VOL. 13 NO. 1,
AUGUST, 2026
E-ISSN 3027-0995
P-ISSN 3026-8265

African Scholar Publications & Research International
www.africanscholarpub.com

Journal of Biotechnology and Agricultural Research (JBAR)

Improving Photosynthetic Efficiency and Climate Resilience in C_4 Crops through Molecular Breeding: A Review

Muhammad Bashir Shuaib[1]; Fatai Ayomide Akande[2]; & Babatunde Afeez Olabuntu[3]

[1]Department of Plant Science and Biotechnology, Muhammad Kamaldeen University, Ilorin. [2]Department of Plant Sciences, University of Cambridge, Cambridge CB@ 3EA, UK. [3]Yam Breeding Unit, International Institute of Tropical Agriculture, Ibadan, Nigeria.
Corresponding Author: mbshuaib01@gmail.com
DOI: https://doi.org/10.70382/ajbar.v13i1.049

Abstract

C_4 crops such as maize, sorghum, pearl millet and sugarcane underpin food, feed and bioenergy production in warm regions, yet heat, drought, high vapour pressure deficit and changing atmospheric CO_2 threaten the efficiency of their photosynthesis. This review synthesises published evidence on the effects of climate stress on the C_4 pathway, the photosynthetic traits associated with resilience, the genetic regulation of those traits, and the use of molecular breeding and genome engineering to improve them. Comparative evidence from maize, sorghum, pearl millet and sugarcane, the role of *Setaria* as a C_4 model, and the translation of leaf-level gains into yield are also considered. The literature indicates that Rubisco content, electron transport, stomatal behaviour, photoprotection and recovery after stress are tractable targets, that genome-wide association studies have begun to link such traits to genomic regions, and that most candidate genes still await functional validation and field testing. Coordinated modification of co-limiting steps, evaluated in both stress and non-stress environments, emerges as the most credible strategy for stabilising yield under climate change. The main gaps are functional validation of candidate genes, studies of combined stresses beyond maize, and field testing of leaf-level gains.

Keywords: C_4 Photosynthesis, CO_2-Concentrating Mechanism, Climate Resilience, Molecular Breeding, Multiplex Genome Editing, Source–Sink Dynamics, *Setaria Viridis*, Water-Use Efficiency.

Introduction

C_4 crops make a major contribution to global food, feed and bioenergy production; in 2022, sugarcane and maize together accounted for about one-third of global primary crop production by quantity (FAO, 2024). Maize and sorghum in particular are central to food and bioenergy systems because of their high productivity and resource-use efficiency, both underpinned by a CO_2-concentrating mechanism (CCM) (Ermakova et al., 2026). Together with sugarcane and pearl millet, these crops supply staple grain, sugar, forage and lignocellulosic biomass across the tropics and subtropics and increasingly in temperate regions, while the wild grass *Setaria viridis* serves as a genetic model for C_4 photosynthesis (Sage & Zhu, 2011).

The agronomic advantage of the C_4 pathway is not fixed. Water limitation, high temperature, elevated vapour pressure deficit (VPD) and shifting atmospheric CO_2 all alter the balance between carbon gain and water loss in C_4 leaves, and each can reduce yield stability in the environments where these crops are most needed. Research on improving photosynthetic efficiency has also been strongly biased towards C_3 species, which leaves the C_4 pathway comparatively underexplored as a target for crop improvement (Sales et al., 2021).

Molecular breeding offers a route to close this gap. Here the term covers the combined use of natural variation, quantitative genetics (quantitative trait locus [QTL] mapping and genome-wide association studies [GWAS]), genomic selection, and genome editing or transgenic modification to improve a defined physiological target. For photosynthesis the target is rarely a single trait controlled by a single gene. It is a set of coordinated traits, including carboxylase activity, electron transport, stomatal behaviour, photoprotection and senescence timing, each of which responds differently to stress.

The review follows the path from physiology to deployment. It begins with the importance of the C_4 pathway for productivity and the effects of climate stress on it, then considers the photosynthetic traits associated with resilience and their genetic regulation. It next examines molecular breeding tools and the engineering of the C_4 pathway, compares the major C_4 crops, and asks how leaf-level gains translate into yield, before identifying knowledge gaps. The overall logic, from climate stress to yield stability, is summarised in Figure 1.

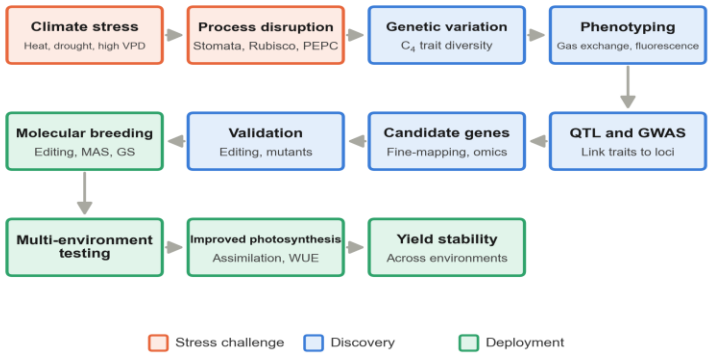


Figure 1. Conceptual framework from climate stress to yield stability. Original schematic synthesised from the literature cited in this review. GS, genomic selection; GWAS, genome-wide association study; MAS, marker-assisted selection; PEPC, phosphoenolpyruvate carboxylase; QTL, quantitative trait locus; VPD, vapour pressure deficit; WUE, water-use efficiency.

