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Opportunities and synthetic biology in agricultural crops

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Abstract

The development of several agronomically significant features in a variety of crops has been largely dependent on conventional breeding methods. The use of contemporary biotechnology techniques expanded and improved trait introduction and development beyond what was feasible with traditional breeding. However, biotic and abiotic conditions that need the production of more genetically complicated features than can be handled by classic genetic engineering or conventional breeding continue to pose challenges to agricultural yields. Therefore, when climate change increases the frequency of biotic and abiotic threats to food and fibre crops, more sophisticated trait development techniques are needed to maintain and enhance yields and production efficiency. Synthetic biology, or SynBio, includes methods for creating new biological components (such as cells, genetic circuits, and enzymes) or redesigning preexisting biological systems to provide new and enhanced functionality. Genetic engineering, which entails transferring individual genes from one creature to another, is "upgraded" by SynBio. Multiple genes, either synthetically created or taken from a foreign organism, may be introduced in a single transgenic event thanks to this method. Additionally, it may make it possible to create new genomes from scratch using a collection of standardised genetic components, which can then be inserted into the intended cell or creature. The introduction of new genes with known functions, artificial genetic variation, topical applications of small RNAs as pesticides, and possibly accelerating the production of new cultivars with elite traits are all new opportunities to advance breeding applications through the use of SynBio technology. The potential use of SynBio to increase agricultural yield and resilience to different obstacles will be shown in this review via case studies. Here, we describe particular ways to use the SynBio toolset to address issues including fungal diseases, insect pests, heat and drought stress, and nutrient uptake in a variety of significant crops.

KEYWORDS

crops, photosynthesis, biotic stress, abiotic stress, and SynBio

1 INTRODUCTION

In agricultural crops like cotton (fibre quality attributes [Campbell et al., 2010; Clement et al., 2015], crop maturity [Campbell et al., 2010; Chen & Du, 2006], and disease resistance [Bell, 1994; Hillocks, 1998; Knight, 1946; Stiller & Wilson, 2014], wheat (dwarfing genes, increased water-use efficiency [WUE], flowering time, and preharvest sprouting; Christy et al., 2018;

Gifford et al., 1984; Sansaloni et al., 2020; Sheehan & Bentley, 2010). Insect and herbicide resistance in broadacre crops are examples of advancements made possible by the use of contemporary biotechnology techniques that surpass the capabilities or effectiveness of traditional breeding (Dill, 2005; Downes et al., 2017). However, biotic and abiotic variables still pose a threat to agricultural output. Furthermore, genetic diversity in cultivated cotton (Iqbal et al., 2001; Wendel et al., 1992), wheat (Sansaloni et al., 2020), and canola (Rahman, 2013) germplasm is becoming limited, and new diversity frequently needs to be sought in close relatives, even though advancements in traditional breeding have yet to reach a ceiling in many crops. Therefore, in order to maintain and increase yields and production efficiency, more sophisticated crop cultivar development techniques like synthetic biology (SynBio) are needed, particularly when climate change increases the frequency of biotic and abiotic threats to food and fibre crops.

2 | WHAT IS SYN BIO?

More than ten years ago, a group of European experts came up with a consensus definition of SynBio: "Synthetic biology is the engineering of biology: the synthesis of complex, biologically based (or inspired) systems, which display functions that do not exist in nature" (Synthetic Biology: Applying Engineering to Biology: Report of a NEST High Level Expert Group; Vancompernelle & Ball, 2005). All levels of biological organization, from molecules to complete organisms, may benefit from this engineering viewpoint. Biological systems may be designed logically and methodically thanks to SynBio (Serrano, 2007). It includes methods for creating new biological components (such as cells, genetic circuits, and enzymes) or redesigning preexisting biological systems to provide new and enhanced functionality. These methods may be used in two subfields: (1) creating nonnatural building blocks to mimic natural functions or generate new ones, and (2) exploiting biological building blocks already in existence to construct combinations not found in nature. Many widely used engineering concepts, like "switch," "rewire," and the "design, test, simulate, learn cycle," have been included into SynBio throughout its development (Figure 1; Liu et al., 2015). Since numerous tools and methods might be labelled as synthetic, the definition of what constitutes SynBio is hotly contested. Additionally, numerous labels have emerged as technology and vocabulary have evolved.

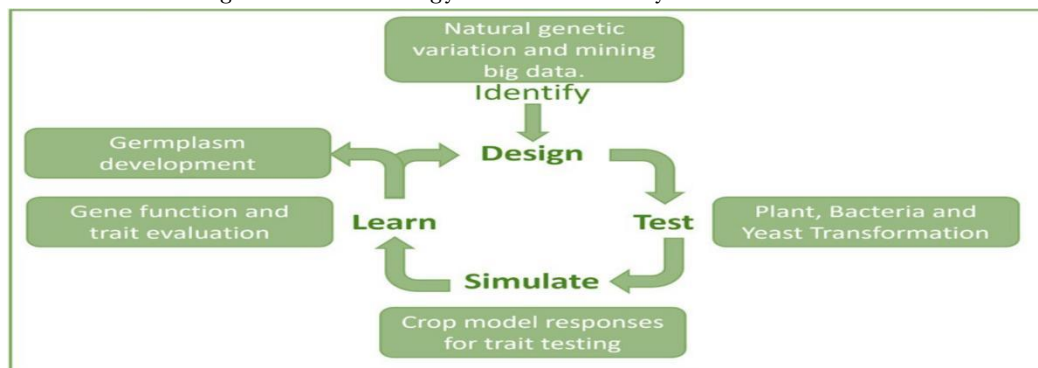


FIGURE 1; The process of designing, testing, simulating, and learning new characteristics for food and fibre crops using synthetic biology. Information from natural variation and big data mining may be used to create novel strategies for increased resistance to biotic and abiotic challenges. High-throughput plant, bacterial, and yeast systems are then used to test these. To ascertain the effect on yield and resource-use efficiency, it is possible to simulate the expected consequence of the modifications for crops. This teaches us how to change crop yield in the most effective manner, and we may start using these qualities or find more variety to include into SynBio design. The development of germplasm is then aided by the selection of valuable characteristics. used in related scientific domains, such as genetic engineering, biotechnology, and SynBio. Single genes or components are transferred or altered in traditional genetic

engineering (Roell & Zurbriggen, 2020; Serrano, 2007). On the other hand, SynBio techniques may create complex multigenic features by simultaneously introducing or modifying numerous genes that are either synthetically created or obtained from donor organisms (Roell & Zurbriggen, 2020). As a result, SynBio “upgrades” genetic engineering’s potential by making it possible to create transgenic material with more intricate alterations more quickly, which is advantageous for the creation of superior agricultural cultivars. For instance, five genes from the NADP-ME biochemical class were added to C4 rice during its early growth (Ermakova et al., 2020b). Traditional genetic engineering, which involves rounds of single gene introduction and subsequent stacking processes, would have required years to achieve this metamorphosis. This intricate change might take place in 6 to 12 months thanks to SynBio procedures like Golden Gate cloning (Ermakova et al., 2020a). Additionally, SynBio can facilitate the creation of new genomes from a collection of standardised genetic components, which may then be introduced into the intended cell or creature (Serrano, 2007). A potential SynBio technique is gene editing, which modifies an organism’s genome without introducing foreign genetic material (Pixley et al., 2019). Another method in the SynBio toolbox that has a lot of promise for use in agriculture (such as biopesticides) is the topical application of double-stranded RNA (dsRNA) to induce gene silencing by RNA interference (RNAi). Similar to this, topical RNA virus transfection may be used on crops to temporarily change agronomic characteristics like blooming time and stress responses by temporarily expressing or

