Received: 11 February 2022 | Accepted: 15 March 2022

DOI: 10.1002/sae2.12014

REVIEW ARTICLE

JOURNAL OF SUSTAINABLE AGRICULTURE AND ENVIRONMENT
Global Initiative of Crop Microbiome and Sustainable Agriculture

Synthetic biology and opportunities within agricultural crops

Demi Sargent[1] | Warren C. Conaty[2] | David T. Tissue[1,3] | Robert E. Sharwood[1]

[1]Hawkesbury Institute for the Environment, Western Sydney University, Richmond, New South Wales, Australia

[2]Australian Cotton Research Institute, CSIRO, Narrabri, New South Wales, Australia

[3]Global Centre for Land Based Innovation, Western Sydney University, Richmond, New South Wales, Australia

Correspondence

Robert E. Sharwood, Hawkesbury Institute for the Environment, Western Sydney University, Richmond, NSW 2753, Australia.
Email: r.sharwood@westernsydney.edu.au

Funding information

Cotton Research and Development Corporation, Grant/Award Numbers: UWS2101, UWS2201

Abstract

Conventional breeding techniques have been integral to the development of many agronomically important traits in numerous crops. The adoption of modern biotechnology approaches further advanced and refined trait development and introduction beyond the scope possible through conventional breeding. However, crop yields continue to be challenged by abiotic and biotic factors that require the development of traits that are more genetically complex than can be addressed through conventional breeding or traditional genetic engineering. Therefore, more advanced trait development approaches are required to maintain and improve yields and production efficiency, especially as climate change accelerates the incidence of biotic and abiotic challenges to food and fibre crops. Synthetic biology (SynBio) 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. SynBio 'upgrades' the potential of genetic engineering, which involves the transfer of single genes from one organism to another. This technology can enable the introduction of multiple genes in a single transgenic event, either derived from a foreign organism or synthetically generated. It can also enable the assembly of novel genomes from the ground up from a set of standardised genetic parts, which can then be transferred into the target cell or organism. New opportunities to advance breeding applications through exploiting SynBio technology include the introduction of new genes of known function, artificially creating genetic variation, topical applications of small RNAs as pesticides and potentially speeding up the production of new cultivars with elite traits. This review will draw upon case studies to demonstrate the potential application of SynBio to improve crop productivity and resistance to various challenges. Here, we outline specific solutions to challenges including fungal diseases, insect pests, heat and drought stress and nutrient acquisition in a range of important crops using the SynBio toolkit.

KEYWORDS

abiotic stress, biotic stress, crops, photosynthesis, SynBio

1 | INTRODUCTION

Conventional breeding techniques have successfully introduced several beneficial agronomic traits into agricultural crops such as 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, 2021; Yang & Zhang, 2010) and canola (pod shattering, herbicide resistance and pathogen resistance; Barbetti et al., 2012; Gan et al., 2016; Kirkegaard et al., 2016). The adoption of modern biotechnology approaches has enabled developments beyond the capacity or efficiency of conventional breeding, such as insect and herbicide resistance in broadacre crops (Dill, 2005; Downes et al., 2017). However, crop yields continue to be challenged by abiotic and biotic factors. In addition, while progress in traditional breeding is yet to reach a ceiling in many crops, 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 with new diversity often having to be sought in close relatives. Therefore, more advanced crop cultivar development approaches such as synthetic biology (SynBio) are required to maintain and improve yields and production efficiency, especially as climate change accelerates the incidence of biotic and abiotic challenges to food and fibre crops.

2 | WHAT IS SYNBIO?

A consensus definition of SynBio was drafted by a group of European experts more than a decade ago: '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; Vancompernolle & Ball, 2005). This engineering perspective may be applied at all levels of biological organisation, from the molecular level to entire organisms. SynBio enables the rational and systematic design of biological systems (Serrano, 2007). 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. These approaches can occur in two subfields: (1) using existing biological building blocks to create combinations not present in nature and (2) create nonnatural building blocks to replicate natural functions or develop novel functions. Through its evolution, SynBio has adopted many of the commonly used engineering terms such as 'switch', 'rewire' and the 'design, test, simulate, learn cycle' (Figure 1; Liu et al., 2015).

Defining what is classified as SynBio is heavily debated as many tools and approaches can be considered synthetic. Furthermore, the evolution of technology and terminology has seen different labels

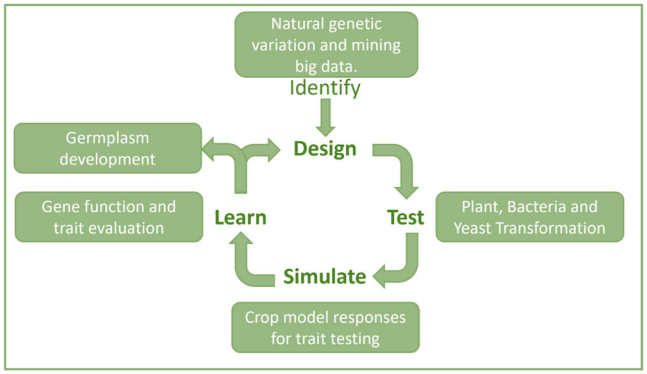

FIGURE 1 The design, test, simulate and learn cycle of developing and introducing novel traits into food and fibre crops through synthetic biology. Natural variation and mining big data sets provide information to design new pathways for improved resilience to abiotic and biotic stresses. These are then tested in high-throughput plant, bacterial and yeast systems. For crops, the intended outcome of the alterations can be modelled to determine the impact on yield and resource-use efficiency. From this we learn the best ways to alter crop productivity and begin to implement incorporation of such traits or return to identify further variation to include in SynBio design. Selected traits of value are then used for germplasm development.

applied to similar scientific fields (i.e., biotechnology, genetic engineering, SynBio). Traditional genetic engineering involves the transfer or modification of single genes or components (Roell & Zurbriggen, 2020; Serrano, 2007). In contrast, SynBio tools are capable of developing complex multigenic traits through the simultaneous introduction or manipulation of multiple genes (Roell & Zurbriggen, 2020), derived from donor organism(s) or synthetically generated. Therefore, SynBio 'upgrades' the potential of genetic engineering, enabling more rapid development of transgenic material with more complex modifications, which is favourable for the development of elite crop cultivars. For example, the initial development of C_4 rice included the introduction of five genes from the NADP-ME biochemical subtype (Ermakova et al., 2020b). This transformation would have taken years through traditional genetic engineering involving 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., 2020a). SynBio can also enable the assembly of novel genomes from a set of standardised genetic parts, which can then be transferred into the target cell or organism (Serrano, 2007). Gene editing is a promising SynBio technology that allows an organism's genome to be modified without the introduction of foreign genetic material (Pixley et al., 2019). Topical application of double-stranded RNA (dsRNA) to elicit gene silencing through RNA interference (RNAi) is another tool within the SynBio toolkit with great potential for agricultural application (e.g., as biopesticides). Topical RNA viral transfection can similarly be applied to crops to transiently alter agronomic traits, such as flowering time and stress responses, by transiently 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.	Mao et al. (2013)
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.	Engler et al. (2009)
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).	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.

silencing regulatory genes (Torti et al., 2021). There are many SynBio tools and techniques suitable for application in agricultural settings (Table 1) with the potential to develop novel agricultural products and significantly improve agricultural management, productivity and sustainability. In extension to this, new artificial promoter development will make it possible to turn genes on and off, depending on the presence of a chemical or biotic and abiotic elicitor (Schreiber & Tissier, 2017).

SynBio offers a range of research applications that can be classified as either 'fundamental' or 'applied'. Significant advancements in understanding fundamental biology have been and continue to be achieved using SynBio. However, there are numerous possible practical applications, from medicine to agriculture. Agricultural industries continue to face severe challenges, particularly those associated with climate change, while demand for agricultural products continues to rise to support a growing population. This challenge could be addressed using SynBio techniques that enable even the most complex biological systems to be efficiently and effectively redesigned.

3 | WHAT IS INSIDE THE SYNBIO TOOLKIT?

3.1 | CRISPR-Cas9 (gene editing)

CRISPR-Cas9 is one of the fastest, easiest and cost-effective gene editing tools (Hayes et al., 2018). It is favoured as an alternative to classical plant breeding and transgenic methods for its simple design and easy construction of DNA constructs (Belhaj et al., 2015). This technique is the application of the Type II CRISPR-Cas system that is involved in the immune system/defence mechanism in bacteria and archaea (Farzadfard et al., 2013; Jinek et al., 2012; Qi et al., 2013). During the recognition (or adaptation) phase of foreign nucleic acids (either from a virus or plasmid) in bacteria, conserved Cas1 and 2 proteins stitch pieces of invading DNA into the bacteria's CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) region, which allows the bacteria to record and recognise the infecting virus (Jinek et al., 2012). These regions are transcribed into crRNA (CRISPR RNA), which are subsequently processed and base pairing is required with transactivating crRNA (tracrRNA), which bind to the Cas9 protein (Mir et al., 2018). The Cas9 ribonucleoprotein then uses this crRNA-tracerRNA molecule as a guide to recognise subsequent matching nucleic acids and through its nuclease activity destroys the invading DNA (Mir et al., 2018). Jinek et al. (2012) discovered that a synthetic guide RNA (gRNA) composed of the crRNA and tracrRNA could identify specific sequences of DNA and cut the DNA at that location. Random repair of the cut can introduce deletions or small random insertions or if a repair template is supplied can edit it to another specific sequence (Jinek et al., 2012). The discovery and development of this technology were recently awarded the Nobel Prize (Ledford & Callaway, 2020). This process can be used to knock out specific genes (e.g., disease-causing genes) or 'fix' genetic errors. This technique can also be modified to promote gene transcription by deactivating Cas9 so it cannot cut DNA and fusing Cas9 with transcriptional activators (Konermann et al., 2015). Gene editing through such technology is viewed favourably in part because single-gene knockouts or single base-pair mutations may not require regulation in a growing number of countries (e.g., Waltz, 2018). The Australian government declared in 2019 that gene-editing techniques in plants and animals that do not introduce new genetic material (i.e., incisions by CRISPR-Cas systems allowed to be repaired naturally without a gRNA) will not be regulated as genetically modified organisms (GMOs; Mallapaty, 2019). Editing techniques that do incorporate new genetic material, such as the introduction of new amino acids or genes, will require regulation by the relevant government authorities to

allow the use of GMOs. In Australia, this is the Office of the Gene Technology Regulator (https://www.ogtr.gov.au/).

3.2 | Golden Gate (gene assembly)

Golden Gate cloning is one example of the toolkit that enables the modular assembly of multiple (upwards of 10) fragments of DNA 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 (Werner et al., 2012). The components (promoters, coding sequences and terminators) are designed to contain recognition sequences to allow precise assembly and unique recognition sites at the ends of the assembled DNA fragments facilitate its precise insertion into the destination vector (Engler et al., 2009). The use of Type IIS (e.g., *Bsa*I) restriction (cutting) enzymes are crucial to this system because they cut outside their recognition region, leaving specific overhangs for ligation (DNA joining) using T4 DNA ligase, and the removal of the recognition sequence after digestion means that the DNA pieces cannot be digested again (Engler et al., 2009). This ensures that only correctly assembled products remain intact and fragments that are no longer required in the final assembled product are removed (Engler et al., 2014). This is a desirable approach for assembling large gene constructs and stacking multiple genes for efficient multitrait transfer with appropriate regulatory sequences. Common design principles enable gene parts to assemble in a modular way to select appropriate regulatory control components for gene expression and provide the ability to incorporate multigene constructs that include whole metabolic pathways into plants.

3.3 | RNAi (gene silencing)

RNAi is a post-transcriptional gene silencing mechanism that is a naturally occurring pathway found in eukaryotic organisms to protect against viruses and/or pathogens producing aberrant RNA molecules (Ashfaq et al., 2020). This process involves the recognition of the aberrant RNA, which is converted into dsRNA (Waterhouse et al., 2001). dsRNA is the elicitor of the RNAi response, which the DICER enzyme cleaves into 21-nucleotide small interfering RNA (siRNA) molecules (Hung & Slotkin, 2021). The siRNA molecules are then bound to an argonaut protein and used as a guide strand to recognise specific regions of messenger RNA (mRNA) for degradation (Fire, 1999) and in some cases the complex can directly inhibit translation of specific genes, effectively silencing them. RNAi has been targeted as a process to silence genes for various agricultural applications, such as inducing sterility or mortality in insect pests when they eat RNAi-producing plants, modifying seed oil composition, suppressing toxin production in edible crop tissues and in suppressing fungal and viral pathogens of plants (Chen et al., 2015; Jørgensen et al., 2005; Kola et al., 2015; Liu et al., 2017; Worrall et al., 2019; Zhang et al., 2015) such as bacterial blight (*Xanthomonas*) infection in cotton (Cox et al., 2017).

3.4 | Gene drives (promoting inheritance of deleterious alleles)

Gene drives increase the frequency of deleterious alleles by inserting enzymes via CRISPR to destroy nondesired genes in chromosomes, thus enabling the desired deleterious gene to be copied and inherited (Bier, 2022). Some types of gene drives can be reversible and spatially restricted (Pixley et al., 2019). This technology could be used to target pests, weeds and diseases (i.e., introduce sterility in insect pests or inhibit seed setting in weeds).

3.5 | Gene synthesis (increasing the speed of cloning)

Gene synthesis has revolutionised the construction of plasmid DNA used for biotechnology and SynBio (e.g., www.genscript.com). Previous gene cloning relied on PCR amplification from various sources that also included the incorporation of restriction sites for cloning and this had to match that of available cloning vectors for protein expression in *Escherichia coli* or other hosts and transformation of plants and algae. Gene synthesis used in combination with Golden Gate cloning and other modular cloning processes has increased the speed at which DNA constructs can be made, drastically reducing the time required for even complex multigene constructs. This has also resulted in the development of gene foundaries that gather appropriate components and assemble them into functioning systems (Chambers et al., 2016). Ultimately, gene synthesis has enabled genes, identified from DNA and transcriptome sequence data, to be cloned. Furthermore, codon modification of sequences to ensure the efficiency of translation of foreign genes between species (Gustafsson et al., 2004) is made easier even for very large genes.

