SYNTHETIC BIOLOGY: UNLEASHING BIOTECHNOLOGY IN AGRICULTURE AND HEALTHCARE

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ABSTRACT

The Synthetic Biology is an emerging technology to use biology as substrate to engineer products of commercial importance. The basic tools for the development include genetic engineering; molecular biology with bioinformatics software. The development of synthetic biology has been pushed by medical and pharmaceutical requirements. Since the rapid developments started from more than a decade ago, synthetic biology has grown substantially and has emerged with many achievements, both in science and application aspects the future for synthetic biology-based therapeutics are promising, with new tools and applications developed in biomedical fields and highly-efficient microbial pharmaceutical production in the twenty-first century. Synthetic biology also involves the modification of living being for essential purpose in agriculture by manufacturing them with new and modified capabilities. This emerging new field of biology is used by researchers to evolve the power of nature to resolve hindrance in agriculture. There are multiple pathways of synthetic biology which can acclimate in different agriculture related applications like plant carbon efficiency, reduce synthetic fertilizer usage by optimizing plant nitrogen and phosphorous utilization, improve the nutritional value of crop plants, and harness the power of photoautotrophic organisms as large-scale production platforms.

Keywords: Synthetic Biology, genetic engineering; molecular biology, bioinformatics software, agriculture, synthetic fertilizer

INTRODUCTION

Synthetic Biology is referred as Synbio/ Synthetic Genomics/ Constructive System Biology. The term Synthetic Biology is anonymous to Bio-engineering. The use of computer assisted biological engineering for the design and construction of new synthetic biological parts, devices and systems which do not exist in nature is termed as Synthetic biology. It is redesigning of biological organisms which incorporates the molecular biology techniques. It mainly introduces synthetically constructed biological parts and is not limited to only the modification of natural organisms.It differs from Recombinant DNA Technology as it involves construction of new life forms with no natural counter -part.

The term Synthetic Biology was first used by Barbara Hobom to describe genetically engineered bacteria. The key fundamental techniques involved in Synthetic Biology include the following:

A) Computational Biology: The prediction of system performance prior to fabrication is an important part of synthetic biology. It mainly relies on the computer modeling systems and uses the approach of engineering cycle for the design of biological candidates.
B) DNA Sequencing: The genome of an organism contains complete instructions for constructing any type of protein cell tissue organ etc. It is also referred as second key technology of synthetic biology as it reads the sequence of the genome of an organism.
C) DNA synthesis: it is the third key technology for Synthetic biology as the genome once sequenced can now to be used to re-write or synthesize all or part of a particular genome.

