in crop improvement.
2. The application of synthetic biology in microorganisms plays a role in sustainable agriculture, such as biofertilization, bio stimulation and biocontrol.

**Eg., $C_4$ rice development** - It requires introduction of five genes from the NADP-ME biochemical subtype (Ermakova et al., 2020). Traditional genetic engineering involves cycles of single gene introduction and subsequent stacking events. SynBio techniques (e.g., Golden Gate cloning) enabled this complex transformation to occur in 6 to 12 months (Ermakova et al., 2020) by staking of 5 genes.

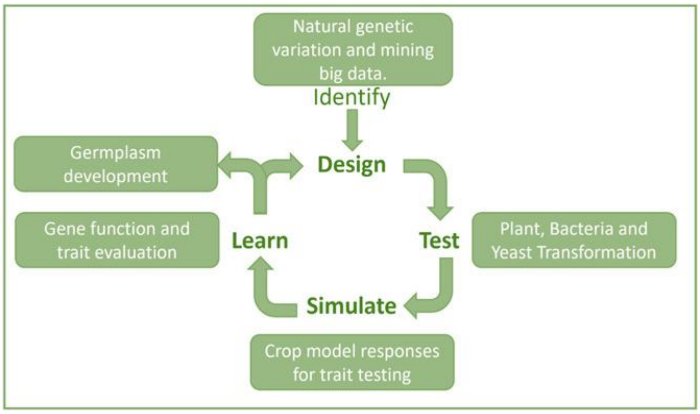



**Fig 1** The design, test, simulate and learn cycle of developing and introducing novel traits into food and fibre crops through synthetic biology.

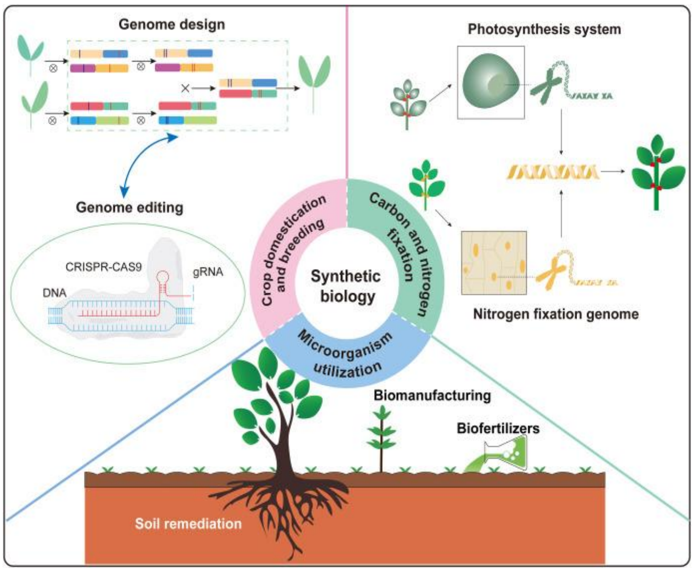



**Fig 2** Synthetic biology opportunities within agricultural crops

**Promising Synbio Solutions To Key Agronomic Challenges**

**1. Photosynthetic carbon assimilation**

- Enhancing productivity and thermotolerance through improving photosynthesis
- Improving crop WUE and drought tolerance

**2. Nutrient acquisition**

- Introducing enzymes
- Enhancing existing microbial activity
- Establishing rhizobium–legume-like interactions

**3. Biopesticides to combat insect pests**

- Controlling insect populations using gene drives
- Fungal diseases

**Enhancing productivity and thermotolerance through improving photosynthesis**

- Rubisco catalyses carbon fixation (carboxylation) inside the chloroplast and is a long-standing target of photosynthetic enhancement for yield gain.
- Carboxylation by Rubisco is aided by its 'helper protein' Rubisco activase, which prevents Rubisco from becoming inactivated and is thermolabile under abiotic stress
- Carbon assimilation and crop biomass and yield have been improved under heat stress by introducing more thermostable Rubisco activase
- Overexpression and manipulation of SBPase (responsible for regeneration of RuBP during the Calvin cycle, the substrate for carboxylation by Rubisco) can enhance photosynthesis under heat stress in transgenic rice

**Nutrient Aquisition**

- More than half of the world's food consumption uses synthetic nitrogen fertilizers.
- Nitrogen fertilizer production consumes much energy.
- More than half of it leaches from the soil, causing not only environmental pollution but also energy consumption.
- Plants cannot fix their nitrogen, introducing biological nitrogen fixation into crops is necessary to reduce our dependence on nitrogen fertilizer and protect the environment while maintaining agricultural productivity.
- Genetic engineering techniques are used to introduce nitrogen-fixing enzyme genes into target organelles such as mitochondria and chloroplasts in plants to help plant nitrogen-fixing enzyme cofactors to complete biosynthesis.
- Expanding the scope of symbiotic nitrogen fixation and establishing new, non-legume crops and rhizobia symbiotic nitrogen fixation systems. The main focus is biotechnology to guide rhizobia to infest non-host plant cells.
- Genetic engineering techniques transfer nitrogen genes to non-legume crops to promote symbiotic nitrogen fixation by non-legumes. An example is the transfer of *nif* genes to plants via vectors.
- Nitrogen fixation cell engineering technology, through cell engineering but the introduction of rhizobia into non-host plant cells as a whole to establish a new symbiotic nitrogen fixation system, this technology can not only expand the range of legume hosts but also transfer symbiotic nodulation capacity to non-legume crops.