3.6 | Regulated and artificial promoters

Understanding how promoters switch on and off in response to environmental cues is important for next-generation solutions. This has important agricultural applications, enabling genes to be activated or deactivated during specific environmental conditions (low or high temperatures; Grover et al., 2013) or disease triggers (Arnaiz et al., 2019). Examples may include water conservation genes that can be activated during the detection of drought stress or a thermotolerant isoform of ribulose-1, 5-bisphosphate carboxylase/oxygenase (Rubisco) activase induced under heatwave conditions (Sharwood, 2017). These are efficient strategies, particularly for genes that may be energetically expensive, as it limits their expression to periods when they are most critically required.

Artificial promoters, such as the transcription activator-like effectors (TALEs) and the synthetic TALE-activated promoter (STAP) systems, may amplify the expression of multiple genes from a single promoter (Boch & Bonas, 2010; Boch et al., 2009; Brückner et al., 2015;

Schreiber & Tissier, 2016, 2017). TALEs are transcription factors that manipulate the transcription of endogenous genes in plants (Schreiber & Tissier, 2016). This system involves expressing the TALE gene activator from the desired promoter, which, in turn, binds to the STAP promoter(s), driving expression of the gene(s) of interest (Brückner et al., 2015). Another example is the expression of dead Cas9 fused to a strong transcriptional activator targeted to specific promoter regions of genes of interest using specific gRNAs. This system has been shown to be a strong activator of gene expression (Xu et al., 2019).

4 | PROMISING SYNBIO SOLUTIONS TO KEY AGRONOMIC CHALLENGES

Conventional plant breeding approaches have been integral in developing agronomically important traits in various crops, improving crop production, productivity and resilience. For example, conventional breeding approaches, including marker-assisted selection, have improved broad disease resistance in rice (Luo et al., 2017; Suh et al., 2013), potato (Haverkort et al., 2016; Zhu et al., 2012) and wheat (Aktar-Uz-Zaman et al., 2017; Liu et al., 2000). Breeding and selection of rice has resulted in the loss of seed dispersal, increased apical dominance, decreased seed dormancy, compact panicles and larger inflorescences and grains throughout its domestication (Doebley et al., 2006; Ishii et al., 2013; Zhu et al., 2013). Breeding maize for leaf architecture traits to enable higher planting densities, and repressed ear prolificacy, inflorescence branching and tillering have improved yields substantially (Duvick, 2005; Tian et al., 2011; Vollbrecht et al., 2005; Wills et al., 2013). Conventional hybridisation and mutation breeding have successfully introduced several beneficial agronomic traits in cotton such as maturity and growth habits suited to a range of season lengths and production regions (Kandhro et al., 2002; Xanthopoulos & Kechagia, 2001), improved fibre quality (Muthusamy & Jayabalan, 2011), photoinsensitivity (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, 1991). In addition to conventional breeding, the adoption of modern biotechnology approaches has generated genetically engineered cotton cultivars with additional traits such as insect and herbicide resistance (Perlak et al., 2001). Despite these advancements over the years, crop yields continue to be challenged by the occurrence of pests, weeds, pathogens, nutrient acquisition and abiotic stresses; thus, introducing novel properties and additional genetic diversity is required. However, the ability to overcome these challenges through conventional breeding is limited by the ability to exploit available genetic diversity in crop germplasm collections (Dwivedi et al., 2007, 2017; Sharwood et al., 2022, in press). Genetic and reproductive barriers such as 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 lack of an effective way to combine multiple desired alleles for complex traits (Lyzenga et al., 2021) remain key limitations for crop breeding programmes to target certain agronomic challenges. Overcoming these limitations for effective and efficient novel trait development is possible using SynBio tools.

4.1 | Insect pests

New, robust solutions to control insect pests and their ability to develop resistance is required to sustain and improve crop production and reduce reliance on insecticides. SynBio technologies present multiple solutions to combat insect pests. Notable success has been achieved historically through the introduction of genes for toxin production in targeted crop tissues. The introduction of the 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 (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) provide effective and relatively specific resistance (Mendelsohn et al., 2003) against Lepidoptera species such as cotton bollworm (*Helicoverpa armigera*), cereal stem borer (*Busseola fusca*) and fall armyworm (*Spodoptera frugiperda*) (Tabashnik et al., 2013). The larvae of these moth species typically feed on plant terminals, reproductive structures and stems (Leigh et al., 1996), thereby potentially reducing crop yield depending on the severity and timing of damage (Sadras, 1995). Following the introduction of *Bt* cotton (data from 1986 to 1995 compared to data from 1996 to 2015), insecticide usage has decreased by 61%–81% and damage losses have reduced by 47%–63% in the USA (Williams, 2015). As a result of the adoption of *Bt* cotton cultivars in Australia, total farm income gain has increased by approximately AUD$180 per hectare and insecticide application has reduced by around 97% since 1992 (Cotton Australia, 2021). *Bt* corn hybrids, widely grown in the United States, 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 susceptible to lodging (Lauer & Wedberg, 1999); however, variation in the improvement does exist. Yield advantages of *Bt* corn over conventional cultivars have been reported in the Philippines as high as 41%, resulting in profitability gains of 15%–86% (Yorobe & Quicoy, 2006).

Although *H. armigera* and other Lepidoptera continue to be controlled in cotton by new *Bt* cultivars (Downes et al., 2016; Tabashnik & Carrière, 2017), resistant individuals are emerging in cotton and other *Bt* crops (Gould, 1998; Tabashnik, 1994; Tabashnik & Carrière, 2017). Some examples include the corn earworm (Tabashnik & Carrière, 2017), pink bollworm to *Bt* cotton in western India (Bagla, 2010), the cereal stem borer to *Bt* corn in South Africa (Kruger et al., 2009) and the fall armyworm to *Bt* corn in Puerto Rico (Matten et al., 2008). The current strategy to delay insect resistance to *Bt* crops is using refuge crops without the *Bt* trait to promote susceptible populations, but this approach has seen varied success (Tabashnik et al., 2008). Additionally, the Cry proteins have a narrow

host specificity and are not expressed in the phloem (Raps et al., 2001); therefore, the commercialized *Bt* traits offer no protection against 'sucking pests' that feed on the phloem such as mirids, whitefly, mites, aphids and thrips. The cessation of insecticides previously used to control *Helicoverpa* spp. has resulted in the increased significance of these secondary insect pests (Wilson et al., 2013, 2018). Further development of crop cultivars with more robust control over a broader range of major insect pests is required. 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 (Ellsworth et al., 2021). Incorporating native host plant genes for resistance against additional sucking pests, such as two-spotted spider mite and silverleaf whitefly, into *Bt* cotton cultivars is also underway and would be highly valuable in conjunction with the ThryvOn trait as thrips can be an early-season predator for mites (Wilson et al., 2018). Silverleaf whitefly (Figure 2) would be a particularly valuable target for both *Bt* and non-*Bt* crops due to its broad host range on different crop species, ability to vector diseases and propensity to develop resistance against insecticides (Da Silva Oliveira et al., 2021; Mayer et al., 2002).

Technologies that enable more rapid development of novel protection is required as crops become exposed to new insect pests. In Australia, 'new' pests, such as the fall armyworm, are emerging from other regions or through the expansion of crops into new production areas, such as cotton in Northern Australia (Wilson et al., 2018). This region is a vastly different breeding target environment than in the more temperate Eastern Australia where cotton is traditionally grown, with different pest species and diseases; therefore, new cultivars may need to be developed specifically for this more tropical production region. The main pests in these Northern areas include the pink bollworm and cluster caterpillar, which contributed to the collapse of the cotton industry in the Ord River during the 1970s (Yeates et al., 2014). The pink bollworm is not effectively controlled by *Bt* cotton due to evolved resistance against two of the three *Bt* genes present in our current cultivars (Mathew et al., 2018). Therefore, the pink bollworm is a significant risk, and thus a valuable target for developing new cotton germplasm.

FIGURE 2 Silverleaf whitefly on a cotton leaf (Photo: Carlos Trapero).

SynBio offers several solutions to target numerous insect pests that affect various *Bt* and non-*Bt* crops. *Bt* crops would be suitable for pilot studies due to their pre-existing transformation platforms and GMO status. SynBio approaches offer the unique ability to rapidly introduce multiple genes simultaneously for more complex protection against existing and new insect pests. 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*. Stacking traits (e.g., through Golden Gate cloning and gene editing to insert them in a single genomic location allowing efficient expression of all the transgenes) 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. This approach limits the number of transformations required to introduce multiple or multifaceted traits such as resistances against a new suite of pests. Although this may be possible through breeding (Miyazaki et al., 2013; Trapero et al., 2016), it can take 20 years or more depending on the source of resistance (unadapted, wild cottons or diploid relatives). Genetic drag (i.e., of unfavourable genes flanking resistance genes) that can occur with breeding from diverse material could be minimised by the more targeted SynBio approaches. Therefore, progress could be more rapid and efficient through SynBio trait-stacking approaches once the resistance genes have been identified and their gene sequences determined.

4.1.1 | Biopesticides to combat insect pests

RNAi technology offers several approaches to combat insect pests. 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. For example, 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 (Mitter et al., 2017; Worrall et al., 2019). However, synthesis of enough dsRNA for large-scale applications requires future development and needs to be affordable to be competitive with existing synthetic chemical pesticides (Zotti et al., 2018). More rapid and efficient synthesis procedures are required for topical RNAi to be viable for agricultural applications.

Alternatively, insecticidal dsRNA molecules can be stably expressed in GM plants along with the use of promoters for tissue-specific expression in the tissues favoured by the pest. Consumption of diet containing dsRNA and siRNAs targeted at whitefly genes such as an actin ortholog, ADP/ATP translocase, α-tubulin, ribosomal protein L9 and V-ATPase A subunit have resulted in significant whitefly mortality (Upadhyay et al., 2011); thus, these dsRNAs might conceivably also be expressed in the target plant. The expression could be temporally controlled or spatially controlled within the plant. Temporal expression includes constitutive (continuous) or inducible

(i.e., upon detection of herbivorous damage) expression. Inducible expression of an insecticidal dsRNA to induce RNAi is possible by introducing a promoter alongside the gene to activate it upon damage by insect pests (Senthil-Kumar & Mysore, 2010). Spatial expression includes ubiquitous (all tissues) or tissue-specific expression. Tissue-specific expression may be particularly valuable for the control of sucking pests that feed on sap. Expressing the most effective of the insecticidal dsRNAs from the in vitro assays with a phloem-specific promoter could enable specific expression in the phloem to affect sucking insect pests such as whitefly (Upadhyay et al., 2011). Phloem-specific promoters have been identified in plants, including those used to protect against bacterial disease (Dutt et al., 2012) and sucking insects (Javaid et al., 2016). Eakteiman et al. (2018) deployed RNAi in *Arabidopsis* with a phloem-specific promoter to target a glutathione *S*-transferase gene, *BtGSTs5*, in whitefly, but with sublethal effects. Full efficacy of RNAi applications will rely on increasing the lethality of these molecules (Shelby et al., 2020), at least to the equivalence of an insecticide if topical or plant expressed dsRNAs are to outcompete chemical insecticides. Ultimately, further fundamental research (e.g., to identify genes for specific and lethal toxin production and promoters for transient or tissue-specific expression) will be required to improve the impact of this approach. The review by Shelby et al. (2020) summarises the strategies and considerations for controlling whitefly and other insect pests using RNAi.

4.1.2 | 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 (Esvelt et al., 2014). However, gene drives in insects are less favourable as controlling the travel of the genetically modified insect population is almost impossible, relative to the control that can be implemented for a GMO plant (Reeves & Phillipson, 2017), so gene technology regulators and governments are taking a cautious approach in regulating gene drive research with applications in the control of insects, particularly those that are vectors for human diseases (Bier, 2022). Targeting the sensory ability of insect pests is also an option, although this study focus is not as common as developing traits and topical applications. For example, 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 (Champer et al., 2016). Given the complex challenge of controlling insect pests, a multifaceted approach incorporating Integrated Pest Management (IPM) strategies is required for optimal control, likely through combining cultivar resistance with management practices and topical applications of environmentally friendly biopesticides.

4.2 | Fungal diseases

Fusarium and *Verticillium* wilt are two of the most devastating diseases in the global cotton industry (Li et al., 2017b) and many other crops including potato (Davis et al., 1996; Johnson & Dung, 2010), peanut (Woodward et al., 2011), safflower (Rao et al., 2014; Urie & Knowles, 1972), tomato (Song et al., 2004), olive (Mercado-Blanco et al., 2003), banana (Ploetz, 2015) and chickpea (Jendoubi et al., 2017). These soil-borne fungi can exist in many different forms in the soil, crop debris, other crops and weeds, and the severity of *Verticillium* wilt tends to worsen with cold, wet conditions (Figure 3; Li et al., 2017b). These diseases can be managed to some degree through integrated agronomic practices, while significant advances towards disease-resistant cotton germplasm have also been achieved through the Commonwealth Scientific and Industrial Research Organisation's cotton breeding programme. Genes encoding anti-fungal proteins and signalling pathways have been reported to

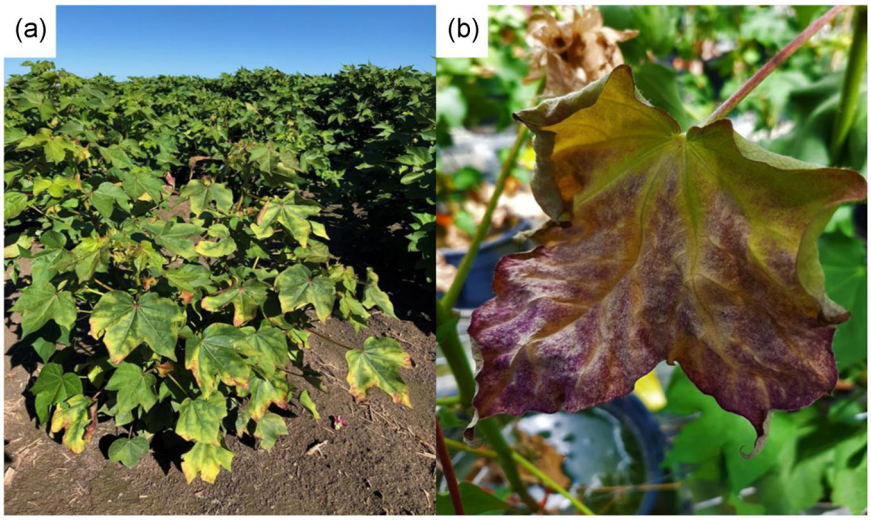

FIGURE 3 *Verticillium* wilt symptoms in cotton (a) whole plant (Photo: Duy Le) and (b) leaf symptoms (Photo: Lucy Egan).

improve cotton's resistance against fungal pathogens such as *Verticillium* and *Fusarium* wilt (Emani et al., 2003; Gaspar et al., 2014; Murray et al., 1999; Parkhi et al., 2010; Tian et al., 2010; Wang et al., 2004). However, it does not appear that any of these studies have resulted in the significant levels of resistance required to be incorporated into cultivars as a GM trait. There is a great need for resistance to these diseases in various crops, and current global activity in this area is high.