C_4 Photosynthesis and Its Importance for Crop Productivity

The productivity of C_4 crops rests on a spatial separation of carbon fixation that concentrates CO_2 around Rubisco. As a result, C_4 photosynthesis saturates at low intercellular CO_2 (C_i) and operates at low stomatal conductance (g_s), which gives high intrinsic water-use efficiency (iWUE, the ratio of assimilation to g_s) (Ghannoum et al., 2025). Water-use efficiency is both a constraint on and a target for crop improvement, and it is a physiologically and genetically complex trait shaped by photosynthesis, stomatal and mesophyll conductances, and canopy structure (Leakey et al., 2019). These properties explain why C_4 species attract interest for water-limited agriculture.

Efficiency does not mean the pathway is fully optimised. In a review of the NADP-malic enzyme (NADP-ME) pathway, Sales et al. (2021) reported that steady-state C_4 photosynthesis under non-stress conditions can be enhanced by increasing Rubisco content or electron transport capacity, and argued from source–sink interactions in maize, sugarcane and sorghum that relieving these bottlenecks is likely to improve yield, particularly as planting density rises. Models of steady-state and dynamic C_4 photosynthesis identify the activity of carboxylases, electron transport, and mesophyll and bundle sheath conductance as limiting steps, and improving C_4 photosynthesis is expected to require coordinated manipulation of several biochemical and regulatory processes rather than a single intervention (Ermakova et al., 2026).

Rubisco content illustrates how modest but practical gains might be achieved. Salesse-Smith, Wang, and Long (2025) simulated a mature sugarcane canopy (leaf area index 5.8) with a three-dimensional canopy model that uses ray tracing to estimate the light reaching each leaf, and represented a 20% increase in Rubisco as a 20% higher maximum carboxylation rate ($V_{c,max}$). Because shaded leaves in the lower canopy are largely light-limited, the simulated gain varied through the canopy and was smaller than in a fully lit leaf (Figure 2). Daily canopy CO_2 uptake rose by 14.4% in sugarcane (C_4) and by about 9% in soybean (C_3); these are simulated values for one canopy, site and date, not measured yields. The authors expect this strategy to raise productivity by about 10% without requiring more nitrogen per unit yield, assuming that the additional carbon is converted into harvested yield, and note that gene editing or breeding could in principle replace transgenic upregulation.