TABLE 1 SynBio tools with potentially valuable applications in agriculture

Technology	Description	References
CRISPR-Cas9	Targeted in vivo gene editing. An efficient tool for silencing, changing or enhancing specific genes or integrating transgenes into a specific location in the genome.	
Golden Gate	Simultaneous and directional in vitro assembly of multiple DNA fragments into a single construct. A valuable tool for stacking multiple genes for complex, multigene traits. Crucial for modular cloning that can be used to exchange promoter and terminator elements.	
RNAi	Targeted gene silencing by RNA-interference (RNAi). Useful for silencing undesirable genes (i.e., toxic compounds in edible tissues) or silencing critical processes in undesired organisms (i.e., infection mechanisms of fungal diseases).Mao et al. (2013)	
Engler et al. (2009)		
Liu et al. (2020) and Niehl et al. (2018)		
Gene drives	Promoting inheritance of deleterious alleles (i.e., lethal or sterile alleles in insect pests). Bier (2022)	
Gene synthesis	Rapid assembly and cloning of identified genes into DNA constructs. www.genscript.com as an example.	
Regulated promoters	Regulated promoters can temporally control gene expression by activating or deactivating downstream genes under specific conditions such as environmental stress or phenological development.Khan et al. (2017) and Schreiber and Tissier (2017)	

Abbreviations: CRISPR, Clustered Regularly Interspaced Short Palindromic Repeats; RNAi, RNA interference; SynBio, synthetic biology.

suppressing genes involved in regulation (Torti et al., 2021). With the potential to create innovative agricultural products and greatly enhance agricultural management, productivity, and sustainability, there are several SynBio instruments and approaches that may be used in agricultural contexts (Table 1). Furthermore, the creation of novel artificial promoters will enable the on/off regulation of genes based on the presence of chemicals or biotic and abiotic

elicitors (Schreiber & Tissier, 2017). A variety of "fundamental" and "applied" research applications are available with SynBio. SynBio has led to and continues to lead to significant advances in our knowledge of basic biology. Nonetheless, there are many potential real-world uses, ranging from agriculture to medical. The need for agricultural goods is rising to sustain a growing population, but agricultural sectors continue to confront significant problems, especially those related to climate change. SynBio methods, which allow for the efficient and successful redesign of even the most complicated biological systems, might be used to overcome this difficulty.

3 | WHAT IS INSIDE THE SYNBIO TOOLKIT?

3.1 | CRISPR-Cas9 (gene editing)

One of the quickest, simplest, and most affordable gene editing methods is CRISPR-Cas9 (Hayes et al., 2018). Because of its straightforward design and ease of building DNA constructs, it is preferred as an alternative to traditional plant breeding and transgenic techniques (Belhaj et al., 2015). The Type II CRISPR-Cas system, which is involved in the immune system and defence mechanism in bacteria and archaea, is used in this method (Farzadfard et al., 2013; Jinek et al., 2012; Qi et al., 2013). Conserved Cas1 and 2 proteins stitch fragments of invasive DNA into the bacteria's CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) region during the recognition (or adaptation) phase of foreign nucleic acids (either from a virus or plasmid) in bacteria. This enables the bacteria to record and identify the infecting virus (Jinek et al., 2012). Base pairing with transactivating crRNA (tracrRNA), which binds to the Cas9 protein, is necessary when these areas are translated into crRNA (CRISPR RNA) and processed (Mir et al., 2018). After identifying consecutive matching nucleic acids using this crRNA-tracrRNA compound as a guide, the Cas9 ribonucleoprotein exploits its nuclease function to eliminate the invading DNA (Mir et al., 2018). Jinek et al. (2012) found that a synthetic guide RNA (gRNA) made of tracrRNA and crRNA could recognise certain DNA sequences and cut the DNA there. If a repair template is provided, it may be edited to a different particular sequence, or random repair of the cut can add deletions or minor random insertions (Jinek et al., 2012). The Nobel Prize was recently given to the development and discovery of this technology (Ledford & Callaway, 2020). This procedure may be used to "fix" genetic mistakes or eliminate certain genes, such as those that cause illness. By deactivating Cas9 to prevent it from cutting DNA and combining Cas9 with transcriptional activators, this method may also be altered to increase gene transcription (Konermann et al., 2015). Because single-gene knockouts or single base-pair alterations would not need to be regulated in an increasing number of nations, gene editing using such technology is seen positively (e.g., Waltz, 2018). In 2019, the Australian government announced that gene-editing methods in plants and animals that do not add new genetic material—for example, CRISPR-Cas system incisions that can be naturally repaired without a gRNA—would not be classified as genetically modified organisms (GMOs; Mallapaty, 2019). The appropriate government authorities will need to regulate editing methods that actually use new genetic material, such as the insertion of new genes or amino acids.

3.2 | Golden Gate (gene assembly)

One toolkit that allows the modular assembly of several (up to ten) DNA fragments into a single vector backbone—a DNA molecule used as a vehicle to transfer genetic material into a cell—in "single tube" reactions without leaving any "scars" at the joins is Golden Gate cloning (Werner et al., 2012). In order to enable exact assembly, the components (promoters, coding sequences, and terminators) are made to include recognition sequences. Additionally, distinct recognition sites at the ends of the constructed DNA fragments enable precise insertion into the target

vector (Engler et al., 2009). Because they cut outside of their recognition region, leaving specific overhangs for ligation (DNA joining) using T4 DNA ligase, and because the recognition sequence is removed after digestion, the DNA fragments cannot be digested again, Type IIS restriction (cutting) enzymes (such as BsaI) are essential to this system (Engler et al., 2009). This guarantees that only properly built goods are kept whole and that parts that are no longer needed for the finished product are eliminated (Engler et al., 2014). With the right regulatory sequences, this is a desirable method for putting together big gene constructs and stacking many genes for effective multitrait transfer. Common design principles allow for the modular assembly of gene components, allowing for the selection of suitable regulatory control elements for gene expression and the incorporation of multigene complexes that include whole metabolic pathways into plants.

3.3 | RNAi (gene silencing)

In eukaryotic species, RNAi is a naturally occurring post-transcriptional gene silencing process that guards against infections and/or viruses that produce abnormal RNA molecules (Ashfaq et al., 2020). In this process, the aberrant RNA is identified and transformed into dsRNA (Waterhouse et al., 2001). The RNAi response is triggered by dsRNA, which is broken down into 21-nucleotide small interfering RNA (siRNA) molecules by the DICER enzyme (Hung & Slotkin, 2021). After being attached to an argonaut protein, the siRNA molecules are used as a guide strand to identify certain messenger RNA (mRNA) areas for destruction (Fire, 1999). In some instances, the complex might directly block the translation of particular genes, therefore silencing them. RNA interference (RNAi) has been used to silence genes for a number of agricultural purposes, including making insect pests sterile or dead when they consume RNAi-producing plants, altering the composition of seed oil, preventing the production of toxins in edible crop tissues, and inhibiting fungal and viral plant pathogens, such as bacterial blight (*Xanthomonas*) infection in cotton (Cox et al., 2017).

| Gene drives (promoting inheritance of deleterious alleles)

By using CRISPR to introduce enzymes that degrade unwanted genes in chromosomes, gene drives enhance the frequency of harmful alleles and allow the intended harmful gene to be duplicated and inherited (Bier, 2022). Certain gene drives have the potential to be both geographically limited and reversible (Pixley et al., 2019). This technique might be used to target illnesses, weeds, and pests (e.g., sterilise insect pests or prevent weeds from establishing seeds).

3.4 | Gene synthesis (increasing the speed of cloning)

The creation of plasmid DNA utilised in biotechnology and SynBio has been transformed by gene synthesis (e.g., www.genscript.com). In order to express proteins in *Escherichia coli* or other hosts and convert plants and algae, previous gene cloning depended on PCR amplification from a variety of sources, which also involved the integration of restriction sites for cloning. The speed at which DNA constructions may be created has been accelerated by the use of gene synthesis in conjunction with Golden Gate cloning and other modular cloning techniques, significantly cutting down on the time needed for even complicated multigene complexes. Additionally, this has led to the creation of gene foundaries, which collect necessary elements and put them together to create functional systems (Chambers et al., 2016). In the end, gene synthesis has made it possible to clone genes that were found using transcriptome and DNA sequence data. Additionally, even for extremely big genes, codon modification of sequences is made easy to guarantee the effectiveness of translation of foreign genes across species (Gustafsson et al., 2004).