Several challenges constrain the development of *Verticillium*- and *Fusarium*-resistant crops. In cotton, cultivars that are more resistant to one of the diseases tend to be susceptible to the other (Li et al., 2017b). Additionally, one of the biggest challenges to the cotton industry is the existence of two pathotypes of *Verticillium* wilt, defoliating and nondefoliating (Li et al., 2017b), which can co-occur (Le et al., 2020) and elicit different responses to different cultivars. Breeding efforts thus far have been unable to develop dual resistance to both pathotypes. There are no cost-effective fungicides identified to date that effectively control *Verticillium* pathotypes, and host plant resistance in combination with management practices is currently the most economical and environmentally friendly approach to managing *Verticillium* wilt (Göre et al., 2017; Li et al., 2017b).

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* if separate resistance genes can be identified. Dual resistance to both *Verticillium* and *Fusarium* would also be highly valuable, albeit an ambitious task, but worth long-term investment. 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). However, these approaches rely on the identification of genes for resistance to introduce or genes to target in the *Verticillium* genome to inhibit infection or survival. Although numerous genes, quantitative trait loci (genomic regions) and proteins have been identified as potential contributors to some level of resistance to *Verticillium* in some 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., 2015, 2018; Zhang et al., 2017), the precise combination of genes and the location of their expression for conferring optimal resistance remain elusive. Further research is required to understand the pathogenicity of key crop diseases and identify genes that may confer resistance. Additionally, the location of their expression (i.e., in the root hairs or xylem) could be critical for effective resistance. This localisation could be aided or fast-tracked through using CRISPR-Cas9 to identify gene functions by targeted knockouts of all the genes within a genetic interval conferring resistance until the exact resistance gene is identified, while Golden Gate cloning would enable gene combinations and expression patterns to be tested.

4.3 | Photosynthetic carbon assimilation

Targeting improved photosynthesis is one of the next frontiers for improving food and fibre crop productivity, resource-use efficiency and abiotic stress tolerance (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). Photosynthetic pathways and abiotic stress responses are highly complex and impact multiple pathways (Figure 4). In some crop scenarios, the photosynthetic rate is poorly linked to yield, but under increasing levels of CO_2, the link is stronger (Long et al., 2006). Therefore, traits that target improved photosynthetic performance and resilience under abiotic stresses will require targeted integration of multiple genes and possibly new reaction pathways. Enhancing photosynthetic pathways would rely on the SynBio toolkit that can efficiently transfer large gene constructs with specified expression patterns. Examples include enhancing photosynthetic enzymes (Sharwood, 2017; Sharwood et al., 2022) improving WUE by introducing novel aquaporins and modifying cellular anatomy to improve mesophyll conductance (the diffusion of CO_2 into photosynthetic chloroplasts; Cousins et al., 2020; Ermakova et al., 2021). Heat-shock proteins (Reddy et al., 2016) and altering root traits (Hu & Xiong, 2014) are also targets for improving crop heat and drought tolerance and WUE that should be considered and could be particularly powerful when combined with photosynthetic enhancements.

4.3.1 | Enhancing productivity and thermotolerance through improving photosynthesis

Improving carbon assimilation and thermotolerance is likely to rely on modifying several key photosynthetic enzymes (in bold below) involved in the Calvin cycle (carbon fixation, reduction and regeneration) and the electron transport chain ('light photosynthesis'). **Rubisco** catalyses carbon fixation (carboxylation) inside the chloroplast and is a long-standing target of photosynthetic enhancement for yield gain (Sharwood, 2017). Carboxylation by Rubisco is aided by its 'helper protein' **Rubisco activase**, which prevents Rubisco from becoming inactivated and is thermolabile under abiotic stress (Kumar et al., 2009; Kurek et al., 2007; Sharwood, 2020). Carbon assimilation and crop biomass and yield have been improved under heat stress by introducing more thermostable Rubisco activase (Kumar et al., 2009; Kurek et al., 2007; Scafaro et al., 2019) or a catalytically superior Rubisco (Long & Ort, 2010; Zhu et al., 2010) or by modifying both Rubisco and Rubisco activase (Qu et al., 2021). **SBPase** is involved in the regeneration of RuBP during the Calvin cycle, the substrate for carboxylation by Rubisco. Overexpression and manipulation of SBPase can enhance photosynthesis under heat stress in transgenic rice (Feng et al., 2007), prevent heat-induced yield reduction in soybean (Köhler et al., 2016) and improve vegetative biomass and seed yield in *Arabidopsis* (Simkin et al., 2017). Overexpression or the introduction of novel SBPases can also improve crop WUE (López-Calcagno et al., 2020). **Cytochrome b_6f** is one of the four major

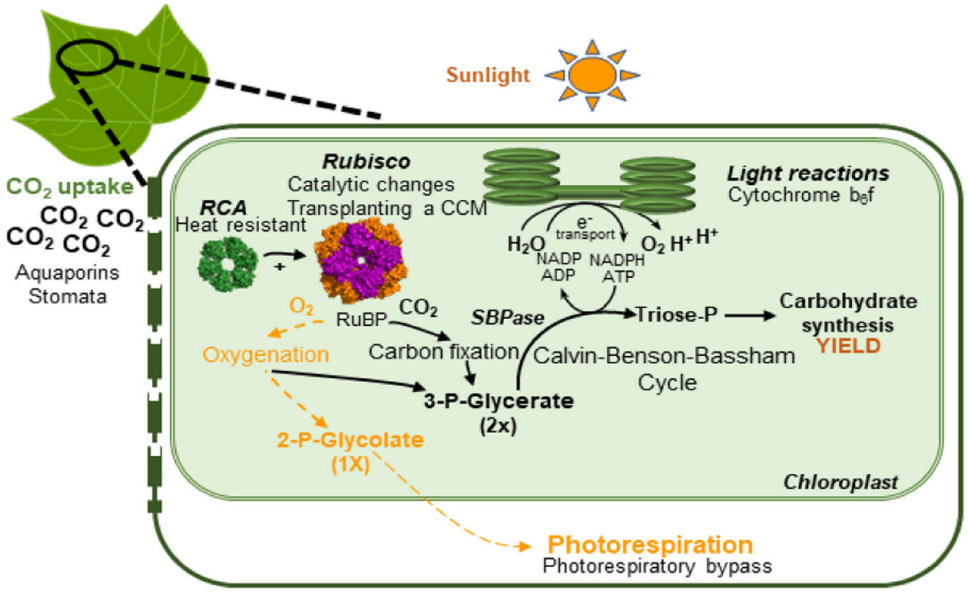

FIGURE 4 Selected strategies for improving carbon assimilation. Engineering elements of the Calvin–Benson–Bassham (CBB) cycle and light reaction systems are intended to improve the synthesis of carbohydrates that are required for plant growth, biomass production and yield. Strategies include improving Rubisco catalysis either through altering catalytic properties through transplanting subunits (Sharwood, 2017) or directed evolution (Zhou & Whitney, 2019) or co-engineering Rubisco into a carbon concentrating mechanism (Sharwood, 2017) and improving Rubisco activase function, which modulates Rubisco activity and is thermolabile (Sharwood, 2017). Additional strategies involve elevating the metabolic flux through the CBB cycle by overexpressing sedoheptulose 1, 7-bisphosphatase (Driever et al., 2017) and overexpressing light reaction components such as cytochrome b_6f (Ermakova et al., 2019). Other strategies include improving CO_2 diffusion through repurposing CO_2 permeable aquaporins (Ermakova et al., 2021) and/or improving stomatal regulation (Lawson & Vialet-Chabrand, 2019), engineering a photorespiratory bypass to remedy inefficiencies associated with Rubisco oxygenation (South et al., 2019) and transplanting CO_2 concentrating mechanisms either in the form of a carboxysome (Long et al., 2018) or a pyrenoid (Atkinson et al., 2020). CCM, carbon-concentrating mechanism.

light-harvesting protein complexes in the chloroplast membrane, involved in electron transport reactions that provides energy for carbon assimilation by the Calvin–Benson–Bassham cycle. Overexpression of light-harvesting complexes such as b_6f offer opportunities to improve crop carbon acquisition (Ermakova et al., 2019; Yamori et al., 2016).

4.3.2 | Improving crop WUE and drought tolerance

Developing more productive, drought stress-resilient and water-use efficient crops are required for productivity to continue in a warmer and drier world. SynBio can facilitate the transfer of novel drought tolerance and improved WUE traits that are not possible through conventional breeding.

Modifying C_3 crops like rice, wheat, canola and cotton to have a more efficient photosynthetic metabolism is one such strategy that is only possible through more advanced technologies offered by SynBio (Depaoli et al., 2014). Crassulacean acid metabolism (CAM) and C_4 photosynthesis are renowned for being more water-use efficient than C_3 photosynthesis (Borland et al., 2014; Depaoli et al., 2014; Ermakova et al., 2020b). This is due to the presence of carbon-concentrating mechanisms (CCMs) that reduce the need for as much stomatal opening, thus transpiring less water while maintaining carbon assimilation rates (Borland et al., 2014). Consequently, CAM plants can use 20%–80% less water to produce the same amount of biomass compared to C_3 and C_4 plants (Antony & Borland, 2009; Von Caemmerer et al., 2012). An added bonus of this mechanism is that its expression can be induced. Facultative CAM species are capable of expressing mRNA encoding for CAM enzymes in response to abiotic stresses, 'switching on' this mechanism when it is most needed (Winter & Holtum, 2014). This could be exploited to develop cotton cultivars capable of switching to more water-use efficient metabolisms when water is scarce. The potential productivity impact of introducing water-conserving mechanisms needs to be carefully considered. Water-preserving traits are likely to be most beneficial in rainfed production systems, particularly if inducing a yield penalty is to be avoided.

Aquaporins are proteins that facilitate the movement of water in plants and/or other substrates like CO_2, silicon, boron and ions (Groszmann et al., 2017; Uehlein et al., 2003). Expression and overexpression experiments revealed the influence of aquaporins on photosynthesis, mesophyll conductance in C_4 plants (Ermakova et al., 2021), stomatal conductance and root hydraulic conductivity, and thus productivity and water use (Sade et al., 2009). Consequently, aquaporins have emerged as another target for developing water-use efficient and drought-resistant crops (Ermakova et al., 2021). However, it is important to note that ectopic expression of

At PIP1;2 and 1;4 did not result in tobacco plants with improved mesophyll conductance (Clarke et al., 2022, in press).

4.3.3 | Photosynthetic enhancement will rely on SynBio

If traits are not found in closely related species suitable for crop breeding, the photosynthetic enhancement will need to rely on SynBio approaches. Photosynthetic manipulation is complex, requiring the introgression or modification of multiple genes to improve flux through the Calvin cycle to enhance the production of carbohydrates (Figure 4). Therefore, rapid and efficient insertion of multiple transgenes into target crops will be paramount (Castilho, 2015; Simkin et al., 2019). SynBio has enabled multiple genetic modifications to occur in a single event, thus enabling improvement of photosynthetic efficiency to improve crop performance, heat and drought resilience and WUE (Kromdijk & Long, 2016; Kubis & Bar-Even, 2019; Ort et al., 2015; Shih et al., 2014; Simkin et al., 2019). Multiple targets, such as Rubisco (either through creating new versions through directed evolution [Zhou & Whitney, 2019] or transplanting foreign large and small subunits [Whitney et al., 2011]), Rubisco activase and possibly also their supporting chaperones (Aigner et al., 2017), would be required to successfully enhance photosynthesis and abiotic stress resilience. Additionally, tissue-specific expression may also be required. This would be particularly important for the installation of a C_4 photosynthetic CCM. Stacking genes through Golden Gate would provide an efficient approach to this multigene modification (Depaoli et al., 2014; Maurino & Weber, 2013). An alternative or addition to gene stacking, gene editing through CRISPR-Cas9 could be used to edit amino acids that confer a catalytic switch, enhancing the photosynthetic activity of enzymes such as Rubisco, which has the small subunit gene coded in the nucleus (Sharwood, 2017; Whitney et al., 2011). This would further include Rubisco activase and other photosynthetic proteins that are transported into the chloroplast.