**Construction of synthetic crop microorganisms to help boost agricultural production**

- Promoting nutrient uptake. A high diversity of bacterial flora contributes to the synergistic uptake of nutrients by crops and promotes crop growth.
- Mitigation of biotic stresses. The diversity of microbial communities is closely related to the disease resistance of plants, and colonies with a high degree of microbial diversity are more effective in resisting the invasion by pathogenic bacteria.
- Mitigation of crop succession barriers. Crop succession barriers are an important scientific problem that limits crop yields. The imbalance of the soil microbial community is one of the key factors leading to crop disorders.
- Reducing chemical fertilizer use. It is important to protect the environment, save energy and improve food security production.

**Construction of synthetic microbiomes in crops**

- **Identification of plant root system-associated fungi**

By identifying root system-associated fungi of agricultural crops, we can effectively apply our findings to promote plant growth, enhance plant disease resistance, and improve plant stress tolerance.

- **Engineering of Crop Microbial Communities**
  - Modify the target genes of microorganisms and genetically alter the characteristics of microorganisms by regulating signaling molecules
  - Isolate target genes from donor organisms and introduce them into recipient bacteria to engineer crop microbial communities to help achieve environmental remediation, crop disease prevention, improved crop nutrient uptake, improved yields and further reduce the use of chemical fertilizers in agricultural production.

**Bioremediation**

- Bioremediation of PAHs (Poly Aromatic Hydrocarbons) mainly refers to relying on microorganisms to degrade the toxic and harmful pollutants in the environment and decompose them into non-toxic and harmless $CO_2$ and $H_2O$.
- *E. coli* engineered bacteria that can degrade organic pollutants using synthetic biology technology

**Cultivation of *E. coli* Engineered Bacteria for Degradation of Organic Pollutants**

**Bio pesticides**

- Biopesticides refer to natural substances from different sources that can control pests.
- Compared with chemical pesticides, biopesticides have the characteristics of low toxicity to non-target organisms, easy degradation, low dosage, and high effect, therefore, the development of biopesticides has a good prospect for development.
- Biopesticides offer a safer alternative to chemical pesticides.
- They are typically derived from living organisms or their byproducts, making them biodegradable and posing minimal risks to non-target organisms and ecosystems.
- By reducing synthetic chemicals dependence, biopesticides contribute to biodiversity preservation and environmental health

**RNAi-based genetic engineering for insect resistance**

- RNAi biopesticides are emerging as highly targeted sprays for the control of specific insect pests (Fletcher et al., 2020).
- These highly specific topical applications reduce the need for chemical pesticides that may be damaging to the environment and nontarget organisms.

The application of RNAi technology in pest control can be achieved by host-induced gene silencing (HIGS) through

1. The generation of transgenic plants expressing dsRNAs, or by
2. spray-induced gene silencing (SIGS) involving foliar application of dsRNAs to the surface of plants.

Bioclays are foliar sprays that are developed by loading dsRNA molecules into layered double hydroxide nanoparticles for more stable delivery of the RNA compared to 'naked RNA' applications

**Generation of transgenic plants expressing dsRNAs**

- Design of small interfering RNA (siRNA), composed of exogenous double-stranded RNA fragments that partially match the mRNA sequence of the target gene.
- When siRNA enters the plant cell, it binds to the mRNA of the target gene. This results in degradation of the target mRNA or blockage of translation, thus inhibiting the expression of the target gene.
- The constructed RNAi vector with siRNA is introduced into the target plant cells, then the transgenic plant will integrate this RNAi vector and express the siRNA.
- The transgenic plant produces siRNAs that interfere with the expression of genes in the target pest.
- After the pest ingests the siRNA, inhibition of the target gene may lead to abnormal growth and development or death of the pest. This is the anti-insect effect.
- **Cry protein** genes from *Bacillus thuringiensis* to develop *Bt* cotton and corn is a prime example of the use of 'traditional' genetic engineering techniques to achieve insect pest resistance against Lepidopteran pests like American Bollworm
- insecticide usage has decreased by 61%–81% and damage losses have reduced by 47%–63% in the USA and 97% since 1992 in Australia
- A new genetically engineered trait has been developed by Bayer—ThryvOn—after an extensive search for hemipteran active *Bt*'s and directed evolution to increase its toxicity to the lygus bug, providing increased protection in cotton against mirids, lygus bugs and thrips

- This trait stacking technology may also enable the development of more complex protection to limit the development of resistance in pests such as *H. armigera*.
- This is also likely to be the most efficient way of developing new cultivars with resistance to multiple insect pests that are not controlled by the current *Bt* traits, such as whitefly and mites.