3.5 | Regulated and artificial promoters

For next-generation solutions, it's critical to comprehend how promoters turn on and off in response to environmental stimuli. This has significant agricultural implications since it allows genes to be triggered or silenced in response to disease triggers (Arnaiz et al., 2019) or certain environmental circumstances (low or high temperatures; Grover et al., 2013). A thermotolerant isoform of ribulose-1, 5-bisphosphate carboxylase/oxygenase (Rubisco) activase

triggered under heatwave conditions or water conservation genes that may be activated after the detection of drought stress are two examples (Sharwood, 2017). These are effective techniques because they restrict the expression of genes to times when they are most desperately needed, especially for genes that may be energetically costly. Transcription activator-like effectors (TALEs) and synthetic TALE-activated promoter (STAP) systems are examples of artificial promoters that can increase the expression of several genes from a single promoter (Boch & Bonas, 2010; Boch et al., 2009; Brückner et al., 2015; Schreiber & Tissier, 2016, 2017). According to Schreiber and Tissier (2016), TALEs are transcription factors that control the transcription of endogenous genes in plants. In this technique, the desired promoter is used to generate the TALE gene activator, which then binds to the STAP promoter or promoters to drive the expression of the desired gene or genes (Brückner et al., 2015). Another example is the production of dead Cas9 coupled to a potent transcriptional activator that uses certain gRNAs to target particular promoter regions of genes of interest. It has been shown that this system is a potent gene expression stimulator (Xu et al., 2019).

4 | PROMISING SYNBIO SOLUTIONS TO KEY AGRONOMIC CHALLENGES

The development of agronomically significant features in a variety of crops has improved agricultural yield, productivity, and resilience thanks in large part to conventional plant breeding techniques. For instance, conventional breeding techniques, such as marker-assisted selection, have enhanced widespread disease resistance in wheat (Aktar-Uz-Zaman et al., 2017; Liu et al., 2000), rice (Luo et al., 2017; Suh et al., 2013), and potatoes (Haverkort et al., 2016; Zhu et al., 2012). Throughout rice's domestication, breeding and selection have led to a reduction in seed dispersion, an increase in apical dominance, a decrease in seed dormancy, compact panicles, and bigger inflorescences and grains (Doebley et al., 2006; Ishii et al., 2013; Zhu et al., 2013). Maize yields have significantly increased as a result of breeding for leaf architectural features that allow for larger planting densities, inhibited ear prolificacy, inflorescence branching, and tillering (Duvick, 2005; Tian et al., 2011; Vollbrecht et al., 2005; Wills et al., 2013). A number of advantageous agronomic traits, including improved fibre quality (Muthusamy & Jayabalan, 2011), photoin sensitivity (Raut et al., 1971), fungal pathogen resistance (Ganesan & Jayabalan, 2006), herbicide tolerance (Rajasekaran et al., 1996), and heat tolerance (Rodriguez-Garay & Barrow, 1988; Trolinder & Shang, 2001), have been successfully introduced into cotton. Adoption of contemporary biotechnology techniques has produced genetically modified cotton cultivars with extra characteristics like insect and herbicide resistance in addition to traditional breeding (Perlak et al., 2001). Despite these developments throughout time, pests, weeds, diseases, nutrient uptake, and abiotic stressors still pose a threat to agricultural yields; thus, new traits and more genetic diversity are needed. However, the capacity to take use of the genetic variety present in agricultural germplasm collections limits the ability to overcome these obstacles via conventional breeding (Dwivedi et al., 2007, 2017; Sharwood et al., 2022, in press). For crop breeding programs to target specific agronomic challenges, genetic and reproductive barriers like interspecific incompatibility (Bedinger et al., 2011; Kitashiba & Nasrallah, 2014), genetic drag (i.e., introducing unfavourable traits along with any new favourable traits; Langridge & Fleury, 2011; Varshney et al., 2014), and the inability to effectively combine multiple desired alleles for complex traits (Lyzenga et al., 2021). SynBio techniques may help overcome these constraints for successful and efficient production of new traits.

4.1 | Insect pests

To maintain and increase agricultural productivity and lessen dependency on pesticides, new, effective methods of controlling insect pests and their capacity to develop resistance are needed. SynBio technologies provide many ways to deal with insect infestations. The insertion of genes for the synthesis of toxins in specific agricultural tissues has traditionally shown notable results. One of the best examples of using 'traditional' genetic engineering methods to produce insect pest resistance is the introduction of *Bacillus thuringiensis* Cry protein genes to create Bt maize and cotton (Carriere et al., 2010; Cousins et al., 1991; Downes et al., 2016; Fitt &

Wilson, 2005; Shelton et al., 2002). These crystalline proteins (Schnepf et al., 1998) offer efficient and comparatively specific defence (Mendelsohn et al., 2003) against Lepidoptera species, including autumn armyworm (*Spodoptera frugiperda*), cereal stem borer (*Busseola fusca*), and cotton bollworm (*Helicoverpa armigera*) (Tabashnik et al., 2013). Depending on the degree and timing of damage, these moth species' larvae may reduce crop output by feeding on plant terminals, reproductive structures, and stems (Leigh et al., 1996; Sadras, 1995). Insecticide use has dropped by 61%–81% and damage losses have fallen by 47%–63% in the USA after the introduction of Bt cotton (data from 1986–1995 compared to data from 1996–2015) (Williams, 2015). Since 1992, the use of insecticides has decreased by around 97% and overall farm revenue gain has grown by about AUD\$180 per hectare due to the introduction of Bt cotton cultivars in Australia (Cotton Australia, 2021). Although there is variation in the improvement, Bt maize hybrids, which are widely grown in the US, produce more grain and above-ground biomass than conventional cultivars (Dillehay et al., 2004; Graeber et al., 1999; Mungai et al., 2005; Subedi & Ma, 2007) and are less vulnerable to lodging (Lauer & Wedberg, 1999). In the Philippines, Bt maize has been shown to exhibit yield benefits over conventional cultivars of up to 41%, leading to profitability improvements of 15% to 86% (Yorobe & Quicoy, 2006). While *H. armigera* and other Lepidoptera remain Resistant individuals are appearing in cotton and other Bt crops (Gould, 1998; Tabashnik, 1994; Tabashnik & Carè, 2017), despite the fact that new Bt cultivars are controlling cotton (Downes et al., 2016; Tabashnik & Carè, 2017). The maize earworm (Tabashnik & Carè, 2017), pink bollworm to Bt cotton in western India (Bagla, 2010), cereal stem borer to Bt corn in South Africa (Kruger et al., 2009), and fall armyworm to Bt corn in Puerto Rico (Matten et al., 2008) are a few instances. Refuge crops devoid of the Bt trait are now used to encourage vulnerable populations in an attempt to postpone insect resistance to Bt crops, however this method has had varying degrees of effectiveness (Tabashnik et al., 2008). Furthermore, commercialised Bt characteristics provide no protection against "sucking pests" that feed on the phloem, such as mirids, whiteflies, mites, aphids, and thrips, since the Cry proteins have a restricted host specificity and are not expressed in the phloem (Raps et al., 2001). These secondary insect pests have become more significant as a consequence of the removal of pesticides that were previously used to manage *Helicoverpa* spp. (Wilson et al., 2013, 2018). It is necessary to continue developing crop cultivars that have stronger control over a wider variety of significant insect pests. After a thorough search for hemipteran active Bt and guided evolution to boost its toxicity to the lygus insect, Bayer created a novel genetically engineered feature called ThryvOn, which increases cotton's defence against mirids, lygus bugs, and thrips (Ellsworth et al., 2021). In addition to the Thryv, natural host plant genes are being included into Bt cotton cultivars to provide resistance against other sucking pests including silverleaf whitefly and two-spotted spider mite. According to Wilson et al. (2018), thrips may be an early-season mite predator. Because of its wide host range on several crop species, capacity to spread illness, and tendency to become resistant to pesticides, the silverleaf whitefly (Figure 2) would be an especially useful target for both Bt and non-Bt crops (Da Silva Oliveira et al., 2021; Mayer et al., 2002). As crops are exposed to new insect pests, technologies that facilitate the faster development of innovative protection are needed. According to Wilson et al. (2018), "new" pests in Australia, such the autumn armyworm, are spreading from other parts of the country or from crops growing in new production areas, including cotton in Northern Australia. New cultivars may need to be created especially for this more tropical producing region since the breeding target environment in this area is quite different from that in the more temperate Eastern Australia where cotton is historically cultivated, with various insect species and illnesses. The pink bollworm and cluster caterpillar are the primary pests in these northern regions, and they had a role in the 1970s collapse of the Ord River cotton industry (Yeates et al., 2014). Due to developed resistance to two of the three Bt genes found in our current cultivars, Bt cotton is unable to effectively control the pink bollworm (Mathew et al., 2018). As a result, the pink bollworm poses a serious problem, making it an excellent target for the development of novel cotton germplasm.