The SynBio toolkit also offers the unique ability of specific regulated promoters to 'switch on' abiotic stress genes that improve CO_2 assimilation (i.e., increase Rubisco or Rubisco activase activity) under high temperatures (Venter, 2007). For example, identification of the promoter elements responsible for the upregulation of different beta and alpha isoforms of Rubisco activase in wheat (Degen et al., 2021) and *Setaria viridis* (Kim et al., 2021) under heat stress could be used to drive the expression of alternative thermotolerant Rubisco activase isoforms from organisms such as *Agave tequilana* and wild rice (Scafaro et al., 2016; Shivhare & Mueller-Cajar, 2017). This would enable improved carbon assimilation at higher temperatures and circumvent yield penalties arising from heatwave conditions or periods of extended heat (Scafaro et al., 2018). Regulated promoters may also be extremely valuable for preventing yield penalties for conservative mechanisms such as water-preserving mechanisms that may hinder yield under prolonged expression. This approach would need to be experimentally tested through trialling a prototype under abiotic stress conditions in the field.

4.4 | Nutrient acquisition

Currently, productivity in western agriculture is sustained by a massive use of fertilisers. The excessive use of fertilisers is environmentally damaging and consumer interest in more sustainable products is increasing. Additionally, this practice is unsustainable due to expected future rising energy costs to produce fertilisers, the low nitrogen-use efficiency of crops and finite availability of macronutrients such as phosphorus (Heuer et al., 2017; Perchlik & Tegeder, 2017; Rogers & Oldroyd, 2014). This challenge can be overcome by improving crop nutrient-use efficiency, uptake or assimilation (Roell & Zurbriggen, 2020). Due to the complexity of these traits and systems, SynBio offers some of the most efficient and effective solutions. Some of the most supported approaches include engineering both crops and their associated microbes to improve the fixation, mobilisation and uptake of macronutrients such as nitrogen and phosphorus (Roell & Zurbriggen, 2020). Symbiotic relationships have evolved between some plant species—most notably, legumes—and nitrogen-fixing bacteria. This interaction delivers around 120 kg/ha of fixed nitrogen directly into the plant's roots (Salvagiotti et al., 2008). Engineering maize to fix the equivalent of 50 kg (N)/ha could substantially improve crop yield (Rogers & Oldroyd, 2014), as demonstrated through modelling (Folberth et al., 2013). In addition to yield improvement, engineering crops to fix their own nitrogen could improve crop nitrogen use efficiency and significantly reduce fertiliser use. Alternatively to crop engineering, crop utilisation of nitrogen and phosphorus could be significantly enhanced in several ways by looking towards microbes.

4.4.1 | Introducing enzymes

Enzymes such as nitrogenases or phytases could be introduced into crops to enable the fixation of essential nutrients. Nitrogenases are enzymes that naturally occur in some bacteria and Archaea, enabling them to fix nitrogen directly from the atmosphere. SynBio approaches could enable these enzymes to be introduced directly into plant cell organelles (Allen et al., 2020, 2017).

4.4.2 | Enhancing existing microbial activity

Introducing new microbes to agricultural soils (identified in nature or engineered synthetic communities) has been raised as an opportunity to improve nutrient assimilation in crops (Chen et al., 2021). However, the difficulty in establishing a foreign microbe into a niche that is likely already occupied by 'local' microbes adapted to local conditions raises concerns around the feasibility of this approach. Instead, approaches that manipulate microbes already present in the

rhizosphere, endosphere or phyllosphere could be more feasible approaches (Bloch et al., 2020). SynBio offers the opportunity to enhance the nitrogen fixation of soil microbes to increase the access of crops to plant-available nitrogen and improve their nutrient use efficiency (Chen et al., 2021; Waltz, 2017). This could be achieved through SynBio in two ways: (i) develop synthetic communities of microbes with specified functions or engineer and reintroduce existing microbes or (ii) engineer crops to release favourable root exudates to manipulate or support microbial activity. The former needs more development due to the issue of controlling the location of genetically engineered material in microbial communities. However, this approach could still be feasible; Bloch et al. (2020) have used gene editing to improve nitrogen fixation by a naturally occurring diazotroph bacterium associated with maize roots that can be applied as a seed coating and reduce fertiliser applications and increase yield. Alternatively, targeting the ability of plants to attract beneficial microbes or manipulate microbial pathways and thus the nitrogen cycle through the production of root exudates (Bardgett et al., 2014; Coskun et al., 2017; Finzi et al., 2015) is potentially a more effective agronomic application that is supplied directly to the plant. This approach will rely on an improved understanding of species-specific interactions between crops and available microbes, and the composition of their root exudates (i.e., carbohydrates, flavonoids and terpenoids identified through metabolomics or transcriptomics) that are required to attract beneficial microbes or stimulate biological nitrogen fixation and belowground nitrogen transfer (Coskun et al., 2017). After this, the genes associated with such exudate components will need to be identified (i.e., through genomics, transcriptomics or CRISPR-Cas9 knockouts of potential targets) to be upregulated or modified through SynBio (i.e., through Golden Gate cloning or CRISPR-Cas9). Optimising root exudate release to improve crop nitrogen use efficiency could also reduce nitrogen loss via leaching, runoff and denitrification, thus mitigating nitrogen pollution from crop production (Coskun et al., 2017).

4.4.3 | Establishing rhizobium–legume-like interactions

Inducing nodule formation and microbe recruitment in nonleguminous crops to mimic the rhizobium–legume symbiosis (Huisman & Geurts, 2020; Rogers & Oldroyd, 2014) could be another opportunity to enhance a crop's nutrient assimilation while improving soil fertility. This would require the expression of particular root exudate compounds such as flavonoids that induce the expression of nodulation factors in local microbes that trigger root nodule formation (Beatty & Good, 2011; Oldroyd et al., 2009). Despite the immense interest and research in this field, developing nodulation in nonleguminous crops is yet to be achieved. Hundreds of genes involved in nodulation in legumes have been identified, but selecting the combination required to induce nodulation in a nonnodulating crop has proven challenging (Huisman & Geurts, 2020). CRISPR-Cas9 and RNAi have been suggested as tools to help narrow this search and identify genes that are required for nodulation, while Golden Gate cloning technology is likely the most efficient approach to transferring the large number of genes that are likely to be required to successfully induce and support nodulation and nitrogen fixation (Huisman & Geurts, 2020). Huisman and Geurts (2020) present a comprehensive review outlining the limitations and requirements for engineering nodulation in crops.

5 | BENEFITS, CONSIDERATIONS AND LIMITATIONS OF SYNBIO IN AGRICULTURE

5.1 | Benefits

SynBio is viewed as a field of biology that could be the key to achieving the 'next Green Revolution' that is required to sustainably feed a growing global population in increasingly challenging circumstances. Traits that have reached their genetic potential through conventional breeding would immensely benefit from SynBio approaches by targeting traits from distantly related species, other organisms or synthetically generated. SynBio can also generate nonbreeding solutions, ranging from topical applications against pests and diseases to generating sterile pests and weeds, and novel properties such as enhanced oil profiles (Figure 5).

SynBio provides a range of tools to develop more complex traits and properties in crops more rapidly than other approaches. A good example of this is C_4 rice, a project that seeks to improve photosynthetic and nitrogen-use efficiency and WUE of rice. This goal requires the conversion of its photosynthetic system from C_3 to C_4, a complex strategy that involves complex 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). Introducing up to 20 genes is required to 'completely rewire' rice metabolism and anatomy (Ermakova et al., 2020b). The construction of such large and complex multigene vectors has largely been enabled by falling gene synthesis costs, synthetic promoter systems and the establishment of complex DNA assembly techniques such as Golden Gate cloning (Ermakova et al., 2020b; Rogers & Oldroyd, 2014). The growth and development of SynBio in the last 5 years has enabled the C_4 rice project to adopt a more rapid cycle of design, test and prototype coupled to the adoption of a rapid *Agrobacterium*-based rice transformation system in a rice cultivar that is fast-flowering, day-neutral, small and an established model for functional genomics (Ermakova et al., 2020b; Li et al., 2017a).

Molecular switches (i.e., regulated promoters) are another tool that can have agronomic applications. Molecular switches activate a specific gene under specific conditions, thus activating a trait or response only when needed. This is an efficient system that can reduce resource waste (i.e., chemical defence production) when not needed. This technology can develop 'Smart Plants' that can adjust to the environment in new ways (Wright & Nemhauser, 2019). This could be a valuable application in targeting abiotic stress resilience

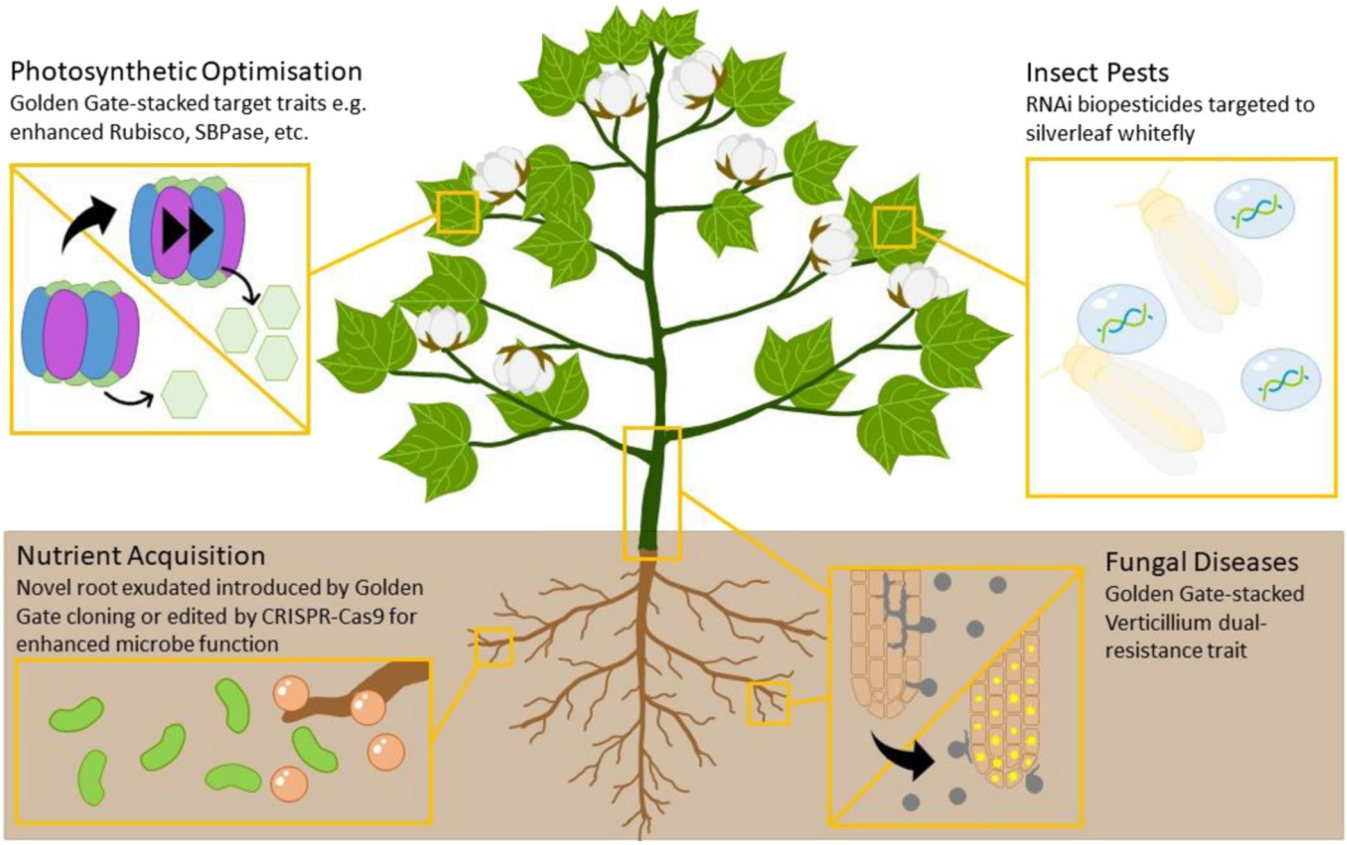

FIGURE 5 A schematic outlining a range of opportunities using synthetic biology to address key agricultural challenges. (Clockwise from top left) Photosynthetic optimisation is possible through the use of Golden Gate cloning to stack traits for the enhancement (i.e., accelerated catalytic rate) of critical photosynthetic proteins such as Rubisco (Engler et al., 2009; Sharwood, 2017). Key insect pests such as the silverleaf whitefly could be specifically targeted by RNAi biopesticides using Bioclay technology (Worrall et al., 2019). Effective resistance against fungal diseases such as *Verticillium* wilt could be achieved in a broad range of host crops using Golden Gate cloning to stack multiple traits for complex resistance, even against dual pathotypes that affect cotton. Nutrient acquisition could be enhanced through multiple strategies, such as Golden Gate cloning-introduced or CRISPR-Cas9-edited genes for the expression of novel root exudates to enhance soil microbe nutrient fixation (Coskun et al., 2017). RNAi, RNA interference.

(Degen et al., 2020) through the development of crop cultivars that 'activate' resilience genes when abiotic stress conditions are first detected.

5.2 | Considerations

Solutions need to be simple, accurate and affordable, addressing the challenges faced by resource-poor farmers and underserved consumers (Pixley et al., 2019). As agriculture becomes more globalised, issues and solutions would benefit from extending beyond an Australian context to be economically viable and impactful. Additionally, equitable access to the benefits of resulting GM crops requires affirmative policies, targeted investments and excellent science (Pixley et al., 2019). Lack of success in some SynBio projects has been due to focusing on humanitarian or environmental sustainability goals that are difficult to monetise (Pixley et al., 2019). Financial benefit to multiple aspects of the value chain needs to be carefully considered to ensure SynBio projects are high value and impactful.

5.3 | Limitations

The biggest limitations constraining the progress of SynBio application is the cost of deregulation (most SynBio applications are considered genetic modification and subject to the same laws as traditional genetic engineering products), a complex patent landscape that needs to be navigated for any potential commercial applications, and social licence. Social acceptance will be a significant challenge to the successful adoption of SynBio and will be similar to that faced by GMO crops over the last three decades. Additionally, successful adoption, application and acceleration of SynBio will rely on investment by various sectors, both public and private.