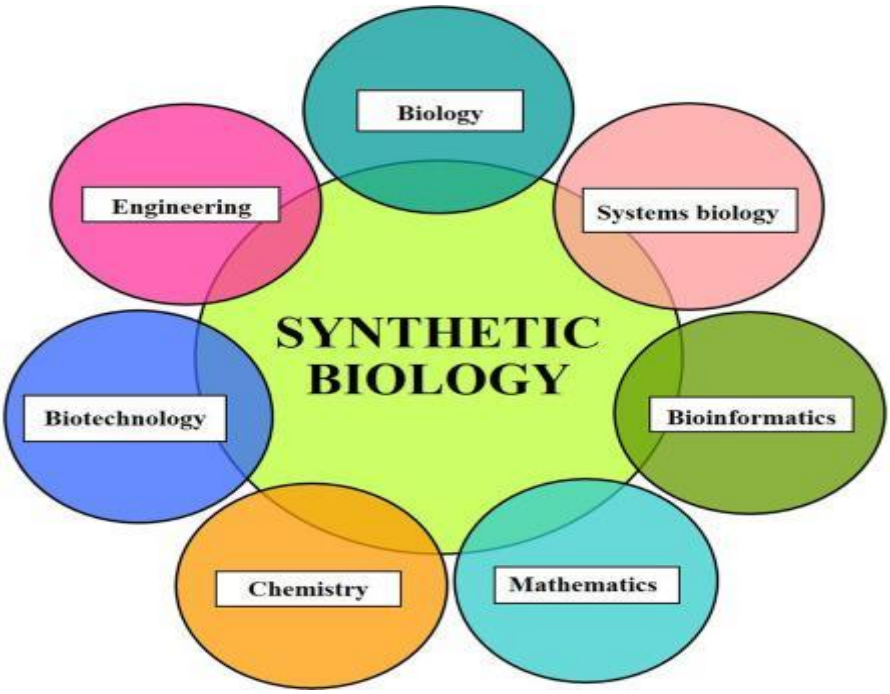

Fig No. 1: Synthetic Biology

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SYNTHETIC BIOLOGY: NOVEL BIOLOGICAL RESEARCH

The ultimate goal of synthetic biology is to develop the products of commercial importance. It is mainly used for the design of engineered biological organisms that process information. It also maintains human health and environment. The researchers mainly focus on the production of vaccines through synthetic microbes. The common example is the use of engineered yeast in the production of artimesinic acid, an antimalarial drug. It also useful in the fabrication of materials and structures like re-engineering of Type III secretion system of *Salmonella typhinurium* to secrete spider silk protein. The other important aspect of Synthetic Biology is the use of synthetically engineered organism to break down biomass into sugars for fuel production. The use of re-engineered algae to produce oil used in transportation and energy infrastructure. It also involves replacement of natural products with synthetically produced equivalents. The best example is the re-engineering of gene encoding isoprene in *E.coli* for the synthetic production of rubber. Other examples are Synthetic insulin produced by yeast and bacteria are used by millions

of diabetics worldwide. Also, Golden rice is rice engineered to produce beta-carotene (precursor of Vitamin A) to prevent vitamin A deficiency in 190 million children and 19 million women across 122 countries. [2]

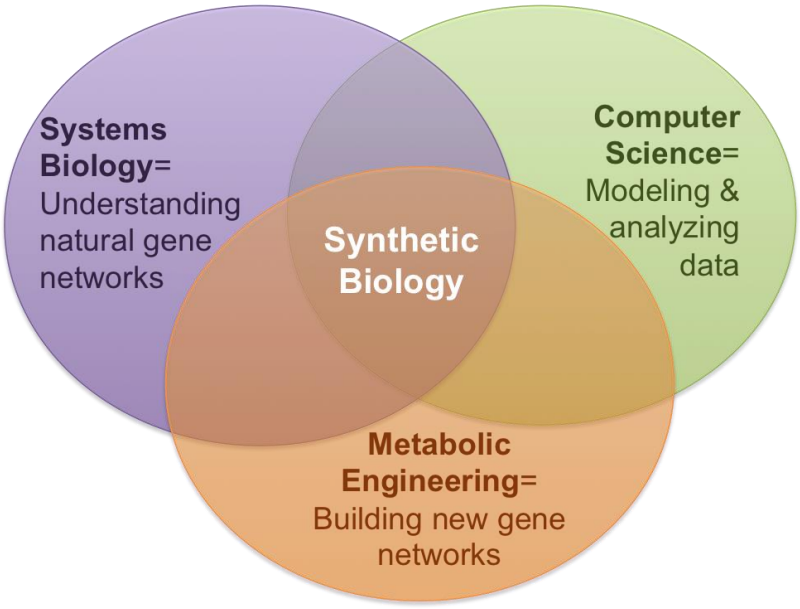

Fig. No. 2: Overview of Synthetic Biology

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APPLICATIONS OF SYNTHETIC BIOLOGY

a) IN MEDICINE AND HEALTHCARE

The advancement in healthcare and medicine includes the integration of heterologous pathways into designer cells to efficiently produce medical agents, the construction of novel genetic circuits for tumor targeting, the controllable release of therapeutic agents in response to specific biomarkers to fight diseases like diabetes and cancer, and enhanced yields of natural products in cell growth media to equal or higher than that of plant or fungal extracts. Research in medicine and pharmacology gains new powers from synthetic biology. By using the engineering paradigm, researchers can create systems that are reliable, strong, and have unique features not seen in nature. [4].The various fields where the role of synthetic biologist is seen are as follows:

(i)Synthetic biology-based vaccines

Vaccines are crucial components of public health and instrumental in reducing the morbidity and mortality of numerous diseases. The fundamental goal of training the human body to respond robustly to a pathogen [4] without causing severe disease requires two main steps: selecting an antigen and[5] delivering it into the body. Current vaccines use either whole (inactivated or live attenuated) microbes or viruses or selected components that are introduced into the body via diverse methods. Numerous innovations in genetics, biochemistry, structural biology, and bioinformatics have resulted in significant advancements in vaccine design and production [6].

(ii)Genomic codon-deoptimized vaccines

The balance between safety and efficacy of vaccines is often difficult to achieve and is compounded by multiple technical challenges. Attenuated live viruses yield highly effective vaccines that offer long-lasting protection. However, no suitable low-virulence species exist for most infectious diseases, and the commonly used method of attenuation through serial culture takes many years and may not produce safe strains [7] . Whole inactivated viruses are easier to generate but frequently lead to short-term protection that is primarily humoral and may even worsen disease outcomes. Alternative methods for generating effective, attenuated live viruses that avoid prolonged culture and minimize reversion to virulent virus are urgently needed. Alternative methods for generating effective, attenuated live viruses that avoid prolonged culture and minimize reversion to virulent virus are urgently needed [8]

The advent of low-cost nucleic acid synthesis has allowed synthetic biologists to reengineer entire viral genomes using large-scale synonymous mutations. This method of viral attenuation [9] uses the degeneracy of triplet codons and the non-random frequencies of specific codons, codon pairs, and di-nucleotides that many species exhibit.

(iii)Therapies based on chimeric antigen receptor (CAR)-T cells

The receptors used in CARs are modified to have regions for both antigen-binding and T cell activation. After being extracted from patients and modified ex vivo to produce a particular CAR, T cells are then reintroduced into the original donor patient to eradicate cancer cells that surface-expressed the target antigen [5]. Three distinct eras of artificial CAR generation have

occurred. While the subsequent generations CARs also have a co-stimulatory domain, such as 4-1BB or CD3ζ, the initial CARs solely have an intracellular CD3ζ domain. Research is also being done on third-generation chimeric receptors for antigens that have several co-stimulatory signaling domains. [10]. The primary benefit of using CAR-based techniques for immunotherapy against cancer is that the single-chain variable fragment (scFv) is produced from the antibodies with affinities that are many orders of significance more than those of traditional TCRs.[11] Furthermore, CARs have the ability to target glycolipids, aberrantly glycosylated proteins, and structural variations that TCRs find difficult to detect. The FDA recently approved CAR-T treatments aimed toward the CD19 protein for therapy of acute lymphoblastic leukemia (ALL) and large B-cell lymphoma (DLBCL). These indications of CAR-T cells' potent anti-tumor therapeutic effects are based on the results of clinical trials. Furthermore, CAR-T applications are beginning to be commercialized. Kymriah, a CD19-targeted CAR T-cell treatment for DLBCL, was created by Novartis and the University of Pennsylvania and was the first to be authorized. [12]. It is difficult to treat multiple myeloma with medication or stem cell transplantation. Preclinical studies show that CAR-T cell therapies are effective for treating multiple myeloma. B cell maturation antigen (BCMA), a protein belonging to the TNF superfamily, is the most promising candidate among the antigens utilized thus far for a multiple myeloma cell-directed CAR-T therapy target. Almost all multiple myeloma patients have 10 BCMA expressed in their cancer cells; this antigen is only expressed on plasma cells and certain types of B cells in somatic cells (4). The first multiple myeloma antigen to be used in a clinical trial using a CAR-T cell approach was BCMA, which caused patients with the cancer to respond systematically [4].