FIGURE 2 A cotton leaf with a silverleaf whitefly (Image: Carlos Trapero). SynBio provides a number of ways to combat a wide range of insect pests that impact both Bt and non-Bt crops. Because of their GMO status and pre-existing transformation platforms, Bt crops would be appropriate for pilot experiments. The special capacity to quickly introduce many genes at once for more sophisticated defence against both current and emerging insect pests is provided by SynBio techniques. The creation of more sophisticated defence to prevent the emergence of resistance in pests like *H. armigera* may also be made possible by this trait stacking technique. The most effective method of creating new cultivars with resistance to several insect pests that are not managed by the current Bt traits, like mites and whiteflies, is probably stacking traits (e.g., by Golden Gate cloning and gene editing to insert them in a single genomic location allowing efficient expression of all the transgenes). This method reduces the amount of changes needed to add many or complex characteristics, including resistance to a new group of pests. Depending on the source of resistance (unadapted, wild cottons or diploid cousins), this may be achievable via breeding (Miyazaki et al., 2013; Trapero et al., 2016), although it may take up to 20 years. The more focused SynBio techniques may reduce genetic drag, or the unfavourable genes that surround resistance genes, which may happen when breeding from different material. Therefore, after the resistance genes have been found and their gene sequences known, SynBio trait-stacking procedures may facilitate faster and more effective advancement.

4.1.1 | Biopesticides to combat insect pests

RNA interference (RNAi) technology provides a number of ways to fight insect infestations. As highly targeted sprays for the management of certain insect pests, RNAi biopesticides are becoming more popular (Fletcher et al., 2020). These very targeted topical treatments lessen the need for chemical pesticides that might harm nontarget creatures and the environment. In contrast to "naked RNA" applications, Bioclays are foliar sprays that are created by loading dsRNA molecules onto layered double hydroxide nanoparticles for more stable RNA delivery (Mitter et al., 2017; Worrall et al., 2019). However, more research is necessary to produce enough dsRNA for widespread use, and it must be reasonably priced to compete with current synthetic chemical pesticides (Zotti et al., 2018). For topical RNAi to be practical for agricultural applications,

faster and more effective synthesis processes are needed. As an alternative, promoters for tissue-specific expression in the pest's preferred tissues may be used in conjunction with the stable production of insecticidal dsRNA molecules in genetically modified plants. According to Upadhyay et al. (2011), eating a diet containing dsRNA and siRNAs that target whitefly genes like an actin ortholog, ADP/ATP translocase, α -tubulin, ribosomal protein L9, and V-ATPase A subunit has significantly increased whitefly mortality. Therefore, it is possible that the target plant also expresses these dsRNAs. Within the plant, the expression may be regulated either geographically or temporally. Constitutive (continuous) and inducible (i.e., upon detection of herbivorous damage) expression are examples of temporal expression. By adding a promoter to the gene, an insecticidal dsRNA may be expressed inducibly to cause RNA interference (RNAi) when it is damaged by insect pests (Senthil-Kumar & Mysore, 2010). Both universal (all tissues) and tissue-specific expression fall under the category of spatial expression. For the management of sucking pests that consume sap, tissue-specific expression could be very helpful. The most potent insecticidal dsRNAs from the in vitro tests might be expressed specifically in the phloem to impact sucking insect pests like whiteflies by using a phloem-specific promoter (Upadhyay et al., 2011). Plants have been shown to contain phloem-specific promoters, such as those that shield against sucking insects (Javaid et al., 2016) and bacterial illness (Dutt et al., 2012). Eakteiman et al. (2018) used RNA interference (RNAi) in *Arabidopsis* with a phloem-specific promoter to target the glutathione S-transferase gene *BtGSTs5* in whiteflies, but the results were sublethal. If topical or plant-expressed dsRNAs are to outcompete chemical insecticides, their lethality must be increased to the equivalent of an insecticide in order for RNAi treatments to be fully effective (Shelby et al., 2020). To increase the effectiveness of this strategy, further basic research will ultimately be needed (e.g., to find genes for selective and fatal toxin synthesis and promoters for transitory or tissue-specific expression). The review by Shelby et al. (2020) provides an overview of the tactics and factors to be taken into account when using RNA interference to manage insect pests, including whiteflies.

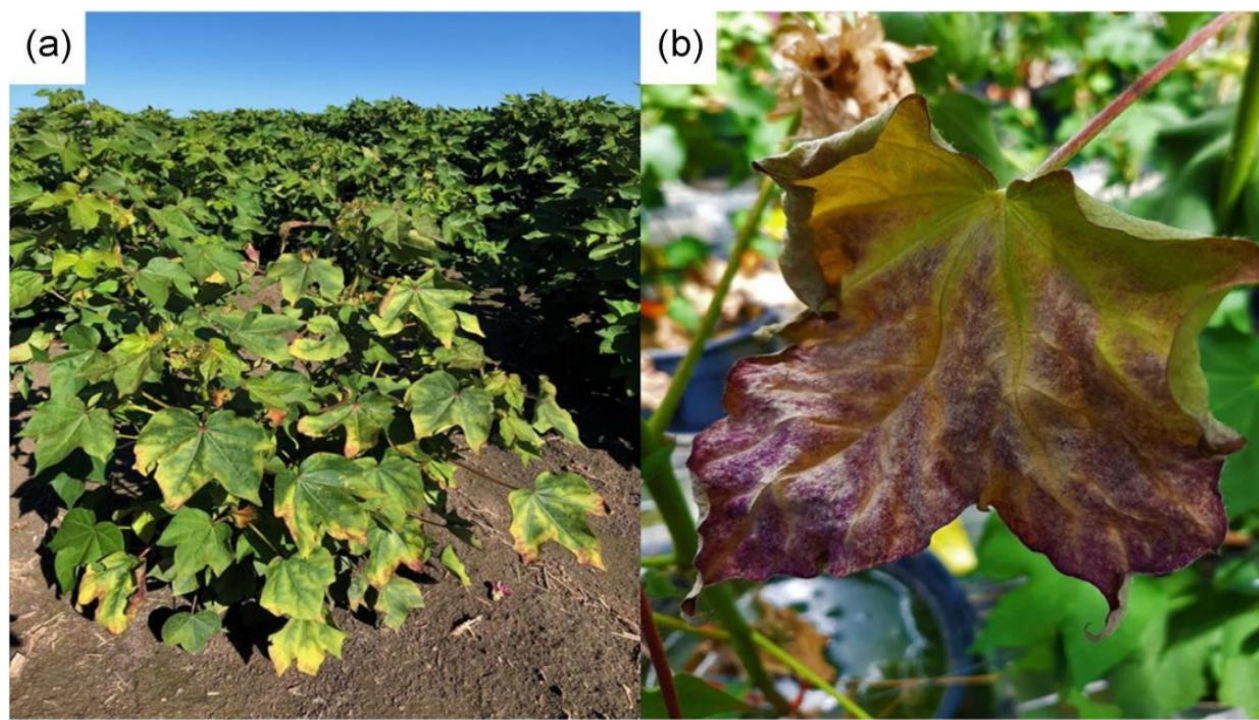
4.1.2 | Controlling insect populations using gene drives