Another significant limitation that has slowed the progress of applying SynBio is the requirement to identify the precise gene(s) required for a targeted function. This is particularly challenging and prolonged when addressing multigenic functions. The development of transgenic plants depends on the optimisation of a suitable transgene transfer and integration procedure. Currently, most key food and fibre crops are predominantly transformed through

Agrobacterium and particle bombardment-mediated gene transfer. Regeneration of plants from transformed tissue of cotton, for example, has seen limited success arising from problems such as somaclonal variation from prolonged culture periods, high frequencies of abnormal embryo development, low conversion rates of somatic embryos into plantlets and high genotype dependency (Mishra et al., 2003; Stelly et al., 1989; Sun et al., 2006). In addition, only a small number of generally older cotton cultivars are amenable to transformation with *Agrobacterium*, but genotype independent protocols are starting to emerge (Chen et al., 2014). Such protocols will allow the transformation of elite cultivars directly and allow new SynBio-developed traits to be added without requiring extensive further breeding.

Finally, any SynBio opportunity requires significant investment, both in time and finances, to yield a technology, product or trait. Many years, likely a decade or longer, of fundamental and proof-of-concept research, is required before the years (likely decades) required to develop a SynBio technology, product or trait. Therefore, committing to a SynBio development opportunity would depend on a partnership or co-investment to invest adequate time, expertise and money into the project to be successful.

6 | CONCLUSIONS

The opportunities offered by SynBio to address various agronomic challenges are 'endless'. However, assessing these opportunities with a *realistic* lens is critical. Despite the substantial efficiency and effectiveness that can be achieved through SynBio techniques, there are numerous challenges that continue to limit the successful application of SynBio. Numerous risks, costs, regulations and public perceptions can limit the uptake of any new technologies. Additionally, developing SynBio technologies, products and traits require substantial investment—decades of research and development, and millions of dollars—to be successful. Ultimately, to be feasible SynBio research ventures need to have a clear value proposition, be highly impactful and have a high benefit to cost ratio.

AUTHOR CONTRIBUTIONS

Demi Sargent: Conceived and contributed to writing the manuscript. **Warren C. Conaty**: Conceived and contributed to writing the manuscript. **David T. Tissue**: Contributed to writing and editing. **Robert E. Sharwood**: Conceived and contributed to writing the manuscript. All authors edited the manuscript.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the Australian Cotton Research and Development Corporation for funding and support (UWS2101 and UWS2201), as well as Cotton Breeding Australia for support. We also acknowledge the following people for valuable discussions that have contributed towards this review: Warwick Stiller (Commonwealth Scientific and Industrial Research Organisation [CSIRO]), Steve Swain (CSIRO), 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). Additional thanks to Danny Llewellyn (CSIRO), Lily Chen (WSU) and Timothy Rhodes (ANU) for your helpful insights and review of the manuscript.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Not applicable for review article.

ETHICS STATEMENT

The authors confirm they have adhered to journal policies regarding ethics.

ORCID

Demi Sargent https://orcid.org/0000-0001-5951-4119
Warren C. Conaty https://orcid.org/0000-0003-1278-153X
David T. Tissue https://orcid.org/0000-0002-8497-2047
Robert E. Sharwood https://orcid.org/0000-0003-4993-3816

REFERENCES

Aigner H, Wilson R, Bracher A, Calisse L, Bhat J, Hartl F, et al. Plant RuBisCo assembly in *E. coli* with five chloroplast chaperones including BSD2. Science. 2017;358:1272–8.
Ainsworth EA, Ort DR. How do we improve crop production in a warming world? Plant Physiol. 2010;154:526–30.
Aktar-Uz-Zaman M, Tuhina-Khatun M, Hanafi MM, Sahebi M. Genetic analysis of rust resistance genes in global wheat cultivars: an overview. Biotechnol Biotechnol Equip. 2017;31:431–45.
Allen RS, Gregg CM, Okada S, Menon A, Hussain D, Gillespie V, et al. Plant expression of NifD protein variants resistant to mitochondrial degradation. Proc Natl Acad Sci USA. 2020;117:23165–73.
Allen RS, Tilbrook K, Warden AC, Campbell PC, Rolland V, Singh SP, et al. Expression of 16 nitrogenase proteins within the plant mitochondrial matrix. Front Plant Sci. 2017;8:287.
Antony E, Borland A. The role and regulation of sugar transporters in plants with Crassulacean acid metabolism. Prog Bot. 2009:127–43.
Arnaiz A, Martinez M, Gonzalez-Melendi P, Grbic V, Diaz I, Santamaria ME. Plant defenses against pests driven by a bidirectional promoter. Front Plant Sci. 2019;10:1173.
Ashfaq MA, Dinesh Kumar V, Soma Sekhar Reddy P, Anil Kumar C, Sai Kumar K, Narasimha Rao N, et al. Post-transcriptional gene silencing: basic concepts and applications. J Biosci. 2020;45:128.
Atkinson N, Mao Y, Chan KX, Mccormick AJ. Condensation of Rubisco into a proto-pyrenoid in higher plant chloroplasts. Nat Commun. 2020;11:6303.
Bagla P. Hardy cotton-munching pests are latest blow to GM crops. Science. 2010;327:1439.
Barbetti MJ, Banga SS, Salisbury PA. Challenges for crop production and management from pathogen biodiversity and diseases under current and future climate scenarios—case study with oilseed Brassicas. Field Crops Res. 2012;127:225–40.
Bardgett RD, Mommer L, De Vries FT. Going underground: root traits as drivers of ecosystem processes. Trends Ecol Evol. 2014;29:692–9.

Beatty PH, Good AG. Future prospects for cereals that fix nitrogen. Science. 2011;333:416–7.

Bedinger PA, Chetelat RT, Mcclure B, Moyle LC, Rose JKC, Stack SM, et al. Interspecific reproductive barriers in the tomato clade: opportunities to decipher mechanisms of reproductive isolation. Sex Plant Reprod. 2011;24:171–87.

Belhaj K, Chaparro-Garcia A, Kamoun S, Patron NJ, Nekrasov V. Editing plant genomes with CRISPR/Cas9. Curr Opin Biotechnol. 2015;32: 76–84.

Bell AA. Mechanisms of disease resistance in *Gossypium species* and variation in *Verticillium dahliae*. In: Constable GA, Forrester NW, editors. Proceeding World Cotton Research Conference 1. Melbourne, Australia: CSIR; 1994. p. 225–35.

Betti M, Bauwe H, Busch FA, Fernie AR, Keech O, Levey M, et al. Manipulating photorespiration to increase plant productivity: recent advances and perspectives for crop improvement. J Exp Bot. 2016; 67:2977–88.

Bier E. Gene drives gaining speed. Nat Rev Genet. 2022;23:5–22.

Bloch SE, Clark R, Gottlieb SS, Wood LK, Shah N, Mak S-M, et al. Biological nitrogen fixation in maize: optimizing nitrogenase expression in a root-associated diazotroph. J Exp Bot. 2020;71:4591–603.

Boch J, Bonas U. Xanthomonas AvrBs3 Family-Type III effectors: discovery and function. Annu Rev Phytopathol. 2010;48:419–36.

Boch J, Scholze H, Schornack S, Landgraf A, Hahn S, Kay S, et al. Breaking the code of DNA binding specificity of TAL-Type III effectors. Science. 2009;326:1509–12.

Borland AM, Hartwell J, Weston DJ, Schlauch KA, Tschaplinski TJ, Tuskan GA, et al. Engineering crassulacean acid metabolism to improve water-use efficiency. Trends Plant Sci. 2014;19:327–38.

Brückner K, Schäfer P, Weber E, Grützner R, Marillonnet S, Tissier A. A library of synthetic transcription activator-like effector-activated promoters for coordinated orthogonal gene expression in plants. Plant J. 2015;82:707–16.

Campbell B, Saha S, Percy R, Frelichowski J, Jenkins JN, Park W, et al. Status of the global cotton germplasm resources. Crop Sci. 2010;50: 1161–79.

Carriere Y, Crowder DW, Tabashnik BE. Evolutionary ecology of insect adaptation to Bt crops. Evol Appl. 2010;3:561–73.

Castilho A. Glyco-engineering. Methods Mol Biol. 2015;1321:v–vii.

Chambers S, Kitney R, Freemont P. The Foundry: the DNA synthesis and construction Foundry at Imperial College. Biochem Soc Trans. 2016; 44:687–8.

Champer J, Buchman A, Akbari OS. Cheating evolution: engineering gene drives to manipulate the fate of wild populations. Nat Rev Genet. 2016;17:146–59.

Chen G, Du X-M. Genetic diversity of source germplasm of upland cotton in China as determined by SSR marker analysis. Acta Genet Sin. 2006;33:733–45.

Chen Q-L, Hu H-W, He Z-Y, Cui L, Zhu Y-G, HE J-Z. Potential of indigenous crop microbiomes for sustainable agriculture. Nat Food. 2021;2:233–40.

Chen Y, Rivlin A, Lange A, Ye X, Vaghchhipawala Z, Eisinger E, et al. High throughput *Agrobacterium tumefaciens*-mediated germline transformation of mechanically isolated meristem explants of cotton (*Gossypium hirsutum* L.). Plant Cell Rep. 2014;33:153–64.

Chen Y, Zhou X-R, Zhang Z-J, Dribnenki P, Singh S, Green A. Development of high oleic oil crop platform in flax through RNAi-mediated multiple FAD2 gene silencing. Plant Cell Rep. 2015;34: 643–53.

Cheng H-Q, Han L-B, Yang C-L, Wu X-M, Zhong N-Q, Wu J-H, et al. The cotton MYB108 forms a positive feedback regulation loop with CML11 and participates in the defense response against *Verticillium dahliae* infection. J Exp Bot. 2016;67:1935–50.

Christy B, Tausz-Posch S, Tausz M, Richards R, Rebetzke G, Condon A, et al. Benefits of increasing transpiration efficiency in wheat under elevated CO_2 for rainfed regions. Global Change Biol. 2018;24: 1965–77.

Clarke VC, Massey B, George AM, Evans JR, Von Caemmerer S, Groszmann M. Mesophyll conductance is unaffected by expression of *Arabidopsis* PIP1 aquaporins in the plasmalemma of *Nicotiana*. J Exp Bot. 2022, in press. https://doi.org/10.1093/jxb/erac065

Clement JD, Constable GA, Stiller WN, Liu SM. Early generation selection strategies for breeding better combinations of cotton yield and fibre quality. Field Crops Res. 2015;172:145–52.

Coskun D, Britto DT, Shi W, Kronzucker HJ. How plant root exudates shape the nitrogen cycle. Trends Plant Sci. 2017;22:661–73.

Cotton Australia. Biotechnology and cotton [online]. https://cottonaustralia.com.au/fact-sheet (2021). Accessed 16 March 2021.

Cousins AB, Mullendore DL, Sonawane BV. Recent developments in mesophyll conductance in C_3, C_4, and crassulacean acid metabolism plants. Plant J. 2020;101(4):816–30. https://doi.org/10.1111/tpj.14664

Cousins Y, Lyon B, Llewellyn D. Transformation of an Australian cotton cultivar: prospects for cotton improvement through genetic engineering. Funct Plant Biol. 1991;18:481–94.

Cox K, Meng F, Wilkins K, Li F, Wang P, Booher N, et al. TAL effector driven induction of a SWEET gene confers susceptibility to bacterial blight of cotton. Nat Commun. 2017;8:15588.

Da Silva Oliveira CE, Hoffmann LV, Toscano LC, Queiroz MS, Zoz T, Witt TW. Resistance of cotton genotypes to silverleaf whitefly (*Bemisia tabaci* [GENNADIUS] Biotype B). Int J Trop Insect Sci. 2021; 41:1697–707.

Davis J, Huisman O, Westermann D, Hafez S, Everson D, Sorensen L, et al. Effects of green manures on *Verticillium* wilt of potato. Phytopathology. 1996;86:444–53.

Degen GE, Orr DJ, Carmo-Silva E. Heat-induced changes in the abundance of wheat Rubisco activase isoforms. New Phytol. 2021; 229:1298–311.

Degen GE, Worrall D, Carmo-Silva E. An isoleucine residue acts as a thermal and regulatory switch in wheat Rubisco activase. Plant J. 2020;103:742–51.

Depaoli HC, Borland AM, Tuskan GA, Cushman JC, Yang X. Synthetic biology as it relates to CAM photosynthesis: challenges and opportunities. J Exp Bot. 2014;65:3381–93.

Dill GM. Glyphosate-resistant crops: history, status and future. Pest Manag Sci. 2005;61:219–24.

Dillehay BL, Roth GW, Calvin DD, Kratochvil RJ, Kuldau GA, Hyde JA. Performance of Bt corn hybrids, their near isolines, and leading corn hybrids in Pennsylvania and Maryland. Agron J. 2004;96:1–12.

Doebley JF, Gaut BS, Smith BD. The molecular genetics of crop domestication. Cell. 2006;127:1309–21.

Dong Q, Magwanga RO, Cai X, Lu P, Nyangasi Kirungu J, Zhou Z, et al. RNA-sequencing, physiological and RNAi analyses provide insights into the response mechanism of the *ABC*-Mediated resistance to *Verticillium dahliae* infection in cotton. Genes. 2019;10:110.

Downes S, Kriticos D, Parry H, Paull C, Schellhorn N, Zalucki MP. A perspective on management of Helicoverpa armigera: transgenic Bt cotton, IPM, and landscapes. Pest Manag Sci. 2017;73:485–92.

Downes S, Walsh T, Tay WT. Bt resistance in Australian insect pest species. Curr Opin Insect Sci. 2016;15:78–83.

Driever SM, Simkin AJ, Alotaibi S, Fisk SJ, Madgwick PJ, Sparks CA, et al. Increased SBPase activity improves photosynthesis and grain yield in wheat grown in greenhouse conditions. Philos Trans R Soc Lond B-Biol Sci. 2017;372:1–10.