(iv) Induced pluripotent stem cells (iPSCs) for medical applications

Through the overexpression of specific genes linked to dedifferentiation, synthetic biology also aids in the creation of human stem cells. Induced pluripotent stem cells are one of the uses. Pluripotent stem cells, or iPSCs, are produced from somatic cell. Four transcriptional factors, including Oct3/4, Sox2, c-Myc, and Klf4, were first used by Yamanaka's lab to transform fibroblasts into embryonic stem (ES)-like cells, which can re-differentiate into blood cells, bone cells or neurons for possible treatment of damages to various tissues and organs. Unlike ES cells, which are created using human embryos, iPSCs do not involve ethical concerns during the

creation process [14] Moreover, autologous somatic cells produce iPSCs that are immune rejection-free. The goal of iPSCs is to be used in therapy development and tissue regeneration. To meet the demand for blood transfusions, iPSCs can be used to generate type O red blood cells. iPSCs can be used to create NK cells, which can be produced in large quantities for cancer patients who require them for immunotherapies, despite their limited availability [13] .Studies on mice reveal that iPSCs have anti-aging properties. It has long been standard practice to chemically induce iPSCs to differentiate into cardiomyocytes. By replicating the genetic codes of the patients from whom they originated, these iPSC-cardiomyocytes enable the creation of models for ischemic heart disease and long QT syndrome. [14] In order to treat malfunctioning mouse retinas, cord-blood cells can be induced into pluripotent stem cells. Redifferentiated iPSCs are then used to treat brain lesions in mice, restoring their motor abilities following therapy [15].

PSCs are effectively used for organ regeneration; for instance, the Yamanaka technique can be used to convert fetal hearts into normal hearts using ex vivo cardiomyocytes. Three distinct cell types, namely iPSCs, endothelial stem cells, and mesenchymal stem cells, can be used to create human "liver buds." The liver buds self-packed into a complex organ for rodent transplantation thanks to the biomimicking processes. It works effectively to metabolize medications [15]. A number of iPSC applications have progressed to the clinical stage. A clinical research plan was approved in Japan, and patients are currently being recruited. For instance, a team at Osaka University created “myocardial sheets” from iPSCs and transplanted them into patients with severe heart failure. Patients are being recruited for phase I clinical trials employing autologous iPSC-differentiated hematopoietic stem cells to treat thalassemia [16].