By increasing the frequency of harmful alleles like sterility or fatal alleles, gene drives (promotion of deleterious alleles) might facilitate efficient and self-sustaining management of insect pest populations (Bier, 2022). Additionally, pesticide-resistant insect populations might be made vulnerable by the application of gene drives (Esvelt et al., 2014). Gene technology regulators and governments are taking a cautious approach to regulating gene drive research with applications in the control of insects, especially those that are vectors for human diseases (Bier, 2022). However, gene drives in insects are less advantageous because controlling the travel of the genetically modified insect population is nearly impossible compared to the control that can be implemented for a GMO plant (Reeves & Phillipson, 2017). Although this research emphasis is less popular than creating characteristics and topical treatments, it is nevertheless possible to target the sensory capacity of insect pests. For instance, the insect pest may be altered such that it can no longer detect a particular crop. In a similar vein, the target crop's chemical profile may be altered (or concealed by a topical treatment) or made "invisible" or "repulsive" to insects (Champer et al., 2016). A multifaceted strategy incorporating Integrated Pest Management (IPM) techniques is necessary for optimal control of insect pests due to their complexity. This is probably achieved by combining cultivar resistance with management techniques and topical applications of eco-friendly biopesticides.

4.2 | Fungal diseases

A number of other crops, such as potatoes (Davis et al., 1996; Johnson & Dung, 2010), peanuts (Woodward et al., 2011), safflowers (Rao et al., 2014; Urie & Knowles, 1972), tomatoes (Song et al., 2004), olives (Mercado-Blanco et al., 2003), bananas (Ploetz, 2017). The severity of *Verticillium* wilt tends to deteriorate under cold, wet circumstances, and these soil-borne fungus may occur in a variety of forms in the soil, crop waste, other crops, and weeds (Figure 3; Li et al., 2017b). The Commonwealth Scientific and Industrial Research Organization's cotton breeding

program has made great progress toward disease-resistant cotton germplasm, and integrated agronomic techniques may help control these diseases to some extent. It has been observed that genes encoding signalling pathways and anti-fungal proteins



strengthen cotton's defence against fungal diseases such *Fusarium* wilt and *Verticillium* (Emani et al., 2003; Gaspar et al., 2014; Murray et al., 1999; Parkhi et al., 2010; Tian et al., 2010; Wang et al., 2004). Nevertheless, none of these trials seem to have produced the substantial levels of resistance needed to be added to cultivars as a genetically modified feature. Resistance to these diseases is much needed in a variety of crops, and there is now a lot of effort in this field worldwide. The development of crops resistant to *Fusarium* and *Verticillium* is hampered by a number of issues. Cultivars of cotton that are more resistant to one of the diseases are often more vulnerable to the other (Li et al., 2017b). The presence of two pathotypes of *Verticillium* wilt, defoliating and nondefoliating (Li et al., 2017b), which may co-occur (Le et al., 2020) and cause distinct reactions to various cultivars, is another major problem for the cotton sector. Dual resistance to both pathotypes has not yet been developed via breeding efforts. According to Göre et al. (2017) and Li et al. (2017b), the most economical and environmentally friendly method of controlling *Verticillium* wilt is currently using host plant resistance in conjunction with management techniques, as there are currently no cost-effective fungicides that effectively control *Verticillium* pathotypes. In addition to creating cotton cultivars with dual resistance to both *Verticillium* pathotypes if distinct resistance genes can be found, trait stacking using Golden Gate cloning may be advantageous for the establishment of complicated and effective resistance in a variety of sensitive crops. Although it would be an enormous endeavour, dual resistance to both *Verticillium* and *Fusarium* would also be very important and worth the long-term investment. Since these technologies have been used to fight powdery mildew (*Blumeria graminis* f. sp. *tritici*) in wheat (Wang et al., 2014) and bacterial blight (*Xanthomonas*) infection in rice (Li et al., 2020), using RNAi technology and/or CRISPR-Cas9 in conjunction with TALENS may also be a successful strategy for developing resistance. These methods, however, depend on identifying genes in the *Verticillium* genome to target or induce resistance in order to prevent infection or survival. While many genes, quantitative trait loci (genomic regions), and proteins have been

found to contribute to some degree of *Verticillium* resistance in certain tomato, potato, and cotton cultivars and species (Cheng et al., 2016; Dong et al., 2019; Duan et al., 2016; Gayoso et al., 2010; Jun et al., 2015; Li et al., 2014, 2018; Liu et al., 2012; Mo et al., 2015; Yang et al., 2018; Zhang et al., 2017). To comprehend the pathogenesis of important agricultural diseases and find genes that can confer resistance, further study is needed. Effective resistance may also depend on where they are expressed, such as in the xylem or root hairs. Using CRISPR-Cas9 to identify genes might help or expedite this localisation. operates by specifically knocking out every gene within a genomic interval that confers resistance until the precise resistance gene is found, while Golden Gate cloning would allow the testing of gene combinations and expression patterns.

| Photosynthetic carbon assimilation

One of the next frontiers for increasing food and fibre crop productivity, resource-use efficiency, and abiotic stress tolerance is focusing on enhanced photosynthesis (Ainsworth & Ort, 2010; Betti et al., 2016; Furbank et al., 2020; Long et al., 2006; Posch et al., 2019; Simkin et al., 2019; Sharwood, 2017). Abiotic stress reactions and photosynthetic processes are quite intricate and affect many pathways (Figure 4). The relationship between photosynthetic rate and yield is weak in certain crop situations, but it becomes stronger as CO₂ levels rise (Long et al., 2006). Therefore, specific integration of many genes and perhaps novel reaction pathways will be needed for characteristics that target enhanced photosynthetic performance and resistance under abiotic stressors. The SynBio toolset, which can effectively transfer huge gene constructs with specific expression patterns, is essential for improving photosynthetic processes. Examples include improving photosynthetic enzymes (Sharwood, 2017; Sharwood et al., 2022), increasing mesophyll conductance (the diffusion of CO₂ into photosynthetic chloroplasts; Cousins et al., 2020; Ermakova et al., 2021), and improving WUE by introducing novel aquaporins. Targets for increasing crop heat and drought resistance and WUE include heat-shock proteins (Reddy et al., 2016) and modifying root characteristics (Hu & Xiong, 2014). These strategies might be especially effective when paired with photosynthetic improvements.