Duan X, Zhang Z, Wang J, Zuo K. Characterization of a novel cotton subtilase gene *GbSBT1* in response to extracellular stimulations and its role in *Verticillium* resistance. PLoS One. 2016;11:e0153988.

Dutt M, Ananthakrishnan G, Jaromin M, Brlansky R, Grosser J. Evaluation of four phloem-specific promoters in vegetative tissues of transgenic citrus plants. Tree Physiol. 2012;32:83–93.

Duvick DN. The contribution of breeding to yield advances in maize (*Zea mays* L.). In: Sparks DL, editor. Advances in agronomy. Amsterdam: Elsevier; 2005. p. 83–145.

Dwivedi SL, Crouch JH, Mackill DJ, Xu Y, Blair MW, Ragot M, et al. The molecularization of public sector crop breeding: progress, problems, and prospects. Adv Agron. 2007;95:163–318.

Dwivedi SL, Scheben A, Edwards D, Spillane C, Ortiz R. Assessing and exploiting functional diversity in germplasm pools to enhance abiotic stress adaptation and yield in cereals and food legumes. Front Plant Sci. 2017;8:1461.

Eakteiman G, Moses-Koch R, Moshitzky P, Mestre-Rincon N, Vassão DG, Luck K, et al. Targeting detoxification genes by phloem-mediated RNAi: a new approach for controlling phloem-feeding insect pests. Insect Biochem Mol Biol. 2018;100:10–21.

Ellsworth PC, Bordini I, Pier N. ThryvOn™ cotton frequently asked questions. Tucson, AZ: The University of Arizona; 2021. https://hdl.handle.net/10150/659845

Emani C, Garcia JM, Lopata-Finch E, Pozo MJ, Uribe P, Kim DJ, et al. Enhanced fungal resistance in transgenic cotton expressing an endochitinase gene from *Trichoderma virens*. Plant Biotechnol J. 2003;1:321–36.

Engler C, Gruetzner R, Kandzia R, Marillonnet S. Golden Gate shuffling: a one-pot dna shuffling method based on Type IIs restriction enzymes. PLoS One. 2009;4:e5553.

Engler C, Youles M, Gruetzner R, Ehnert T-M, Werner S, Jones JDG, et al. A Golden Gate modular cloning toolbox for plants. ACS Synth Biol. 2014;3:839–43.

Ermakova M, Arrivault S, Giuliani R, Danila F, Alonso-Cantabrana H, Vlad D, et al. Installation of C_4 photosynthetic pathway enzymes in rice using a single construct. Plant Biotechnol J. 2020a;19:575–88.

Ermakova M, Danila FR, Furbank RT, Von Caemmerer S. On the road to C_4 rice: advances and perspectives. Plant J. 2020b;101:940–50.

Ermakova M, Lopez-Calcagno PE, Raines CA, Furbank RT, Von Caemmerer S. Overexpression of the Rieske FeS protein of the cytochrome b_6f complex increases C_4 photosynthesis in *Setaria viridis*. Commun Biol. 2019;2:314.

Ermakova M, Osborn H, Groszmann M, Bala S, Bowerman A, Mcgaughey S, et al. Expression of a CO_2-permeable aquaporin enhances mesophyll conductance in the C_4 species *Setaria viridis*. eLife. 2021;10:e70095.

Esvelt KM, Smidler AL, Catteruccia F, Church GM. Concerning RNA-guided gene drives for the alteration of wild populations. eLife. 2014;3:e03401.

Farzadfard F, Perli SD, Lu TK. Tunable and multifunctional eukaryotic transcription factors based on CRISPR/Cas. ACS Synth Biol. 2013;2:604–13.

Feng L, Wang K, Li Y, Tan Y, Kong J, Li H, et al. Overexpression of SBPase enhances photosynthesis against high temperature stress in transgenic rice plants. Plant Cell Rep. 2007;26:1635–46.

Finzi AC, Abramoff RZ, Spiller KS, Brzostek ER, Darby BA, Kramer MA, et al. Rhizosphere processes are quantitatively important components of terrestrial carbon and nutrient cycles. Global Change Biol. 2015;21:2082–94.

Fire A. RNA-triggered gene silencing. TIG. 1999;15:358–63.

Fitt GP, Wilson L. Integration of Bt cotton in IPM systems: an Australian perspective. In: Second international symposium on biological control of arthropods. Washington DC: USDA. 2005. Vol. 1, p. 382–9.

Fletcher SJ, Reeves PT, Hoang BT, Mitter N. A perspective on RNAi-based biopesticides. Front Plant Sci. 2020;11:51.

Folberth C, Yang H, Gaiser T, Abbaspour KC, Schulin R. Modeling maize yield responses to improvement in nutrient, water and cultivar inputs in sub-Saharan Africa. Agric Syst. 2013;119:22–34.

Furbank RT, Sharwood R, Estavillo GM, Silva-Perez V, Condon AG. Photons to food: genetic improvement of cereal crop photosynthesis. J Exp Bot. 2020;71:2226–38.

Gan Y, Blackshaw RE, May WE, Vera C, Johnson EN. Yield stability and seed shattering characteristics of *Brassica juncea* canola in the Northern Great Plains. Crop Sci. 2016;56:1296–305.

Ganesan M, Jayabalan N. Isolation of disease-tolerant cotton (*Gossypium hirsutum* L. cv. SVPR 2) plants by screening somatic embryos with fungal culture filtrate. Plant Cell Tissue Organ Cult. 2006;87:273–84.

Gaspar YM, Mckenna JA, Mcginness BS, Hinch J, Poon S, Connelly AA, et al. Field resistance to *Fusarium oxysporum* and *Verticillium dahliae* in transgenic cotton expressing the plant defensin *NaD1*. J Exp Bot. 2014;65:1541–50.

Gayoso C, Pomar F, Novo-Uzal E, Merino F, Martínez De Ilárduya Ó. The Ve-mediated resistance response of the tomato to *Verticillium dahliae* involves H_2O_2, peroxidase and lignins and drives PALgene expression. BMC Plant Biol. 2010;10:1–19.

Gifford RM, Thorne JH, Hitz WD, Giaquinta RT. Crop productivity and photoassimilate partitioning. Science. 1984;225:801–8.

Göre ME, Erdoğan O, Altin N. Searching for resistance sources to *Verticillium* wilt of cotton in seedlings from *Gossypium* spp. Trop Plant Pathol. 2017;42:28–31.

Gould F. Sustainability of transgenic insecticidal cultivars: integrating pest genetics and ecology. Annu Rev Entomol. 1998;43:701–26.

Graeber JV, Nafziger ED, Mies DW. Evaluation of transgenic, Bt-containing corn hybrids. J Prod Agric. 1999;12:659–63.

Groszmann M, Osborn HL, Evans JR. Carbon dioxide and water transport through plant aquaporins. Plant Cell Environ. 2017;40:938–61.

Grover A, Mittal D, Negi M, Lavania D. Generating high temperature tolerant transgenic plants: achievements and challenges. Plant Sci. 2013;205–6:38–47.

Gustafsson C, Govindarajan S, Minshull J. Codon bias and heterologous protein expression. Trends Biotechnol. 2004;22:346–53.

Haverkort AJ, Boonekamp PM, Hutten R, Jacobsen E, Lotz LAP, Kessel GJT, et al. Durable late blight resistance in potato through dynamic varieties obtained by cisgenesis: scientific and societal advances in the DuRPh Project. Potato Res. 2016;59:35–66.

Hayes KR, Hosack GR, Dana GV, Foster SD, Ford JH, Thresher R, et al. Identifying and detecting potentially adverse ecological outcomes associated with the release of gene-drive modified organisms. J Responsible Innov. 2018;5:S139–58.

Heuer S, Gaxiola R, Schilling R, Herrera-Estrella L, López-Arredondo D, Wissuwa M, et al. Improving phosphorus use efficiency: a complex trait with emerging opportunities. Plant J. 2017;90:868–85.

Hibberd JM, Covshoff S. The regulation of gene expression required for C_4 photosynthesis. Annu Rev Plant Biol. 2010;61:181–207.

Hibberd JM, Sheehy JE, Langdale JA. Using C_4 photosynthesis to increase the yield of rice—rationale and feasibility. Curr Opin Plant Biol. 2008;11:228–31.

Hillocks RJ. Cotton diseases control: contrasting approaches. In: Gillham FM, editor. Proceedings of the world cotton research conference 2, new frontiers in cotton research, Athens. 1998. p. 69–74.

Hu H, Xiong L. Genetic engineering and breeding of drought-resistant crops. Annu Rev Plant Biol. 2014;65:715–41.

Huisman R, Geurts R. A roadmap toward engineered nitrogen-fixing nodule symbiosis. Plant Commun. 2020;1:100019.

Hung Y-H, Slotkin RK. The initiation of RNA interference (RNAi) in plants. Curr Opin Plant Biol. 2021;61:102014.

Iqbal M, Reddy O, El-Zik K, Pepper A. A genetic bottleneck in the 'evolution under domestication' of upland cotton *Gossypium hirsutum* L. examined using DNA fingerprinting. Theor Appl Genet. 2001;103:547–54.

Ishii T, Numaguchi K, Miura K, Yoshida K, Thanh PT, Htun TM, et al. OsLG1 regulates a closed panicle trait in domesticated rice. Nature Genet. 2013;45:462–5.

Javaid S, Amin I, Jander G, Mukhtar Z, Saeed NA, Mansoor S. A transgenic approach to control hemipteran insects by expressing insecticidal genes under phloem-specific promoters. Sci Rep. 2016;6:1–11.

Jendoubi W, Bouhadida M, Boukteb A, Béji M, Kharrat M. Fusarium wilt affecting chickpea crop. Agriculture. 2017;7:23.

Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA, Charpentier E. A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. Science. 2012;337:816–21.

Johnson DA, Dung JK. *Verticillium* wilt of potato—the pathogen, disease and management. Can J Plant Pathol. 2010;32:58–67.

Jørgensen K, Bak S, Busk PK, Sørensen C, Olsen CE, Puonti-Kaerlas J, et al. Cassava plants with a depleted cyanogenic glucoside content in leaves and tubers. Distribution of cyanogenic glucosides, their site of synthesis and transport, and blockage of the biosynthesis by RNA interference technology. Plant Physiol. 2005;139:363–74.

Jun Z, Zhang Z, Gao Y, Zhou L, Fang L, Chen X, et al. Overexpression of *GbRLK*, a putative receptor-like kinase gene, improved cotton tolerance to *Verticillium* wilt. Sci Rep. 2015;5:1–12.

Kandhro MM, Laghari S, Sial MA, Nizamani GS. Performance of early maturing strains of cotton (*Gossypium hirsutum* L.) developed through induced mutation and hybridization. Asian J Plant Sci. 2002;1:581–2.

Khan Z, Khan SH, Mubarik MS, Sadia B, Ahmad A. Use of TALEs and TALEN technology for genetic improvement of plants. Plant Mol Biol Report. 2017;35:1–19.

Kim SY, Slattery RA, Ort DR. A role for differential Rubisco activase isoform expression in C_4 bioenergy grasses at high temperature. GCB Bioenergy. 2021;13:211–23.

Kirkegaard JA, Lilley JM, Morrison MJ. Drivers of trends in Australian canola productivity and future prospects. Crop Pasture Sci. 2016;67: i–ix.

Kitashiba H, Nasrallah JB. Self-incompatibility in Brassicaceae crops: lessons for interspecific incompatibility. Breed Sci. 2014;64:23–37.

Knight RL. Breeding cotton resistant to blackarm disease. Emp J Exp Agric. 1946;14:161–74.

Köhler IH, Ruiz-Vera UM, Vanloocke A, Thomey ML, Clemente T, Long SP, et al. Expression of cyanobacterial FBP/SBPase in soybean prevents yield depression under future climate conditions. J Exp Bot. 2016; 68:715–26.

Kola VSR, Renuka P, Madhav MS, Mangrauthia SK. Key enzymes and proteins of crop insects as candidate for RNAi based gene silencing. Front Physiol. 2015;6:119.

Konermann S, Brigham MD, Trevino AE, Joung J, Abudayyeh OO, Barcena C, et al. Genome-scale transcriptional activation by an engineered CRISPR-Cas9 complex. Nature. 2015;517:583–8.

Kromdijk J, Long SP. One crop breeding cycle from starvation? How engineering crop photosynthesis for rising CO_2 and temperature could be one important route to alleviation. Proc R Soc B. 2016;283: 20152578.

Kruger M, Van Rensburg J, Van Den Berg J. Perspective on the development of stem borer resistance to Bt maize and refuge compliance at the Vaalharts irrigation scheme in South Africa. Crop Prot. 2009;28:684–9.

Kubis A, Bar-Even A. Synthetic biology approaches for improving photosynthesis. J Exp Bot. 2019;70:1425–33.

Kumar A, Li C, Portis AR. *Arabidopsis thaliana* expressing a thermostable chimeric Rubisco activase exhibits enhanced growth and higher rates of photosynthesis at moderately high temperatures. Photosynth Res. 2009;100:143–53.

Kurek I, Chang TK, Bertain SM, Madrigal A, Liu L, Lassner MW, et al. Enhanced thermostability of *Arabidopsis* Rubisco activase improves photosynthesis and growth rates under moderate heat stress. Plant Cell. 2007;19:3230–41.

Langdale JA. C_4 cycles: past, present, and future research on C_4 photosynthesis. Plant Cell. 2011;23:3879–92.

Langridge P, Fleury D. Making the most of 'omics' for crop breeding. Trends Biotechnol. 2011;29:33–40.

Lauer J, Wedberg J. Grain yield of initial Bt corn hybrid introductions to farmers in the Northern corn belt. J Prod Agric. 1999;12:373–6.

Lawson T, Vialet-Chabrand S. Speedy stomata, photosynthesis and plant water use efficiency. New Phytol. 2019;221:93–8.