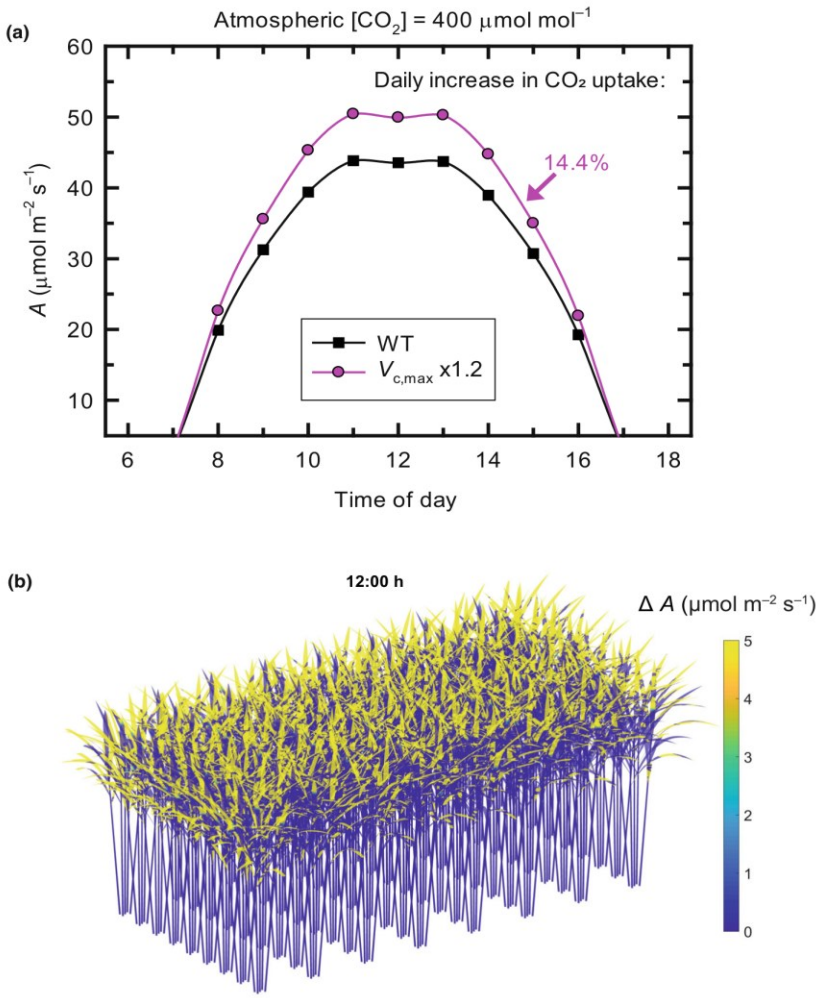


Figure 2. Simulated effect of a 20% increase in Rubisco on canopy photosynthesis in sugarcane. (a) Diurnal course of canopy net CO_2 assimilation (A, µmol m^{-2} s^{-1}) at an atmospheric [CO_2] of 400 µmol mol^{-1} for the wild type (WT) and with the maximum Rubisco carboxylation rate increased by 20% ($V_{c,max}$ × 1.2); simulated daily canopy CO_2 uptake increases by 14.4%. (b) Gain in leaf net CO_2 assimilation (ΔA, µmol m^{-2} s^{-1}) across a modelled sugarcane canopy (*Saccharum officinarum*, RB86-7515; row spacing 100 cm, leaf area index 5.8) at 12:00 h; colours show the spatial variation in ΔA through the canopy (colour scale 0–5 µmol m^{-2} s^{-1}). Values are model outputs, not measurements. Reproduced from Salesse-Smith, Wang, and Long (2025), *New Phytologist*, Figure 3, under a CC BY 4.0 licence (https://creativecommons.org/licenses/by/4.0/); resized, with no other changes.

Atmospheric CO_2 adds a further layer. Because the CCM brings Rubisco close to CO_2 saturation at the current CO_2 concentration ([CO_2]), C_4 crops were long assumed to respond little to elevated [CO_2], and the direct effect on C_4 assimilation is generally small when water is ample (Ghannoum et al., 2025; Silva et al., 2020). In maize grown under free-air CO_2 enrichment (FACE) at 550 µmol mol^{-1} in a season without water stress, photosynthesis, biomass and yield were unchanged, yet g_s was 34% lower and soil moisture up to 31% higher (Leakey et al., 2006). The effect is therefore largely indirect: lower g_s reduces transpiration, raises iWUE and can conserve soil water, which may delay and lessen drought stress

(Ghannoum et al., 2025). Consistent with this, elevated [CO_2] increased final biomass by 24% and grain yield by 41% in the drought treatment of one season in FACE maize, with no growth response under ample water (Manderscheid et al., 2014). In FACE sorghum, g_s was 32–37% lower on a seasonal-mean basis and shoot biomass under reduced irrigation was 15% higher (Wall et al., 2001). These observations do not by themselves establish whether stomata acclimate to long-term elevated [CO_2]. With longer exposure, photosynthetic capacity can decline as carbohydrates accumulate in leaves, and genes such as that encoding phosphoenolpyruvate carboxylase (PEPC) are regulated differently among species and genotypes (Silva et al., 2020), although no such feedback was detected in FACE maize (Leakey et al., 2006). The benefit under water limitation is nevertheless conditional: in glasshouse sorghum under combined water and heat stress, elevated [CO_2] had little or a negative effect on photosynthesis, because leaves were warmer and evaporative cooling was reduced, although biomass loss was prevented (Al-Salman et al., 2023).

These C_4-specific questions sit within a broader programme of photosynthesis improvement that includes CO_2-concentrating strategies (Croce et al., 2024; Figure 3).