4.2.1 | Enhancing productivity and thermotolerance through improving photosynthesis

Modifying a number of important photosynthetic enzymes (highlighted below) involved in the Calvin cycle (carbon fixation, reduction, and regeneration) and the electron transport chain (also known as "light photosynthesis") is probably necessary to improve carbon absorption and thermotolerance. Rubisco has long been a focus of photosynthetic improvement for yield increase because it catalyses carbon fixation (carboxylation) inside the chloroplast (Sharwood, 2017). Rubisco's "helper protein," Rubisco activase, aids in carboxylation by keeping Rubisco from deactivating and making it thermolabile under abiotic stress (Kumar et al., 2009; Kurek et al., 2007; Sharwood, 2020). By adding additional thermostable materials, crop biomass, yield, and carbon assimilation have all improved under heat stress. either a catalytically superior Rubisco (Long & Ort, 2010; Zhu et al., 2010) or Rubisco activase (Kumar et al., 2009; Kurek et al., 2007; Scafaro et al., 2019), or by altering both Rubisco and Rubisco activase (Qu et al., 2021). RuBP, the substrate for Rubisco's carboxylation, is regenerated by SBPase during the Calvin cycle. SBPase overexpression and modification may prevent heat-induced yield decline in soybeans (Köhler et al., 2016), increase vegetative biomass and seed output in *Arabidopsis* (Simkin et al., 2017), and improve photosynthesis under heat stress in transgenic rice (Feng et al., 2007). Crop WUE may also be enhanced by overexpression or the introduction of new SBPases (López-Calcano et al., 2020). One of the four main cytochrome b6f

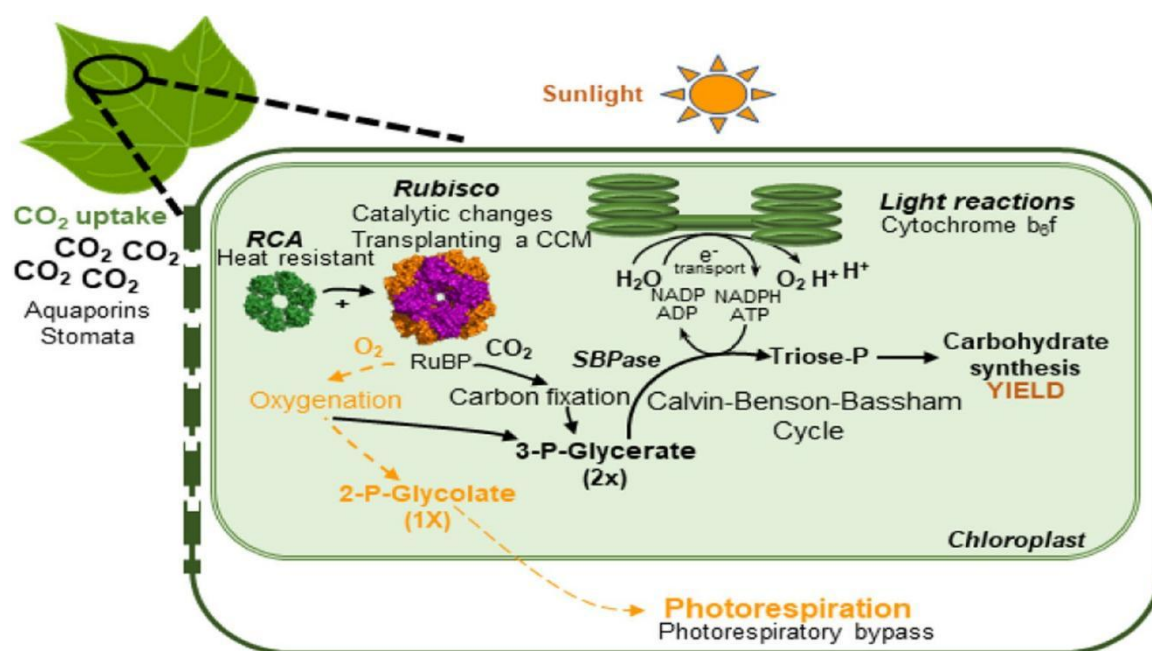


FIGURE 4 specific tactics to enhance carbon absorption. The goal of engineering components of light reaction systems and the Calvin-Benson-Bassham (CBB) cycle is to enhance the synthesis of carbohydrates, which are necessary for plant development, biomass production, and yield. Strategies include co-engineering Rubisco into a carbon concentrating mechanism (Sharwood, 2017), improving Rubisco activase function, which modulates Rubisco activity and is thermolabile (Sharwood, 2017), or changing catalytic properties through subunit transplantation (Sharwood, 2017) or directed evolution (Zhou & Whitney, 2019). Other tactics include overexpressing light reaction components including cytochrome b₆f (Ermakova et al., 2019) and sedoheptulose 1, 7-bisphosphatase (Driever et al., 2017) to increase the metabolic flow via the CBB cycle. Other tactics include engineering a photorespiratory bypass to address inefficiencies related to Rubisco oxygenation (South et al., 2019), transplanting CO₂ concentrating mechanisms in the form of a carboxysome (Long et al., 2018) or a pyrenoid (Atkinson et al., 2020), and improving stomatal regulation (Lawson & Vialet-Chabrand, 2019). CCM stands for carbon-concentrating mechanism. The Calvin-Benson-Bassham cycle uses light-harvesting protein complexes in the chloroplast membrane to carry out electron transport processes that provide energy for carbon absorption. Opportunities to enhance agricultural carbon collection are provided by over-expression of light-harvesting complexes such as b₆f (Ermakova et al., 2019; Yamori et al., 2016).

4.2.2 | Improving crop WUE and drought tolerance

For production to continue in a warmer, drier environment, more productive, drought-stressed, and water-efficient crops must be developed. Novel drought tolerance and enhanced WUE features that are not achievable via traditional breeding may be transferred with the help of SynBio. One such tactic that is only achievable with the more sophisticated technology provided by SynBio is the modification of C₃ crops, such as rice, wheat, canola, and cotton, to have a more effective photosynthetic metabolism (Depaoli et al., 2014). It is well known that C₄ photosynthesis and crassulacean acid metabolism (CAM) are more water-efficient than C₃ photosynthesis (Borland et al., 2014; Depaoli et al., 2014; Ermakova et al., 2020b). This is because carbon-concentrating mechanisms (CCMs) lower the amount of stomatal opening required, allowing for reduced water transpiration while maintaining rates of carbon absorption (Borland et al., 2014). As a result, compared to C₃ and C₄ plants, CAM plants may generate the same amount of biomass using 20%–80% less water (Antony & Borland, 2009; Von Caemmerer et al., 2012). This technique also has the

advantage of being able to stimulate its expression. In response to abiotic stressors, facultative CAM species may produce mRNA encoding for CAM enzymes, "switching on" this mechanism when it is most required (Winter & Holtum, 2014). This might be used to create cotton cultivars that, in times of water scarcity, can transition to more water-efficient metabolisms. It is important to carefully assess the possible productivity effect of implementing water-conserving systems. In rainfed production systems, water-preserving features are probably most advantageous, especially if a yield penalty is to be avoided. Proteins called aquaporins help plants transfer water and/or other substrates like silicon, boron, CO₂, and ions (Groszmann et al., 2017; Uehlein et al., 2003). Aquaporins have an impact on photosynthesis, mesophyll conductance in C₄ plants (Ermakova et al., 2021), stomatal conductance and root hydraulic conductivity, and therefore productivity and water utilisation (Sade et al., 2009), according to expression and overexpression studies. As a result, aquaporins are now another target for creating crops that are drought-resistant and water-efficient (Ermakova et al., 2021). It's crucial to remember that ectopic expression of At PIP1;2 and 1;4 did not produce tobacco plants with enhanced mesophyll conductance (Clarke et al., 2022, in press).