Le DP, Gregson A, Tran TT, Jackson R. Co-occurrence of defoliating and non-defoliating pathotypes of *Verticillium dahliae* in field-grown cotton plants in New South Wales, Australia. Plants. 2020;9:750.

Ledford H, Callaway E. Pioneers of revolutionary CRISPR gene editing win chemistry Nobel. Nature. 2020;586:346–7.

Leigh T, Roach S, Watson T, King E, Phillips J, Coleman R. Biology and ecology of important insect and mite pests of cotton. Memphis, TN: The Cotton Foundation; 1996.

Li C, He X, Luo X, Xu L, Liu L, Min L, et al. Cotton WRKY1 mediates the plant defense-to-development transition during infection of cotton by *Verticillium dahliae* by activating *JASMONATE ZIM-DOMAIN1* expression. Plant Physiol. 2014;166:2179–94.

Li C, Li W, Zhou Z, Chen H, Xie C, Lin Y. A new rice breeding method: CRISPR/Cas9 system editing of the Xa13 promoter to cultivate transgene-free bacterial blight-resistant rice. Plant Biotech J. 2020; 18:313–5.

Li G, Jain R, Chern M, Pham NT, Martin JA, Wei T, et al. The sequences of 1504 mutants in the model rice variety Kitaake facilitate rapid functional genomic studies. Plant Cell. 2017a;29:1218–31.

Li X, Pei Y, Sun Y, Liu N, Wang P, Liu D, et al. A cotton cyclin-dependent kinase E confers resistance to *Verticillium dahliae* mediated by jasmonate-responsive pathway. Front Plant Sci. 2018;9:642.

Li X, Zhang YN, Ding C, Xu W, Wang X. Temporal patterns of cotton *Fusarium* and *Verticillium* wilt in Jiangsu coastal areas of China. Sci Rep. 2017b;7:12581.

Liu J, Liu D, Tao W, Li W, Wang S, Chen P, et al. Molecular marker-facilitated pyramiding of different genes for powdery mildew resistance in wheat. Plant Breed. 2000;119:21–4.

Liu Q, Wu M, Zhang B, Shrestha P, Petrie J, Green AG, et al. Genetic enhancement of palmitic acid accumulation in cotton seed oil through RNAi down-regulation of *ghKAS2* encoding β-ketoacyl-ACP synthase II (KASII). Plant Biotech J. 2017;15:132–43.

Liu R, Bassalo MC, Zeitoun RI, Gill RT. Genome scale engineering techniques for metabolic engineering. Metab Eng. 2015;32:143–54.

Liu S, Jaouannet M, Dempsey DMA, Imani J, Coustau C, Kogel K-H. RNA-based technologies for insect control in plant production. Biotech Adv. 2020;39:107463.

Liu S-P, Zhu Y-P, Xie C, Jue D-W, Hong Y-B, Chen M, et al. Transgenic potato plants expressing StoVe1 exhibit enhanced resistance to *Verticillium dahliae*. Plant Mol Biol Report. 2012;30:1032–9.

Long BM, Hee WY, Sharwood RE, Rae BD, Kaines S, Lim YL, et al. Carboxysome encapsulation of the CO2-fixing enzyme Rubisco in tobacco chloroplasts. Nat Commun. 2018;9:3570.

Long SP, Ort DR. More than taking the heat: crops and global change. Curr Opin Plant Biol. 2010;13:240–7.

Long SP, Zhu X-G, Naidu SL, Ort DR. Can improvement in photosynthesis increase crop yields? Plant Cell Environ. 2006;29:315–30.

López-Calcagno PE, Brown KL, Simkin AJ, Fisk SJ, Vialet-Chabrand S, Lawson T, et al. Stimulating photosynthetic processes increases productivity and water-use efficiency in the field. Nat Plants. 2020; 6:1054–63.

Luo Y, Ma T, Zhang A, Ong KH, Luo Z, Li Z, et al. Marker-assisted breeding of Chinese elite rice cultivar 9311 for disease resistance to rice blast and bacterial blight and tolerance to submergence. Mol Breed. 2017; 37:106.

Lyzenga WJ, Pozniak CJ, Kagale S. Advanced domestication: harnessing the precision of gene editing in crop breeding. Plant Biotech J. 2021; 19:660–70.

Mallapaty S. Australian gene-editing rules adopt 'middle ground'. Nature. 2019.
Mao Y, Zhang H, Xu N, Zhang B, Gou F, Zhu J-K. Application of the CRISPR-Cas system for efficient genome engineering in plants. Mol Plant. 2013;6:2008–11.
Mathew LG, Ponnuraj J, Mallappa B, Chowdary LR, Zhang J, Tay WT, et al. ABC transporter mis-splicing associated with resistance to Bt toxin Cry2Ab in laboratory- and field-selected pink bollworm. Sci Rep. 2018;8:13531.
Matten SR, Head GP, Quemada HD. How governmental regulation can help or hinder the integration of Bt crops within IPM programs. In: Romeis J, Shelton AM, Kennedy GG, editors. Integration of insect-resistant genetically modified crops within IPM programs. Progress in biological control. Dordrecht: Springer; 2008. Vol. 5. https://doi.org/10.1007/978-1-4020-8373-0_2
Maurino VG, Weber AP. Engineering photosynthesis in plants and synthetic microorganisms. J Exp Bot. 2013;64:743–51.
Mayer RT, Inbar M, Mckenzie CL, Shatters R, Borowicz V, Albrecht U, et al. Multitrophic interactions of the silverleaf whitefly, host plants, competing herbivores, and phytopathogens. Arch Insect Biochem Physiol. 2002;51:151–69.
Mendelsohn M, Kough J, Vaituzis Z, Matthews K. Are Bt crops safe? Nat Biotechnol. 2003;21:1003–9.
Mercado-Blanco J, Rodríguez-Jurado D, Parrilla-Araujo S, Jiménez-Díaz R. Simultaneous detection of the defoliating and nondefoliating *Verticillium dahliae* pathotypes in infected olive plants by duplex, nested polymerase chain reaction. Plant Dis. 2003;87:1487–94.
Mir A, Edraki A, Lee J, Sontheimer EJ. Type II-C CRISPR-Cas9 biology, mechanism, and application. ACS Chem Biol. 2018;13:357–65.
Mishra R, Wang H-Y, Yadav NR, Wilkins TA. Development of a highly regenerable elite Acala cotton (*Gossypium hirsutum* cv. Maxxa)—a step towards genotype-independent regeneration. Plant Cell Tissue Organ Cult. 2003;73:21–35.
Mitter N, Worrall EA, Robinson KE, Li P, Jain RG, Taochy C, et al. Clay nanosheets for topical delivery of RNAi for sustained protection against plant viruses. Nat Plants. 2017;3:1–10.
Miyazaki J, Stiller WN, Wilson LJ. Identification of host plant resistance to silverleaf whitefly in cotton: Implications for breeding. Field Crops Res. 2013;154:145–52.
Mo H, Wang X, Zhang Y, Zhang G, Zhang J, Ma Z. Cotton polyamine oxidase is required for spermine and camalexin signalling in the defence response to *Verticillium dahliae*. Plant J. 2015;83:962–75.
Mungai NW, Motavalli PP, Nelson KA, Kremer RJ. Differences in yields, residue composition and N mineralization dynamics of Bt and non-Bt maize. Nutr Cycl Agroecosys. 2005;73:101–9.
Murray F, Llewellyn D, Mcfadden H, Last D, Dennis ES, Peacock WJ. Expression of the *Talaromyces flavus* glucose oxidase gene in cotton and tobacco reduces fungal infection, but is also phytotoxic. Mol Breed. 1999;5:219–32.
Muthusamy A, Jayabalan N. In vitro induction of mutation in cotton (*Gossypium hirsutum* L.) and isolation of mutants with improved yield and fiber characters. Acta Physiol Plant. 2011;33:1793–801.
Niehl A, Soininen M, Poranen MM, Heinlein M. Synthetic biology approach for plant protection using dsRNA. Plant Biotech J. 2018;16:1679–87.
Oldroyd GE, Harrison MJ, Paszkowski U. Reprogramming plant cells for endosymbiosis. Science. 2009;324:753–4.
Ort DR, Merchant SS, Alric J, Barkan A, Blankenship RE, Bock R, et al. Redesigning photosynthesis to sustainably meet global food and bioenergy demand. Proc Natl Acad Sci USA. 2015;112:8529–36.
Parkhi V, Kumar V, Campbell LM, Bell AA, Shah J, Rathore KS. Resistance against various fungal pathogens and reniform nematode in transgenic cotton plants expressing *Arabidopsis* NPR1. Transgenic Res. 2010;19:959–75.
Perchlik M, Tegeder M. Improving plant nitrogen use efficiency through alteration of amino acid transport processes. Plant Physiol. 2017;175:235–47.
Perlak FJ, Oppenhuizen M, Gustafson K, Voth R, Sivasupramaniam S, Heering D, et al. Development and commercial use of Bollgard® cotton in the USA—early promises versus today's reality. Plant J. 2001;27:489–501.
Pixley KV, Falck-Zepeda JB, Giller KE, Glenna LL, Gould F, Mallory-Smith CA, et al. Genome editing, gene drives, and synthetic biology: will they contribute to disease-resistant crops, and who will benefit? Annu Rev Phytopathol. 2019;57:165–88.
Ploetz RC. *Fusarium* wilt of banana. Phytopathology. 2015;105:1512–21.
Posch BC, Kariyawasam BC, Bramley H, Coast O, Richards RA, Reynolds MP, et al. Exploring high temperature responses of photosynthesis and respiration to improve heat tolerance in wheat. J Exp Bot. 2019;70:5051–69.
Qi LS, Larson MH, Gilbert LA, Doudna JA, Weissman JS, Arkin AP, et al. Repurposing CRISPR as an RNA-guided platform for sequence-specific control of gene expression. Cell. 2013;152:1173–83.
Qu Y, Sakoda K, Fukayama H, Kondo E, Suzuki Y, Makino A, et al. Overexpression of both Rubisco and Rubisco activase rescues rice photosynthesis and biomass under heat stress. Plant Cell Environ. 2021;44:2308–20.
Rahman H. Breeding spring canola (*Brassica napus* L.) by the use of exotic germplasm. Can J Plant Sci. 2013;93:363–73.
Rajasekaran K, Grula JW, Anderson DM. Selection and characterization of mutant cotton (*Gossypium hirsutum* L.) cell lines resistant to sulfonylurea and imidazolinone herbicides. Plant Sci. 1996;119:115–24.
Rao VN, Sastry RK, Craufurd P, Meinke H, Parsons D, Rego T, et al. Cropping systems strategy for effective management of *Fusarium* wilt in safflower. Field Crops Res. 2014;156:191–8.
Raps A, Kehr J, Gugerli P, Moar WJ, Bigler F, Hilbeck A. Immunological analysis of phloem sap of *Bacillus thuringiensis* corn and of the nontarget herbivore *Rhopalosiphum padi* (Homoptera: Aphididae) for the presence of Cry1Ab. Mol Ecol. 2001;10:525–33.
Raut R, Jain H, Panwar R. Radiation-induced photo-insensitive mutants in cotton. Curr Sci. 1971;40:383–4.
Reddy PS, Chakradhar T, Reddy RA, Nitnavare RB, Mahanty S, Reddy MK. Role of heat shock proteins in improving heat stress tolerance in crop plants. In: Asea A, Kaur P, Calderwood S, editors. Heat shock proteins and plants. Heat shock proteins. Cham: Springer; 2016. Vol. 10. https://doi.org/10.1007/978-3-319-46340-7_14
Reeves RG, Phillipson M. Mass releases of genetically modified insects in area-wide pest control programs and their impact on organic farmers. Sustainability. 2017;9:59.
Rodriguez-Garay B, Barrow JR. Pollen selection for heat tolerance in cotton. Crop Sci. 1988;28:857–9.
Roell M-S, Zurbriggen MD. The impact of synthetic biology for future agriculture and nutrition. Curr Opin Biotechnol. 2020;61:102–9.
Rogers C, Oldroyd GE. Synthetic biology approaches to engineering the nitrogen symbiosis in cereals. J Exp Bot. 2014;65:1939–46.
Sade N, Gebretsadik M, Seligmann R, Schwartz A, Wallach R, Moshelion M. The role of tobacco aquaporin1 in improving water use efficiency, hydraulic conductivity, and yield production under salt stress. Plant Physiol. 2009;152:245–54.
Sadras VO. Compensatory growth in cotton after loss of reproductive organs. Field Crops Res. 1995;40:1–18.
Salvagiotti F, Cassman KG, Specht JE, Walters DT, Weiss A, Dobermann A. Nitrogen uptake, fixation and response to fertilizer N in soybeans: a review. Field Crops Res. 2008;108:1–13.
Sansaloni C, Franco J, Santos B, Percival-Alwyn L, Singh S, Petroli C, et al. Diversity analysis of 80,000 wheat accessions reveals consequences and opportunities of selection footprints. Nat Commun. 2020;11:4572.

Scafaro AP, Atwell BJ, Muylaert S, Reusel BV, Ruiz GA, Van Rie J, et al. A thermotolerant variant of Rubisco activase from a wild relative improves growth and seed yield in rice under heat stress. Front Plant Sci. 2018;9:1663.

Scafaro AP, Bautsoens N, den Boer B, Van Rie J, Gallé A. A conserved sequence from heat-adapted species improves Rubisco activase thermostability in wheat. Plant Physiol. 2019;181:43–54.

Scafaro AP, Galle A, Van Rie J, Carmo-Silva E, Salvucci ME, Atwell BJ. Heat tolerance in a wild *Oryza* species is attributed to maintenance of Rubisco activation by a thermally stable Rubisco activase ortholog. New Phytol. 2016;211:899–911.

Schnepf E, Crickmore N, Van Rie J, Lereclus D, Baum J, Feitelson J, et al. *Bacillus thuringiensis* and its pesticidal crystal proteins. Microbiol Mol Biol Rev. 1998;62:775–806.