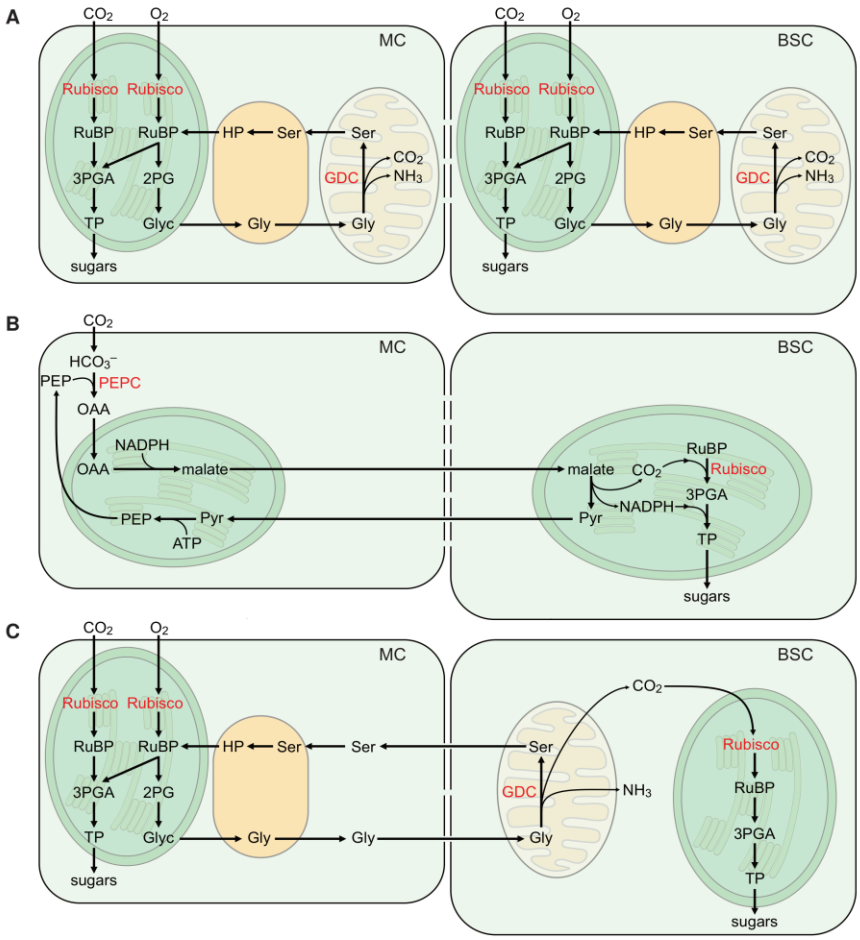


Figure 3. Schematic representation of C_3, C_4 and C_3–C_4 intermediate photosynthesis in mesophyll cells (MC) and bundle sheath cells (BSC). (A) C_3 photosynthesis and photorespiration, with a complete photorespiratory pathway in both cell types. (B) C_4 photosynthesis, drawn for the NADP-malic enzyme subtype: in the mesophyll, CO_2 (as HCO_3^-) is fixed by phosphoenolpyruvate carboxylase (PEPC) onto

PEP to form OAA, which is reduced to malate; malate moves to the bundle sheath, where it is decarboxylated to release CO_2 around Rubisco (the photorespiratory pathway is not shown). (C) C_3–C_4 intermediate photosynthesis, in which photorespiration is shared between the cell types and glycine decarboxylation is restricted to the bundle sheath. Enzymes are shown in red. 2PG, 2-phosphoglycolate; 3PGA, 3-phosphoglycerate; GDC, glycine decarboxylase; Gly, glycine; Glyc, glycolate; HP, hydroxypyruvate; OAA, oxaloacetate; PEP, phosphoenolpyruvate; Pyr, pyruvate; RuBP, ribulose 1,5-bisphosphate; Ser, serine; TP, triose phosphate. Reproduced from Croce et al. (2024), *The Plant Cell*, Figure 8, under a CC BY 4.0 licence (https://creativecommons.org/licenses/by/4.0/); resized, with no other changes.

C_4 Photosynthesis Under Climate Stress

The C_4 pathway is efficient under many conditions, but climate change alters the variables on which that efficiency depends. Although iWUE is generally higher in C_4 than in C_3 plants, future scenarios of rising [CO_2], higher temperatures and increased aridity may challenge this advantage, and the physiological, anatomical and environmental factors controlling iWUE need to be balanced against productivity and resilience. Proposed responses include breeding or engineering Rubisco isoforms with greater thermostability, modifying PEPC expression or regulation to protect against thermal and osmotic stress, and using leaf width as an integrative trait that links anatomy, morphology and water use (Ghannoum et al., 2025).

Experimental work in maize illustrates why combined stresses need to be studied together. Correia et al. (2021) compared two genotypes, B73 and P0023, that differ in drought and heat tolerance, grown at 25 °C and then kept at 25 °C or moved to 38 °C (at a VPD of 2.5–4.0 kPa) for 5 days under well-watered or water-deficit conditions (Figure 4). They hypothesised that resilience would depend on efficient regulation of photosynthetic traits, including the C_4 CCM, and concluded that limited transpiration and synchronised regulation of carbon assimilation metabolism were key traits for drought and heat tolerance. When water deficit coincided with high temperature, limited transpiration saved water and acted as a drought-avoidance mechanism.