4.2.3 | Photosynthetic enhancement will rely on SynBio

The photosynthetic improvement will have to depend on SynBio techniques if features are not present in closely related species that are suited for crop breeding. In order to optimise flow through the Calvin cycle and increase the generation of carbohydrates, photosynthetic manipulation is complicated and requires the introduction or change of many genes (Figure 4). Thus, it will be crucial to introduce many transgenes into target crops quickly and effectively (Castilho, 2015; Simkin et al., 2019). In order to improve crop performance, heat and drought resilience, and WUE, SynBio has made it possible for multiple genetic modifications to take place in a single event (Kromdijk & Long, 2016; Kubis & Bar-Even, 2019; Ort et al., 2015; Shih et al., 2014; Simkin et al., 2019). To effectively improve photosynthesis and abiotic stress resilience, several targets would be needed, including Rubisco (either by producing new versions through directed evolution [Zhou & Whitney, 2019] or transplanting foreign large and small subunits [Whitney et al., 2011]), Rubisco activase, and potentially also their supporting chaperones (Aigner et al., 2017). Furthermore, tissue-specific expression could be necessary. For the installation of a C₄ photosynthetic CCM, this would be very crucial. An effective method for this multigene alteration would be to stack genes using Golden Gate (Depaoli et al., 2014; Maurino & Weber, 2013). In addition to gene stacking, CRISPR-Cas9 gene editing could be used to modify amino acids that provide a catalytic switch, increasing the photosynthetic activity of enzymes like Rubisco, whose small subunit gene is encoded in the nucleus (Sharwood, 2017; Whitney et al., 2011). Rubisco activase and other photosynthetic proteins that are carried into the chloroplast would also fall under this category. Additionally, at high temperatures, the SynBio toolbox provides the special capacity of some controlled promoters to "switch on" abiotic stress genes that enhance CO₂ absorption (i.e., increase Rubisco or Rubisco activase activity) (Venter, 2007). For instance, alternative thermotolerant Rubisco activase isoforms from organisms like *Agave tequilana* and wild rice (Scafaro et al., 2016; Shivhare & Mueller-Cajar, 2017) could be expressed by identifying the promoter elements responsible for the upregulation of various beta and alpha isoforms of Rubisco activase in wheat (Degen et al., 2021) and *Setaria viridis* (Kim et al., 2021). This would avoid yield penalties brought on by heatwave conditions or lengthy periods of heat and allow for better carbon absorption at higher temperatures (Scafaro et al., 2018). Additionally, regulated promoters may be very helpful in avoiding yield penalties for conservative systems, such water-preserving mechanisms, which might impede yield during extended expression. A prototype would need to be tested experimentally in the field under abiotic stress conditions.

|Nutrient acquisition

Fertilisers are being used extensively in western agriculture to maintain production. The overuse of fertilisers is harming the environment, and consumers are becoming increasingly interested in

sustainable goods. Furthermore, the poor nitrogen-use efficiency of crops, the limited supply of macronutrients like phosphorus, and anticipated future increases in energy costs to generate fertilisers make this approach unsustainable (Heuer et al., 2017; Perchlik & Tegeder, 2017; Rogers & Oldroyd, 2014). Enhancing crop nutrient-use efficiency, absorption, or assimilation may help overcome this obstacle (Roell & Zurbriggen, 2020). SynBio provides some of the most effective and efficient solutions because of the intricacy of these characteristics and systems. Engineering crops and the microorganisms that are associated with them to enhance the fixation, mobilisation, and absorption of macronutrients like phosphorus and nitrogen is one of the most popular strategies (Roell & Zurbriggen, 2020). Certain plant species, most notably legumes, have developed symbiotic interactions with nitrogen-fixing bacteria. The plant's roots get around 120 kg/ha of fixed nitrogen directly from this contact (Salvagiotti et al., 2008). According to simulations (Folberth et al., 2013), engineering maize to fix the equivalent of 50 kg (N)/ha might significantly increase crop output (Rogers & Oldroyd, 2014). Engineering crops to fix their own nitrogen might enhance crop nitrogen usage efficiency and drastically lower fertiliser consumption in addition to increasing yield. By focusing on microorganisms, crop utilisation of nitrogen and phosphorus might be greatly improved in a number of ways as an alternative to crop engineering.

4.2.4 | Introducing enzymes

To facilitate the fixation of vital nutrients, crops might be treated with enzymes like phytases or nitrogenases. Certain bacteria and archaea naturally have enzymes called nitrogenases that allow them to fix nitrogen straight from the atmosphere. These enzymes might be directly inserted into plant cell organelles using SynBio techniques (Allen et al., 2020, 2017).

4.2.5 | Enhancing existing microbial activity

It has been suggested that adding additional bacteria to agricultural soils—either naturally occurring or artificially created—could enhance crops' ability to absorb nutrients (Chen et al., 2021). However, questions about the viability of this strategy are raised by the challenge of introducing a foreign microbe into a niche that is probably already inhabited by "local" microorganisms that have adapted to local circumstances. Alternatively, methods that work with microorganisms already found in the phyllosphere, endosphere, or rhizosphere could be more practical (Bloch et al., 2020). In order to increase crops' availability to plant-available nitrogen and boost their nutrient usage efficiency, SynBio provides the chance to improve soil microorganisms' nitrogen fixation (Chen et al., 2021; Waltz, 2017). SynBio might do this in two ways: (i) create artificial microbial communities with specific roles or modify and reintroduce preexisting organisms; or (ii) modify crops to generate beneficial root exudates to control or enhance microbial activity. Because of the problem of regulating the position of genetically modified material in microbial communities, the former requires further improvement. This strategy could still work, however, since Bloch et al. (2020) employed gene editing to enhance nitrogen fixation by a naturally occurring diazotroph bacteria linked to maize roots that can be used as a seed coating to lower fertiliser requirements and boost yield. Alternatively, a more successful agronomic application that is given directly to the plant might focus on the capacity of plants to draw beneficial microbes or control microbial pathways and, consequently, the nitrogen cycle through the production of root exudates (Bardgett et al., 2014; Coskun et al., 2017; Finzi et al., 2015). According to Coskun et al. (2017), this strategy will rely on a better understanding of the species-specific interactions between crops and available microbes as well as the composition of their root exudates (i.e., carbohydrates, flavonoids, and terpenoids identified through metabolomics or transcriptomics) that are necessary to attract beneficial microbes or stimulate biological nitrogen fixation and belowground nitrogen transfer. The next step is to identify the genes linked to these exudate components (e.g., by transcriptomics, genomics, or CRISPR-Cas9 knockouts of possible targets) in order to modify or upregulate them using SynBio (e.g., Golden Gate cloning or CRISPR-Cas9). By reducing nitrogen loss via leaching, runoff, and denitrification, optimising root exudate release to increase crop nitrogen use efficiency might help mitigate nitrogen pollution from crop production (Coskun et al., 2017).

4| BENEFITS, CONSIDERATIONS AND LIMITATIONS OF SYNBIO IN AGRICULTURE

4.3 | Benefits

SynBio is seen as a branch of biology that may hold the key to the “next Green Revolution,” which is necessary to feed the world’s expanding population sustainably under more difficult conditions. SynBio techniques, which target qualities from distantly related species, other animals, or synthetically manufactured traits, would greatly benefit traits that have attained their genetic potential via traditional breeding. Additionally, SynBio may provide nonbreeding solutions, such as topical treatments for illnesses and pests, sterile weeds and pests, and unique qualities including improved oil profiles (Figure 5). Compared to previous methods, SynBio offers a variety of instruments to help crops acquire more complex characteristics and attributes more quickly. An excellent illustration of this is the C4 rice project, which aims to increase rice’s WUE and photosynthetic and nitrogen-use efficiency. Its photosynthetic system must be changed from C3 to C4 in order to achieve this goal. This is a complicated strategy that requires intricate anatomical and biochemical changes that took millions of years to evolve naturally (Ermakova et al., 2020b; Hibberd & Covshoff, 2010; Hibberd et al., 2008; Langdale, 2011; Sedelnikova et al., 2018). To “completely rewire” rice metabolism and morphology, up to 20 genes must be introduced (Ermakova et al., 2020b). Falling gene synthesis costs, synthetic promoter systems, and the development of sophisticated DNA assembly methods like Golden Gate cloning have all contributed to the creation of such huge and intricate multigene vectors (Ermakova et al., 2020b; Rogers & Oldroyd, 2014). The C4 rice project has been able to adopt a faster cycle of design, test, and prototype in conjunction with the adoption of a rapid *Agrobacterium*-based rice transformation system in a rice cultivar that is small, fast-flowering, day-neutral, and an established model for functional genomics thanks to the growth and development of SynBio over the past five years (Ermakova et al., 2020b; Li et al., 2017a). Another technique with potential agronomic uses is molecular switches, often known as controlled promoters. Molecular switches only activate a characteristic or reaction when necessary by activating a particular gene under certain circumstances. When not in use, this effective method may minimise resource waste (such as the creation of chemical defences). “Smart Plants” that can adapt to their surroundings in novel ways may be created using this technology (Wright & Nemhauser, 2019). This might be a useful tool for improving resistance to abiotic stress.