**Controlling insect populations using gene drives**

- Gene drives (promotion of deleterious alleles) can enable effective and self-sustaining control of insect pest populations by increasing the frequency of deleterious alleles such as sterility or lethal alleles (Bier, 2022). Gene drives could also be used to revert pesticide-resistant insect populations back to susceptible
- the insect pest could be modified to remove its ability to sense a target crop. Similarly, the chemical profile of the target crop could be modified (or masked by a topical application) or make it 'invisible' or 'repulsive' to the insect

**Fungal diseases**

- Trait stacking through Golden Gate cloning could be beneficial for the development of complex and effective resistance in a range of susceptible crops, as well as developing cotton cultivars with dual resistance to both pathotypes of *Verticillium.*
- Using RNAi technology and/or CRISPR-Cas9 in combination with TALENS could also be an effective approach to developing resistance, as these technologies have been used to combat bacterial blight (*Xanthomonas*) infection in rice (Li et al., 2020) and powdery mildew (*Blumeria graminis* f. sp. *tritici*) in wheat (Wang et al., 2014)

**Reducing Anti nutritional factors in plants**

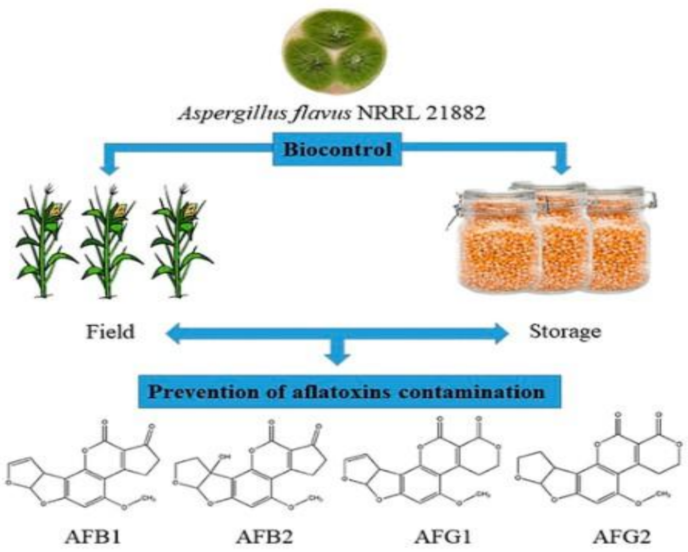
**Biocontrol of Non-Aflatoxigenic *Aspergillus flavus***

- Aflatoxins contaminate many commercial value crops, including corn, oilseeds, rice, and nuts.
- Aflatoxin contamination in cereals can occur during the pre-harvest or post-harvest stages. High temperatures and humidity can stimulate fungal growth in fields and storage rooms.
- Most potent mutagenic, teratogenic, and carcinogenic compounds among mycotoxins, and long-term exposure can cause severe disease in humans, poultry, fish, and cattle.

**Development of Non-Aflatoxigenic *Aspergillus flavus* Strains**

- Isolated several non-aflatoxin strains, including K49, F3W4, NRRL 58,974, NRRL 58,976, NRRL 58,988
- Application of non-aflatoxigenic *Aspergillus flavus* strains to soil as infected barley, rice, wheat grain cultures or directly inoculate corn cobs by injection.

Non-aflatoxigenic *aspergillus flavus*: a promising biological control agent against aflatoxin contamination of corn. (Lavkor *et al*., 2023).




**Photoautotrophic systems as bio-production platforms**

- Due to the low cost, high scalable biomass yield, and absence of endotoxin synthesis, photoautotrophic organisms are well suited for systems for large-scale production of immunotherapeutic, biopharmaceuticals, and biofuels.
- Efficient green cell factories, can be used to produce drugs and cosmetics. Aquatic phototrophic organisms, such as some algae, are also relevant for biofuel production.
- In addition to phototrophic organisms, such as artificial leaves and synthetic photosynthetic cells, fully synthetic or biohybrid systems can also use solar energy as a driving force. Eg. Artemisinin, extracted and isolated from the plant *Artemisia annua*, is currently the drug of choice for treating malaria.

**Improved Saccharification Efficiency**

- Improving glycosylation efficiency is important for improving biofuel production
- TALEN-mediated mutation of multiple caffeic acid O-methyltransferase alleles in sugarcane

## Conclusion

Traditional genetic engineering involves the transfer or modification of single genes or components. In contrast, SynBio tools are capable of developing complex multigenic traits through the simultaneous introduction or manipulation of multiple genes, derived from donor organism(s) or synthetically generated. Hence SynBio enables the rational and systematic design of biological systems. With the current average growth 35% globally, the impact of synthetic biology in future agriculture and nutrition is expected to pronounce in areas of improvement of plant growth and production, nutritional value, photo autotrophic synthesis of biofuels, immune therapeutics, soil microbial activity, reduction in fertilizer usage, biopesticides, bioremediation.

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