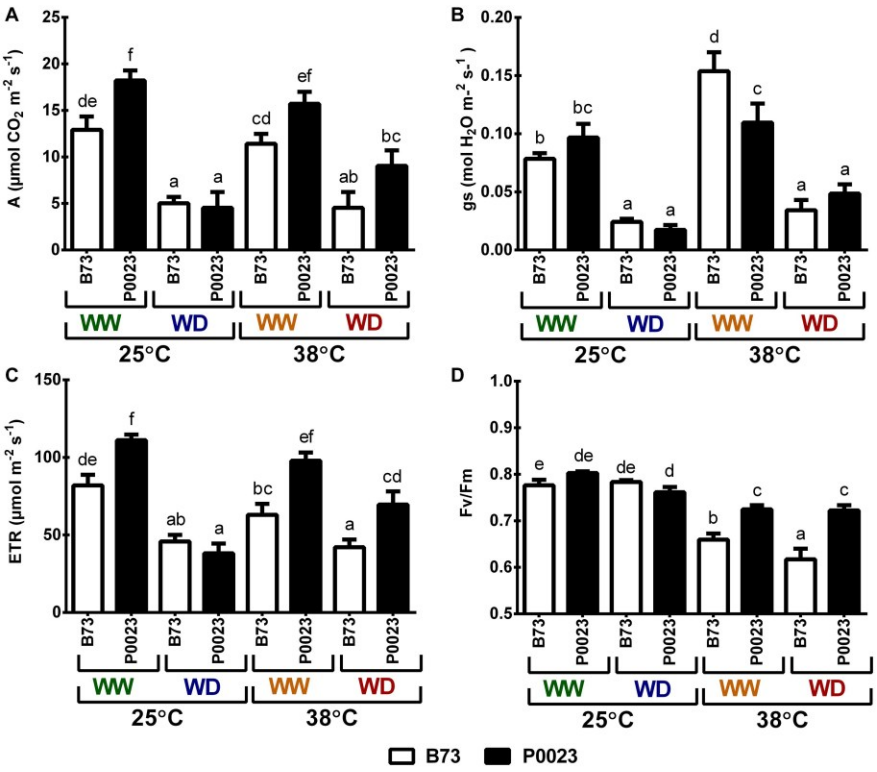


Figure 4. Steady-state photosynthesis in two maize genotypes, B73 (open bars) and P0023 (filled bars), grown under well-watered (WW) or water-deficit (WD) conditions and acclimated to 25 or 38 °C. (A) Net CO_2 assimilation (A, µmol CO_2 m^{-2} s^{-1}); (B) stomatal conductance (g_s, mol H_2O m^{-2} s^{-1}); (C) electron transport rate (ETR, µmol m^{-2} s^{-1}); (D) maximum quantum yield of photosystem II photochemistry (F_v/F_m). Measurements were made at a photosynthetic photon flux density of 600 µmol m^{-2} s^{-1} and 400 µmol CO_2 mol^{-1} on fully expanded leaves of 4-week-old plants. Values are means ± standard error of the mean (SEM; n = 5); different letters denote significant differences between treatments (Duncan test, $p < 0.05$). Reproduced from Correia et al. (2021), *Frontiers in Plant Science*, Figure 1, under a CC BY 4.0 licence (https://creativecommons.org/licenses/by/4.0/); resized, with no other changes.

Salesse-Smith, Wang, and Long (2025) further proposed increasing leaf Rubisco content as a near-term strategy for future-proofing agriculture under rising [CO_2]. The evidence so far comes from single-site trials: in transgenic sorghum with higher Rubisco content, vegetative biomass increased by 15.5% in a one-season field trial, but grain yield did not (Salesse-Smith, Adar, et al., 2025). In maize, higher Rubisco content has also been reported to increase biomass under chilling in controlled environments (as reviewed by Salesse-Smith, Wang, & Long, 2025), but whether such gains persist under heat or drought in the field has yet to be tested.

Photosynthetic Traits Associated with Climate Resilience

Breeding for resilience requires traits that can be measured reliably, vary among genotypes, and relate to performance under stress. Photosynthetic metabolism under stressful growth conditions has been proposed as a basis for crop breeding and yield improvement (Morales et al., 2020), and four groups of

traits recur in the C_4 literature: gas exchange and stomatal behaviour, electron transport and photoprotection, recovery after stress, and forage or biomass quality under stress.

Gas exchange traits link carbon gain to water loss. In finger millet (*Eleusine coracana*), stomatal conductance and electron transport both declined with rising VPD and increased with photosynthetically active radiation, whereas elevated CO_2 reduced stomatal conductance while electron transport remained stable, and genotypes differed in stomatal sensitivity to CO_2 (Gupta et al., 2025). In maize, combined gas exchange characteristics, chlorophyll fluorescence and response curves have been evaluated as selection traits for temperature tolerance (Ramazan et al., 2021). Both studies go beyond single-point measurements, using either response curves (Ramazan et al., 2021) or measurements across changing field conditions (Gupta et al., 2025).

Chlorophyll fluorescence reports on photosystem II efficiency and energy dissipation. Combining gas exchange, fluorescence and yield data allowed six spring wheat cultivars released in different eras to be compared under drought (Guan et al., 2015), and infrared and fluorescence imaging has been used to monitor drought tolerance in an apple rootstock (Gao et al., 2021). Photosynthetic responses to fluctuating light and chilling have likewise been explored in tomato as a route to climate-resilient crops (Rayaprolu et al., 2025). These C_3 examples, one of them a woody species, are useful mainly as methodological precedents, because the C_4 CCM changes how photosynthesis relates to C_i and to light.

Resistance and recovery are distinct properties. Qi et al. (2021) exposed maize to drought, flooding and rewatering in greenhouse and field experiments and found that both drought and flooding hampered photosynthetic capacity, particularly when severe or prolonged. Most photosynthetic traits recovered after rewatering with few compensatory responses, and rewatering often produced high photosynthetic resilience after severe late-season drought, with the response depending strongly on drought severity and duration. Associations between chlorophyll concentration and functional photosynthetic activity were stronger after tasselling than before, which points to leaf age and senescence as modifiers of the response, and coordinated changes in chlorophyll content, gas exchange and fluorescence parameters appeared to contribute to resistance and resilience.

In forage and bioenergy grasses, photosynthetic performance is only part of the outcome. Water deficit, alone or combined with warming, reduced leaf photosynthesis in a field-grown C_4 tropical forage grass, and both warming and water deficit decreased forage quality and digestibility (Habermann et al., 2019), so resilience traits for such species need to be evaluated against nutritional or processing quality as well as biomass. For breeders, the practical consequence is that resilience traits should be defined by function, for example maintenance of assimilation at high VPD or speed of recovery after rewatering, rather than by a single instrument reading, and validated across environments.

Genetic Regulation of Photosynthetic Performance In C_4 Crops

The genetic control of C_4 photosynthesis is only partly understood, and forward-genetic studies of natural variation in photosynthesis more generally have made limited progress (van Bezouw et al., 2019). Quantitative genetics and selective breeding are underexplored routes to identify regulators of the C_4 syndrome, through natural intraspecific variation, hybridisation between C_3 and C_4 species, artificial selection and GWAS (Simpson et al., 2022). Such approaches could also guide the engineering of the C_4 pathway into C_3 crops.