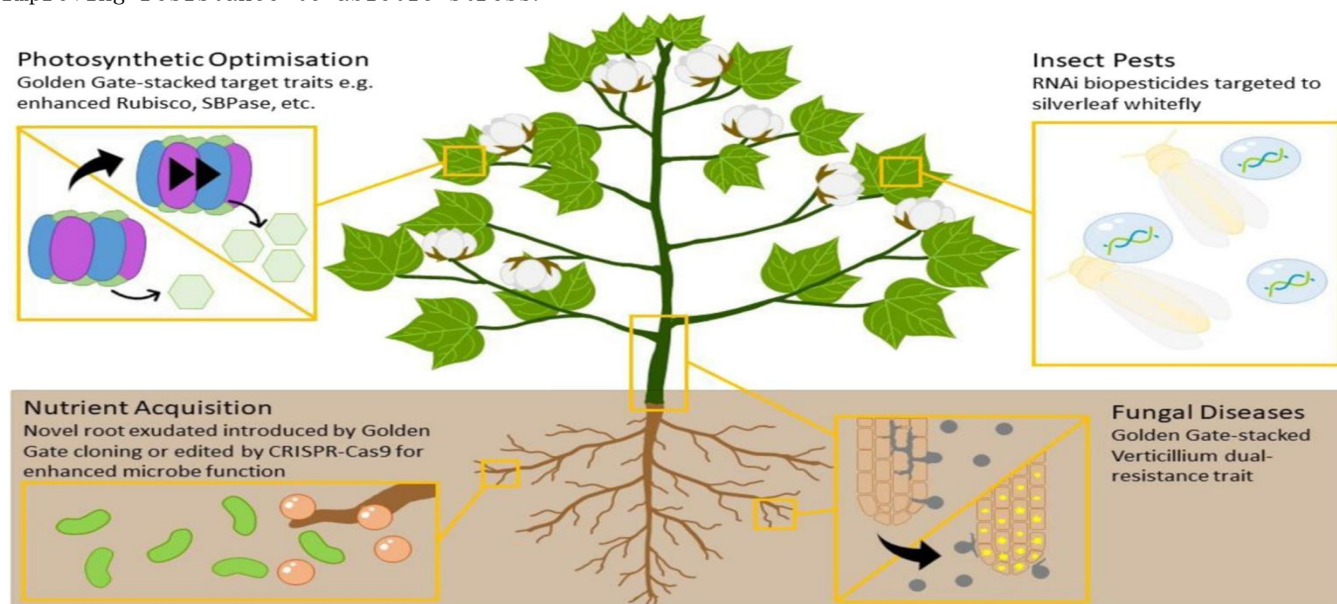


FIGURE 5 An outline of potential applications of synthetic biology to major agricultural problems. (From top left, clockwise) Golden Gate cloning may be used to stack characteristics for the increase (i.e., faster catalytic rate) of important photosynthetic proteins like Rubisco, allowing for photosynthetic optimisation (Engler et al., 2009; Sharwood, 2017). Using Bioclay technology, RNAi biopesticides might target important insect pests as the silverleaf whitefly (Worrall et al., 2019). Using Golden Gate cloning to stack numerous characteristics for complicated resistance, especially against dual pathotypes that impact cotton, effective resistance against fungal diseases like *Verticillium* wilt might be established in a wide variety of host crops. Numerous techniques, such as CRISPR-Cas9-edited genes for the development of new root exudates to improve soil microbial nutrient fixation or Golden Gate cloning-introduced genes, might improve nutrient acquisition (Coskun et al., 2017). RNA interference, or RNAi. (Degen et al., 2020) by creating crop cultivars that, upon the first detection of abiotic stress conditions, "activate" resilience genes.

4.4 | Considerations

In order to solve the problems that resource-poor farmers and underprivileged consumers encounter, solutions must be straightforward, precise, and reasonably priced (Pixley et al., 2019). In order for problems and solutions to be both economically feasible and significant, they would benefit from going outside an Australian setting as agriculture becomes increasingly globalised. Furthermore, positive policies, focused investments, and top-notch research are necessary for fair access to the advantages of ensuing GM crops (Pixley et al., 2019). Some SynBio initiatives have failed because they have concentrated on difficult-to-monetize environmental sustainability or humanitarian aims (Pixley et al., 2019). To make sure SynBio initiatives are high value and significant, the financial benefits to all parts of the value chain must be carefully taken into account.

| Limitations

The cost of deregulation (the majority of SynBio applications are classified as genetic modification and are therefore subject to the same laws as traditional genetic engineering products), a complicated patent landscape that must be negotiated for any potential commercial applications, and social licence are the main obstacles impeding the advancement of SynBio application. Similar to the difficulties GMO crops have had over the last three decades, social acceptability will be a major obstacle to the effective implementation of SynBio. Additionally, funding from a variety of public and commercial sectors will be necessary for SynBio's effective acceptance, application, and acceleration. The need to pinpoint the exact gene or genes needed for a desired function is another major obstacle that has impeded the advancement of using SynBio. When dealing with multigenic functions, this is more difficult and time-consuming. Optimising an appropriate transgene transfer and integration process is essential for the creation of transgenic plants. Nowadays, the majority of important food and fibre crops are primarily altered via gene transfer mediated by *Agrobacterium* and particle bombardment. For instance, somaclonal variation from extended culture times, high frequencies of aberrant embryo development, low conversion rates of somatic embryos into plantlets, and high genotype dependency have all contributed to the limited success of regenerating plants from transformed cotton tissue (Mishra et al., 2003; Stelly et al., 1989; Sun et al., 2006). Furthermore, only a few usually older cotton cultivars may be transformed using *Agrobacterium*; nevertheless, genotype-independent methods are beginning to appear (Chen et al., 2014). These procedures will enable the immediate transformation of elite cultivars and the addition of new SynBio-developed features without the need for significant further breeding. Lastly, in order to produce a technology, product, or characteristic, each SynBio possibility need a substantial time and financial commitment. Before the years (probably decades) needed to establish a SynBio technology, product, or characteristic, many years of basic and proof-of-concept research are needed. As a result, committing to a SynBio development potential would need a partnership or co-investment in order to provide sufficient time, money, and experience for the project to succeed.

5 | CONCLUSIONS

SynBio offers “endless” opportunity to tackle different agricultural difficulties. But it’s crucial to evaluate these chances realistically. The practical use of SynBio is still constrained by a number of issues, despite the significant efficacy and efficiency that may be attained using SynBio procedures. The adoption of any new technology may be constrained by a number of hazards, expenses, laws, and public attitudes. Millions of dollars and decades of research and development are also necessary for the effective development of SynBio technology, products, and features. In the end, SynBio research projects must be highly influential, have a clear value proposition, and have a high benefit to cost ratio in order to be viable. Tom Walsh (CSIRO), Brajesh Singh (WSU), Sharon Downes (CSIRO), Iain Wilson (CSIRO), Duy Le (NSW DPI), Aphrika Gregson (NSW DPI), Blake Palmer (NSW DPI), Brendan Choat (WSU), Jonathan Plett (WSU), Oliver Knox (UNE), Craig Wood (CSIRO), Lucy Egan (CSIRO), Shiming Liu (CSIRO), and Eleonora Egidi (WSU). We also appreciate Timothy Rhodes (ANU), Lily Chen (WSU), and Danny Llewellyn (CSIRO) for their insightful comments and manuscript review.

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