Schreiber T, Tissier A. Chapter fourteen—libraries of synthetic TALE-activated promoters: methods and applications. In: O'Connor SE, editor. Methods in enzymology. United States: Humana Press; 2016. Vol. 576, p. 361–78.

Schreiber T, Tissier A. Generation of dTALEs and libraries of synthetic TALE-activated promoters for engineering of gene regulatory networks in plants. Methods Mol Biol. 2017;1629:185–204.

Sedelnikova OV, Hughes TE, Langdale JA. Understanding the genetic basis of C_4 Kranz anatomy with a view to engineering C_3 crops. Annu Rev Genet. 2018;52:249–70.

Senthil-Kumar M, Mysore K. RNAi in plants: recent developments and applications in agriculture. In: Catalano AJ, editor. Gene silencing: theory, techniques and applications. Hauppauge: Nova Science Publisher; 2010. p. 183–99.

Serrano L. Synthetic biology: promises and challenges. Mol Syst Biol. 2007;3:158.

Sharwood RE. Engineering chloroplasts to improve Rubisco catalysis: prospects for translating improvements into food and fiber crops. New Phytol. 2017;213:494–510.

Sharwood RE. Mix-and-match Rubisco subunits. Nat Plants. 2020;6: 1199–200.

Sharwood RE, Quick WP, Sargent D, Estavillo G, Silva-Perez V, Furbank RT. Mining for allelic gold: finding genetic variation in photosynthetic traits in crops and wild relatives. J Exp Bot. 2022, in press. Accepted for publication. https://doi.org/10.1093/jxb/erac081

Sheehan H, Bentley A. Changing times: opportunities for altering winter wheat phenology. Plants People Planet. 2021;3:113–23.

Shelby EA, Moss JB, Andreason SA, Simmons AM, Moore AJ, Moore PJ. Debugging: strategies and considerations for efficient RNAi-mediated control of the whitefly *Bemisia tabaci*. Insects. 2020;11: 723.

Shelton AM, Zhao J-Z, Roush RT. Economic, ecological, food safety, and social consequences of the deployment of Bt transgenic plants. Annu Rev Entomol. 2002;47:845–81.

Shih PM, Zarzycki J, Niyogi KK, Kerfeld CA. Introduction of a synthetic CO_2-fixing photorespiratory bypass into a cyanobacterium. J Biol Chem. 2014;289:9493–500.

Shivhare D, Mueller-Cajar O. In vitro characterization of thermostable CAM Rubisco activase reveals a Rubisco interacting surface loop. Plant Physiol. 2017;174:1505–16.

Simkin AJ, Lopez-Calcagno PE, Davey PA, Headland LR, Lawson T, Timm S, et al. Simultaneous stimulation of sedoheptulose 1, 7-bisphosphatase, fructose 1, 6-bisphophate aldolase and the photorespiratory glycine decarboxylase-H protein increases CO_2 assimilation, vegetative biomass and seed yield in Arabidopsis. Plant Biotech J. 2017;15:805–16.

Simkin AJ, López-Calcagno PE, Raines CA. Feeding the world: improving photosynthetic efficiency for sustainable crop production. J Exp Bot. 2019;70:1119–40.

Song W, Zhou L, Yang C, Cao X, Zhang L, Liu X. Tomato *Fusarium* wilt and its chemical control strategies in a hydroponic system. Crop Protect. 2004;23:243–7.

South PF, Cavanagh AP, Liu HW, Ort DR. Synthetic glycolate metabolism pathways stimulate crop growth and productivity in the field. Science. 2019;363:eaat9077.

Stelly DM, Altman D, Kohel R, Rangan T, Commiskey E. Cytogenetic abnormalities of cotton somaclones from callus cultures. Genome. 1989;32:762–70.

Stiller WN, Wilson IW. Australian cotton germplasm resources. In: Abdurakhmonov IY, editor. *World cotton germplasm resources*. London: IntechOpen; 2014. https://doi.org/10.5772/58414

Subedi KD, Ma BL. Dry matter and nitrogen partitioning patterns in Bt and non-Bt near-isoline maize hybrids. Crop Sci. 2007;47:1186–92.

Suh J-P, Jeung J-U, Noh T-H, Cho Y-C, Park S-H, Park H-S, et al. Development of breeding lines with three pyramided resistance genes that confer broad-spectrum bacterial blight resistance and their molecular analysis in rice. Rice. 2013;6:5.

Sun H-J, Uchii S, Watanabe S, Ezura H. A highly efficient transformation protocol for Micro-Tom, a model cultivar for tomato functional genomics. Plant Cell Physiol. 2006;47:426–31.

Tabashnik BE. Evolution of resistance to *Bacillus thuringiensis*. Annu Rev Entomol. 1994;39:47–79.

Tabashnik BE, Brévault T, Carrière Y. Insect resistance to Bt crops: lessons from the first billion acres. Nat Biotechnol. 2013;31:510–21.

Tabashnik BE, Carrière Y. Surge in insect resistance to transgenic crops and prospects for sustainability. Nat Biotechnol. 2017;35:926–35.

Tabashnik BE, Gassmann AJ, Crowder DW, Carriére Y. Insect resistance to Bt crops: evidence versus theory. Nat Biotechnol. 2008;26: 199–202.

Tian F, Bradbury PJ, Brown PJ, Hung H, Sun Q, Flint-Garcia S, et al. Genome-wide association study of leaf architecture in the maize nested association mapping population. Nat Genet. 2011;43: 159–62.

Tian J, Zhang X, Liang B, Li S, Wu Z, Wang Q, et al. Expression of baculovirus anti-apoptotic genes p35 and op-iap in cotton (*Gossypium hirsutum* L.) enhances tolerance to *Verticillium* wilt. PLoS One. 2010;5:e14218.

Torti S, Schlesier R, Thümmler A, Bartels D, Römer P, Koch B, et al. Transient reprogramming of crop plants for agronomic performance. Nat Plants. 2021;7:159–71.

Trapero C, Wilson IW, Stiller WN, Wilson LJ. Enhancing integrated pest management in GM cotton systems using host plant resistance. Front Plant Sci. 2016;7:500.

Trolinder NL, Shang X. In vitro selection and regeneration of cotton resistant to high temperature stress. Plant Cell Rep. 1991;10: 448–52.

Uehlein N, Lovisolo C, Siefritz F, Kaldenhoff R. The tobacco aquaporin NtAQP1 is a membrane CO_2 pore with physiological functions. Nature. 2003;425:734–7.

Upadhyay SK, Chandrashekar K, Thakur N, Verma PC, Borgio JF, Singh PK, et al. RNA interference for the control of whiteflies (*Bemisia tabaci*) by oral route. J Biosci. 2011;36:153–61.

Urie A, Knowles P. Safflower introduction resistant to *Verticillium* wilt. Crop Sci. 1972;12:545–6.

Vancompernolle K, Ball P. Report of a NEST High-level Expert Group. In: EUR 21796—synthetic biology—applying engineering to biology. Luxemburg: Office for Official Publications of the European Communities; 2005.

Varshney RK, Terauchi R, Mccouch SR. Harvesting the promising fruits of genomics: applying genome sequencing technologies to crop breeding. PLoS Biol. 2014;12:e1001883.

Venter M. Synthetic promoters: genetic control through *cis* engineering. Trends Plant Sci. 2007;12:118–24.

Vollbrecht E, Springer PS, Goh L, Buckler IV, ES, Martienssen R. Architecture of floral branch systems in maize and related grasses. Nature. 2005;436:1119–26.

Von Caemmerer S, Quick WP, Furbank RT. The development of C_4 rice: current progress and future challenges. Science. 2012;336:1671–2.

Waltz E. A new crop of microbe startups raises big bucks, takes on the establishment. Nat Biotechnol. 2017;35:1120–22.

Waltz E. With a free pass, CRISPR-edited plants reach market in record time. Nat Biotechnol. 2018;36:6–7.

Wang Y, Chen D, Wang D, Huang Q, Yao Z, Liu F, et al. Over-expression of *Gastrodia* anti-fungal protein enhances *Verticillium* wilt resistance in coloured cotton. Plant Breed. 2004;123:454–9.

Wang Y, Cheng X, Shan Q, Zhang Y, Liu J, Gao C, et al. Simultaneous editing of three homoeoalleles in hexaploid bread wheat confers heritable resistance to powdery mildew. Nat Biotechnol. 2014;32: 947–51.

Waterhouse PM, Wang M-B, Lough T. Gene silencing as an adaptive defence against viruses. Nature. 2001;411:834–42.

Wendel JF, Brubaker CL, Percival AE. Genetic diversity in *Gossypium hirsutum* and the origin of upland cotton. Am J Bot. 1992;79: 1291–310.

Werner S, Engler C, Weber E, Gruetzner R, Marillonnet S. Fast track assembly of multigene constructs using Golden Gate cloning and the MoClo system. Bioengineered. 2012;3:38–43.

Whitney SM, Sharwood RE, Orr D, White SJ, Alonso H, Galmés J. Isoleucine 309 acts as a C_4 catalytic switch that increases ribulose-1, 5-bisphosphate carboxylase/oxygenase (rubisco) carboxylation rate in Flaveria. Proc Natl Acad Sci USA. 2011;108:14688–93.

Williams M. Cotton insect losses [online]. https://www.entomology.msstate.edu/resources/cottoncrop.asp (2015). Accessed 20 Apr 2016.

Wills DM, Whipple CJ, Takuno S, Kursel LE, Shannon LM, Ross-Ibarra J, et al. From many, one: genetic control of prolificacy during maize domestication. PLoS Genet. 2013;9:e1003604.

Wilson L, Downes S, Khan M, Whitehouse M, Baker G, Grundy P, et al. IPM in the transgenic era: a review of the challenges from emerging pests in Australian cotton systems. Crop Pasture Sci. 2013;64: 737–49.

Wilson LJ, Whitehouse MEA, Herron GA. The management of insect pests in Australian cotton: an evolving story. Annu Rev Entomol. 2018;63: 215–37.

Winter K, Holtum JAM. Facultative crassulacean acid metabolism (CAM) plants: powerful tools for unravelling the functional elements of CAM photosynthesis. J Exp Bot. 2014;65:3425–41.

Woodward J, Wheeler T, Cattaneo M, Russell S, Baughman T. Evaluation of soil fumigants for management of *Verticillium* wilt of peanut in Texas. Plant Health Progress. 2011;12:16.

Worrall EA, Bravo-Cazar A, Nilon AT, Fletcher SJ, Robinson KE, Carr JP, et al. Exogenous application of RNAi-inducing double-stranded RNA inhibits aphid-mediated transmission of a plant virus. Front Plant Sci. 2019;10:10.

Wright RC, Nemhauser J. Plant synthetic biology: quantifying the "known unknowns" and discovering the "unknown unknowns". Plant Physiol. 2019;179:885–93.

Xanthopoulos F, Kechagia U. Improvement of two locally adapted cotton cultivars in earliness by induced mutations. Aust J Agric Res. 2001; 52:523–7.

Xu X, Gao J, Dai W, Wang D, Wu J, Wang J. Gene activation by a CRISPR-assisted trans enhancer. eLife. 2019;8:e45973.

Yamori W, Kondo E, Sugiura D, Terashima I, Suzuki Y, Makino A. Enhanced leaf photosynthesis as a target to increase grain yield: insights from transgenic rice lines with variable Rieske FeS protein content in the cytochrome b_6/f complex. Plant Cell Environ. 2016; 39:80–7.

Yang C-L, Liang S, Wang H-Y, Han L-B, Wang F-X, Cheng H-Q, et al. Cotton major latex protein 28 functions as a positive regulator of the ethylene responsive factor 6 in defense against *Verticillium dahliae*. Mol Plant. 2015;8:399–411.

Yang J, Zhang J. Crop management techniques to enhance harvest index in rice. J Exp Bot. 2010;61:3177–89.

Yang Y, Chen T, Ling X, Ma Z. Gbvdr6, a gene encoding a receptor-like protein of cotton (*Gossypium barbadense*), confers resistance to Verticillium wilt in *Arabidopsis* and upland cotton. Front Plant Sci. 2018;8:2272.

Yeates S, Strickland G, Grundy P. Can sustainable cotton production systems be developed for tropical northern Australia? Crop Pasture Sci. 2014;64:1127–40.

Yorobe JM, Quicoy CB. Economic impact of Bt corn in the Philippines. Philipp Agric Sci. 2006;89:258.

Zhang J, Khan SA, Hasse C, Ruf S, Heckel DG, Bock R. Full crop protection from an insect pest by expression of long double-stranded RNAs in plastids. Science. 2015;347:991–4.

Zhang W, Zhang H, Liu K, Jian G, Qi F, Si N. Large-scale identification of *Gossypium hirsutum* genes associated with *Verticillium dahliae* by comparative transcriptomic and reverse genetics analysis. PLoS One. 2017;12:e0181609.

Zhou Y, Whitney S. Directed evolution of an improved Rubisco; in vitro analyses to decipher fact from fiction. Int J Mol Sci. 2019;20:20.

Zhu S, Li Y, Vossen JH, Visser RGF, Jacobsen E. Functional stacking of three resistance genes against Phytophthora infestans in potato. Transgenic Res. 2012;21:89–99.

Zhu X-G, Long SP, Ort DR. Improving photosynthetic efficiency for greater yield. Annu Rev Plant Biol. 2010;61:235–61.

Zhu Z, Tan L, Fu Y, Liu F, Cai H, Xie D, et al. Genetic control of inflorescence architecture during rice domestication. Nat Commun. 2013;4:2200.

Zotti M, Dos Santos EA, Cagliari D, Christiaens O, Taning CNT, Smagghe G. RNA interference technology in crop protection against arthropod pests, pathogens and nematodes. Pest Manag Sci. 2018;74:1239–50.

How to cite this article: Sargent D, Conaty WC, Tissue DT, Sharwood RE. Synthetic biology and opportunities within agricultural crops. J Sustain Agric Environ. 2022;1:89–107. https://doi.org/10.1002/sae2.12014