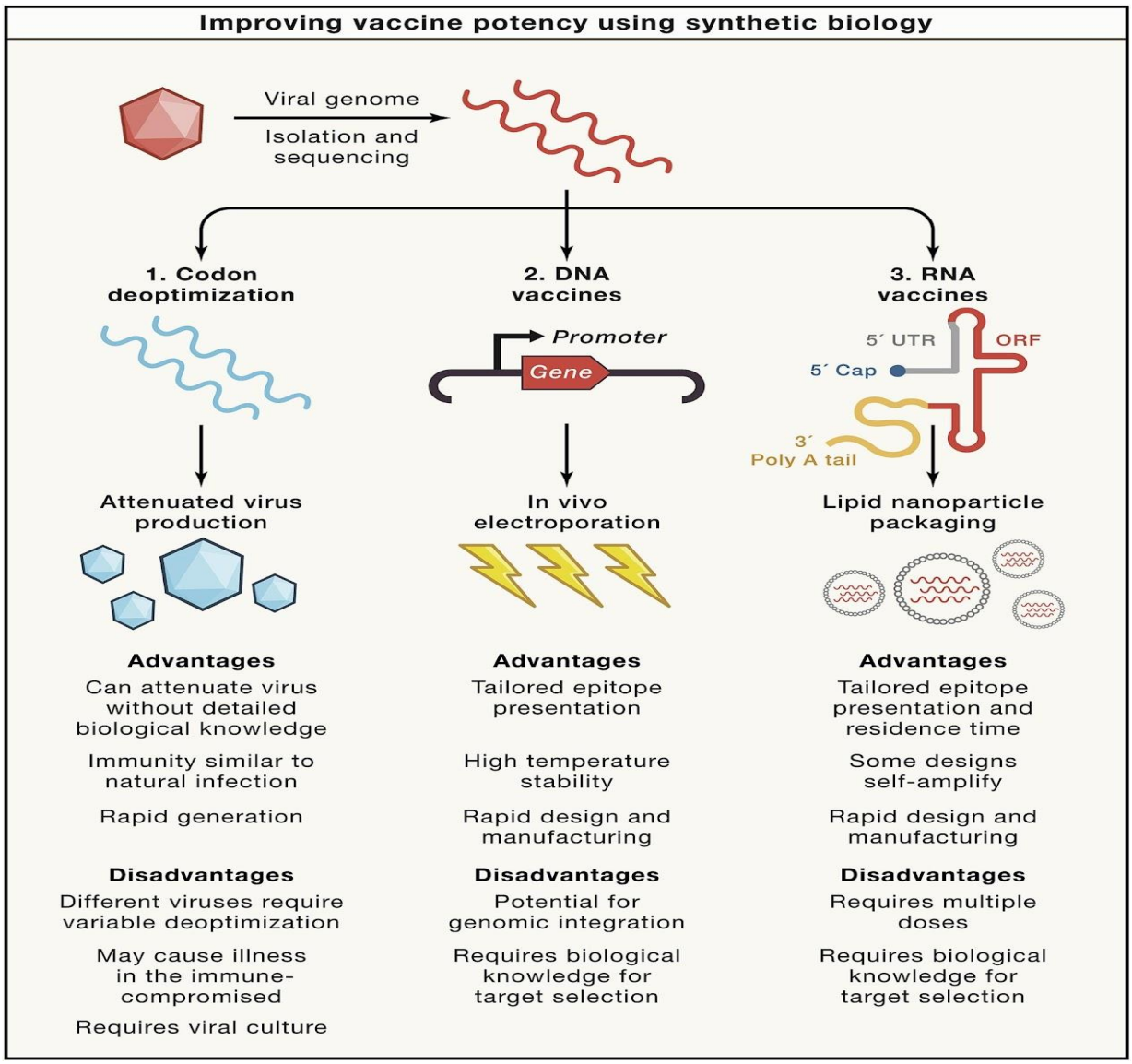

Fig. No. 3: Synthetic biology and vaccine design [4]

b) IN AGRICULTURE

The synthetic biology can contribute in improvement of agriculture field through various ways like by enhancing the nutritional value of the food, by improving the variety if microbes' linkage with plant root, making plant resistant against climate, pests, weeds etc. In this review article we mainly focused in one of the branches of synthetic biology which immensely contributes in every perspective specially in agriculture and plant biotechnology is CRISPR-Cas. CRISPR is an abbreviation of (clustered regularly interspaced short palindromic repeats)-Cas stand for

(CRISPR associated protein) and they combine form a mechanism. CRISPR (clustered regularly interspaced short palindromic repeats)–Cas (CRISPR-associated protein) is an adaptive phage immunity system in archaea and bacteria. As they rely on DNA–RNA recognition and binding for sequence-specific nucleic acid cleavage, CRISPR–Cas9 and other CRISPR–Cas systems can be easily programmed to introduce DSBs at any desired target site at minimal cost [17]

(i) CRISPR–Cas in crop upgrade and breeding

CRISPR-Cas has become a very useful technology in agriculture because of its unmatched capacity to accurately modify plant genomes,. It has transformed the present breeding processes in addition to aiding in the development of novel kinds with desirable features. Furthermore, CRISPR-Cas has made it possible to quickly domesticate new species. Unless otherwise noted, SpCas9 was utilized for genome editing in the majority of the investigations included in this section.