Sorghum provides an example of association mapping applied to photosynthesis under stress. Ortiz et al. (2017) studied this C_4 species, which is sensitive to cold spring conditions at northern latitudes, and ran a GWAS for seven gas exchange and chlorophyll fluorescence traits across three consecutive temperature treatments: control (28/24 °C day/night), cold (15/15 °C) and recovery (28/24 °C). Cold

stress significantly reduced photosynthetic CO_2 uptake (Figure 5), and 143 unique genomic regions were associated with at least one trait in a given treatment or with derived variables. Candidate genes within those intervals had predicted functions related to carotenoids, phytohormones, thioredoxin, photosystem I components and antioxidants, which suggests that photoprotection and redox regulation contribute to cold tolerance of photosynthesis; the authors highlighted the most promising of these regions for future validation rather than presenting them as confirmed causal genes. The same toolkit has been applied at the agronomic end of the trait chain, through GWAS of grain yield components in diverse sorghum germplasm (Boyles et al., 2016).

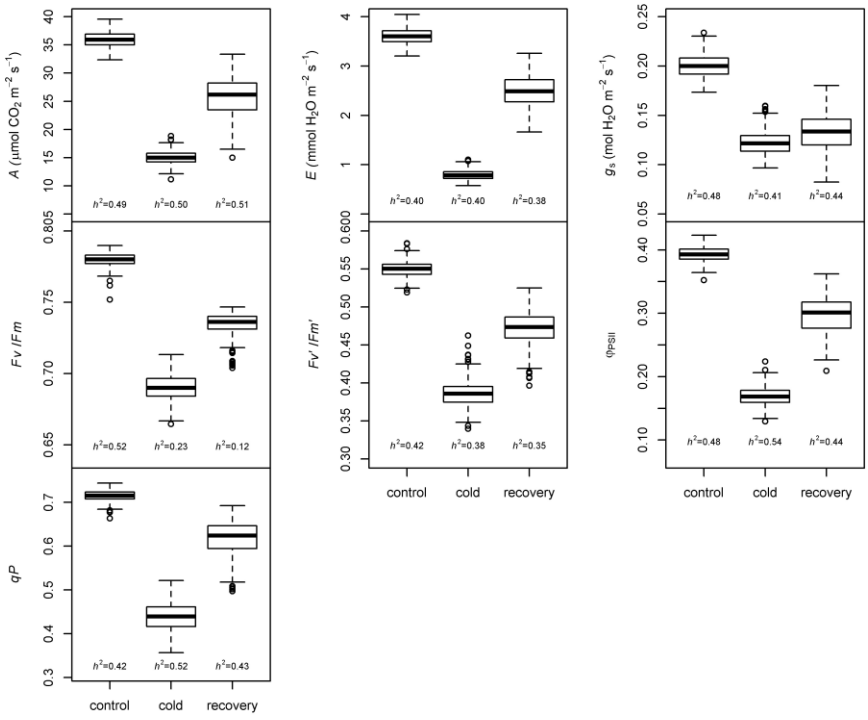


Figure 5. Gas exchange and chlorophyll fluorescence traits of sorghum (*Sorghum bicolor*) genotypes during the control (28/24 °C), cold (15/15 °C) and recovery (28/24 °C) periods. Box plots of best linear unbiased predictors for net photosynthesis (A, μmol CO_2 m^{-2} s^{-1}), transpiration (E, mmol H_2O m^{-2} s^{-1}), stomatal conductance (g_s, mol H_2O m^{-2} s^{-1}), maximum quantum yield of photosystem II (PSII) (F_v/F_m), efficiency of energy capture by open PSII reaction centres (F_v'/F_m'), effective quantum yield of PSII (Φ_{PSII}) and photochemical quenching (qP); h^2, heritability in each period. Reproduced from Ortiz et al. (2017), *Journal of Experimental Botany*, Figure 1, under a CC BY 4.0 licence (https://creativecommons.org/licenses/by/4.0/); resized, with no other changes.

Wild species complement crop GWAS. Genomic regions associated with C_4 photosynthetic activity and leaf anatomy have been sought in the grass *Alloteropsis semialata* (Alenazi et al., 2024), which extends the search for loci beyond cultivated species.

The pipeline from trait to gene depends on pairing precise phenotypes with dense genotypes. Converging phenomics and genomics has been proposed as a way to study natural variation in photosynthetic efficiency (van Bezouw et al., 2019), and multi-scale remote-sensing phenomics integrated with multi-omics has been reviewed for drought and heat tolerance in climate-smart

agriculture (Liang et al., 2025). A wider review of pathways, regulation and biotechnological applications for enhancing photosynthetic efficiency (Khan et al., 2025) is complemented by integrated multi-omics and breeding strategies for abiotic stress tolerance in *Brassica* crops (Peng et al., 2026) and in soybean (Kim et al., 2026). The *Brassica* and soybean studies concern C_3 species and are useful mainly as models for combining omics layers with breeding.

Taken together, these studies indicate that photosynthetic performance in C_4 crops is polygenic, environment-dependent and measurable. Mapping under contrasting treatments is especially informative because it separates loci that act under non-stress conditions from those that act under stress or during recovery (Ortiz et al., 2017).