(ii) In increasing yield

Among the many variables influencing yield, one useful strategy to raise cereal production is to modify cytokinin homeostasis. In rice, modifying the C terminus of Oryza sativa LOGL5, which encodes an enzyme that activates cytokines, increased crop production under a range of environmental circumstances[18] .Similarly, high-yield phenotypes in wheat were produced by knocking down the gene encoding the enzyme cytokinin oxidase/dehydrogenase (CKX), which catalyzes the degradation of cytokinin[19]. Rice cultivars with higher yields and tiller numbers while preserving grain quality were created by deleting the gene encoding amino acid permease 3, which is important in nutrient partitioning[20]. Crop plants with higher yields have also resulted from CRISPR-Cas-mediated editing of other genes, such as *O. sativa* PIN5b (controlling panicle size), *O. sativa* GS3 (regulating grain size), *Triticum aestivum* GW2, *O. sativa* GW2, and *O. sativa* GW5 (regulating grain weight)[21-23]. Researchers have edited CLV [24] and ENO [25], which regulate meristem size, to enhance the output of fruit crops in addition to grains.

(iii)**Improving quality**

A crop's other qualities, aside from yield, are as important for agricultural output. Lower amylose content grains are better for cooking and eating, and they are used extensively in the adhesives and textile industries. Granule-bound starch synthase 1 (GBSS1) is essential for the manufacture

of amylose. Lower amylose content grains are better for cooking and eating, and they are used extensively in the adhesives and textile industries. Granule-bound starch synthase 1 (GBSS1) is essential for the manufacture of amylose. Therefore, using CRISPR–Cas9 to disrupt GBSS1, waxy maize variants were produced in 12 elite inbred lines. [26]

(iv)Disease resistance

Plant protection against biotic stress may be achieved more successfully by employing CRISPR-Cas to alter host susceptibility factors, as opposed to inserting dominant resistance genes, which tend to encourage the reciprocal evolution of resistance in pathogens.The deadly pathogen *Xanthomonas oryzaepv. oryzae*, which causes bacterial blight, poses a serious danger to the world's rice crop.A set of bacterial stimuli can trigger the transcription of the SWEET genes during an infection; the products of these genes are essential for the susceptibility to illness.Researchers have created rice lines with broad-spectrum resistance to *X. oryzaepv. oryzae* by utilizing CRISPR-Cas to alter the promoter region of *O. sativa* SWEET11, *O. sativa* SWEET13, and *O. sativa* SWEET14 [27,28].Similar to this, in citrus, Xanthomonas citri subsp. citri can be resistant when Citrus × sinensis LOB1's promoter region is targeted [29].

Blumeriagraminis f. sp. tritici is a biotrophic fungus that can produce powdery mildew in wheat. Plants with improved resistance to *B. graminis f. sp. tritici*, were produced by simultaneous CRISPR–Cas mutation of the three wheat homologues of enhanced disease resistance 1 (EDR1), a gene encoding a MAPK kinase kinase that suppresses defense responses to powdery mildew [30].Similar to how a wheat variety with broad-spectrum resistance to powdery mildew was created by simultaneously mutating all three *mildew-resistance locus O* (MLO) homologues, targeting *Solanum lycopersicum* MLO1 in tomato using CRISPR-Cas conferred tolerance to *Oidiumneolycopersici*, which causes powdery mildew in tomato[31]. CRISPR-Cas9 may be engineered to cleave plant DNA viruses' genomes and impart viral resistance because it can produce double-strand breaks (DSBs). By employing this strategy, scientists have created plant immune systems that are resistant to geminivirus diseases [32] and Caulimovirus [33]

(v) Herbicide resistance

Creating herbicide-resistant germplasms becomes a financially viable strategy to preserve high agricultural output and stop soil degradation as weed issues spread around the world. When compared to traditional transgenic techniques that incorporate foreign herbicide-resistant genes, like bar, which encodes phosphinothricin N-acetyltransferase into crops, CRISPR-Cas is a desirable method due to its transgene-free, fast, and flexible editing of herbicide-targeted genes to confer endogenous resistance.A crucial enzyme in the manufacture of branched-chain amino acids, acetolactate synthase (ALS) is the target of herbicides like imidazolinone and sulfonylurea. Herbicide tolerance in ALS can be achieved by precise amino acid changes, according to research on naturally occurring point mutations in the ALS gene [34]. Therefore, rice was given herbicide resistance while still exhibiting ALS activity when certain base transitions were introduced into *O. sativa* ALS by the application of CBEs [35].Similar techniques were applied to other species as well, introducing specific ALS mutations by HDR to give herbicide resistance [36].Coenzyme acetyl A carboxylase, also known as ACCase, is an important target for herbicides and an enzyme involved in lipid production. The *O. sativa* ACCase gene was mutated by an ABE to substitute C2186R, resulting in rice variants that were haloxyfop-tolerant-(methyl) [37]. More than 100 plant varieties produced by genome editing technologies have been approved by the US Department of Agriculture as not regulated, allowing commercial cultivation in the country, even though the majority of these varieties are still in the experimental stage. These include high-oil content camelina [38], powdery mildew resistant wheat [39], and oleic acid-enriched soybean varieties produced by disruption of Glycine max FAD2.

CHALLENGES AND FUTURE SCOPE:

Synthetic Biology recreates systems with emergent properties of evolution, inheritance and genetics. It is also similar to Biomimetic chemistry. The biology is complex process but often context dependent. The synthetic organism threatens the biological diversity and impact the conservation and sustainability if not used properly. It has the capability to create organisms that never existed before and their complexity increase over time. Therefore there is need to establish proper regulations and safeguards for the proper evolvement of this technology in practice. The potential risks are real and the technological are not limited to beneficial use only. The major risk is the accidental release of a synthetic organism with unintended determinal effects on

environment and human health [40]. The other risk is the creation of unpredictable microorganism which might have unpredictable impact on environment and human health [41]. However the creation always leads to the applications like biofuel, vaccines, pharmaceuticals, food products etc if managed properly.So it is dual sense technologies which causeboth harm and benefits. For example, there is huge value in our ability to engineer viruses to be more effective and specific shuttles for gene therapies of devastating inherited disorders; however, engineering viruses may also lead to the creation of even more deadly pathogens by those intent on harm. The Synthetic biologist mainly works on standardization of biological parts, application of designed proteins, product synthesis and study of genomics. Commercial firms that sell synthetic DNA (oligonucleotides, genes, or genomes) to users are DNA synthesis companies, including ATG:biosynthetics, Blue Heron Biotechnology, DNA 2.0, GENEART and Genomatica [42]. Scientists and engineers will need to design organisms that remain stable; this could be achieved through efforts that prevent the organisms from being able to evolve new traits or that cause them to lose their designed traits. However, whereas it is relatively easy to predict what a synthetic organism will do in its intended environment, it is far more difficult to predict how it will evolve after multiple generations of exposure to environmental pressures or interaction with other organisms.

CONCLUSION

Synthetic Biology has tremendous potential in all areas of life science including our daily lives. So, it is importamt in both academic applied industrial sectors. However with proper development some ethical issues arise at an early stage in its development. So there occurs need to resolve issues at early stages that may prevent its misuse. The transformation of synthetic biology into an emerging engineering discipline requires foundation tools with applications in basic and applied research. The l influences in human health, energy and sustainability is aoption to make era of synthetic biology should last forever overcoming its societal challenges. Even so, the future for synthetic biology-based therapeutics are promising, with new tools and applications developed in biomedical fields and highly-efficient microbial pharmaceutical production in the twenty-first century.

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