Molecular Breeding for Improved Photosynthesis

Molecular breeding converts loci and candidate genes into selectable variation. The toolkit now available for millets includes marker-assisted selection (MAS), GWAS, genomic selection (GS), genetic engineering, omics technologies, speed breeding and machine learning (Figure 6), yet these species remain underutilised because of limited genomic resources, harsh growing conditions and insufficient technological support (A. Kumar et al., 2026). The same authors note that clustered regularly interspaced short palindromic repeats (CRISPR)/Cas systems, including multiplex CRISPR/Cas, add the possibility of targeted modification of stress resilience and yield traits. The integration of molecular breeding with genome editing and omics approaches has likewise been proposed as a way to accelerate climate-resilient agriculture (Mersha et al., 2026).

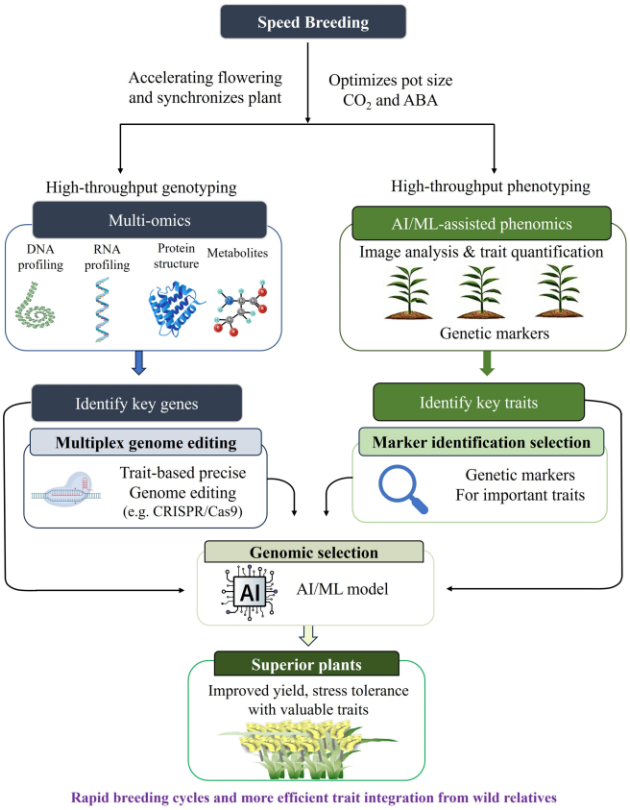


Figure 6. Integration of omics technologies with speed breeding and machine learning in millet breeding. Speed breeding is combined with high-throughput genotyping (multi-omics: DNA and RNA

profiling, protein structure, metabolites) and high-throughput phenotyping (AI/ML-assisted phenomics). Key genes are targeted by multiplex genome editing (e.g. CRISPR/Cas9) and key traits by marker identification and selection; both feed genomic selection supported by AI/ML models to deliver plants with improved yield and stress tolerance. AI/ML, artificial intelligence/machine learning. Reproduced from A. Kumar et al. (2026), *Frontiers in Plant Science*, Figure 6, under a CC BY 4.0 licence (https://creativecommons.org/licenses/by/4.0/); resized, with no other changes.

The tools are being adapted to crops with different genetic constraints. Genomics-assisted precision breeding for drought tolerance has been reviewed for sugarcane, a C_4 crop in which drought is a central constraint (Tripathy, 2022). In forage grasses, molecular insights on stress responses and agronomic traits are being integrated for breeding (Achon & Enciso-Maldonado, 2026), an approach that links photosynthetic and quality traits of the kind affected by warming and water deficit in C_4 tropical grasses (Habermann et al., 2019). Rice is a C_3 species, but new breeding techniques for boosting grain yield (Hussain et al., 2022) and multi-trait plant-type modification in a breeding-by-design framework (Habde et al., 2026) show how several traits can be combined in one ideotype, a logic that could be applied to C_4 cereals.

Because photosynthesis depends on many coordinated processes, single-gene modification is often insufficient (Ermakova et al., 2026; Li et al., 2026). CRISPR-mediated multiplex genome editing has been reviewed as a means of transforming plant breeding for crop improvement and polygenic trait engineering (U. Kumar et al., 2025). In C_4 crops, multiplexing could in principle target several co-limiting steps at once, such as carboxylase activity, electron transport and stomatal patterning.

A practical pipeline therefore links five steps: phenotyping resilience traits under defined stress; mapping them by GWAS or QTL analysis; validating candidate genes by expression analysis, mutants or transgenic lines; deploying validated alleles by MAS, GS or editing; and testing improved lines in target environments. The weakest links are validation and multi-environment testing, because many association results remain at the level of candidate regions awaiting functional confirmation.

Engineering C_4 Photosynthesis for Climate Resilience

Where breeding relies on existing variation, engineering creates new variation. Traditional plant breeding is most likely reaching a plateau, and synthetic biology offers the potential to build novel pathways in predictable directions and so accelerate improvements in photosynthetic efficiency (Batista-Silva et al., 2020). Targets can be organised by process: light capture and photoprotection, enzymes of the Calvin–Benson–Bassham cycle, CO_2-concentrating strategies, and CO_2 delivery into leaves, with the effects of selected strategies on nitrogen-use efficiency and canopy photosynthesis analysed alongside (Croce et al., 2024). Effects on nitrogen-use efficiency and canopy photosynthesis matter because a leaf-level gain that costs nitrogen or does not scale to the canopy may not improve yield.

Better knowledge of C_4 function offers new ways to improve existing C_4 crops and bioenergy species, for example by developing varieties with ultra-high water- and nitrogen-use efficiencies, although knowledge of the genes controlling Kranz anatomy lags far behind knowledge of the enzymes of the C_4 cycle (Sage & Zhu, 2011). A related question for engineered systems is whether C_3 crops equipped with the C_4 CCM can reach their full yield potential, an outcome that depends on the wider physiological and canopy context (Driever & Kromdijk, 2013).

General reviews address genetic manipulation of photosynthesis to enhance crop productivity under changing environmental conditions (A. Kumar et al., 2023) and the enhancement of photosynthesis and plant productivity through genetic modification (Nazari et al., 2024). Both are broad in scope rather

than C_4-specific, so their conclusions need to be checked against the C_4 CCM before being applied to maize, sorghum or sugarcane.Genome editing can modify native genes and, once the editing construct has been segregated away, leave no foreign DNA in the plant. Two book chapters address its use for photosynthesis. CRISPR/Cas systems for enhancing photosynthesis have been considered in the context of climate resilience and food production (Barupal & Kumar, 2024), and genome editing for improving photosynthesis in crop plants has been reviewed (Bukhat et al., 2025). In some jurisdictions, editing may also face fewer regulatory barriers than transgenic strategies (Namata et al., 2025), and it could in principle offer a transgene-free route to the Rubisco increase discussed above (Salesse-Smith, Wang, & Long, 2025).

Technical barriers to editing differ among C_4 crops. In maize and sorghum, genotype-independent delivery of editing reagents is still a research priority rather than an established method (Namata et al., 2025). Sorghum is particularly recalcitrant to tissue culture and regeneration, with strong genotype dependence; morphogenic regulators have raised its transformation frequency, but their cassettes had to be removed before plants could be regenerated (Fontanet-Manzaneque et al., 2025). Most millets still lack genotype-independent transformation (A. Kumar et al., 2026), and CRISPR editing in pearl millet remains at the proof-of-concept stage (Mersha et al., 2026). In highly polyploid sugarcane, many gene copies must be mutated together to change a trait (Eid et al., 2021). Low editing efficiency and regeneration bottlenecks also constrain multiplex editing (U. Kumar et al., 2025), and none of the studies reviewed here reports a C_4 photosynthetic trait improved by genome editing and tested in the field.

Engineered increases in light capture or electron flow that are not matched by carbon-fixation capacity can raise the risk of oxidative damage under high light, heat or drought, because photo-oxidative stress arises from an imbalance between light absorption and carbon utilisation, against which plants deploy systems-level resilience (Li et al., 2026). Gains realised only under optimal conditions could therefore be lost during stress if photoprotective and antioxidant capacity do not scale with them.

The common design principle is that engineering should target co-limiting steps together and be tested under stress as well as under optimal conditions. Figure 7 relates the C_4 CCM, the physiological traits that breeders can select, and the molecular approaches used to modify them.

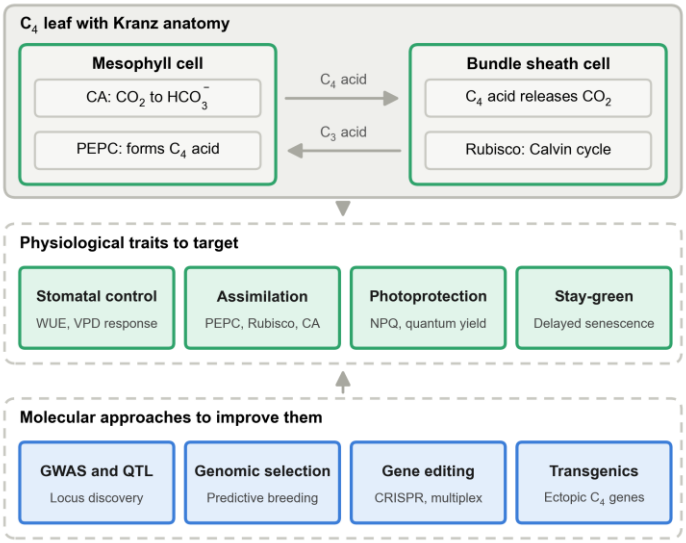


Figure 7. The C_4 CO_2-concentrating mechanism (NADP-ME type, simplified), the physiological traits breeders can target, and the molecular approaches used to modify them. Original schematic; not reproduced from a published figure. Pathway after Sales et al. (2021) and Ermakova et al. (2026); traits after Fontanet-Manzaneque et al. (2025), Gupta et al. (2025), Qi et al. (2021) and Ramazan et al. (2021); molecular approaches after A. Kumar et al. (2026), U. Kumar et al. (2025), Mersha et al. (2026) and Yadav & Mishra (2020). CA, carbonic anhydrase; NPQ, non-photochemical quenching; PEPC, phosphoenolpyruvate carboxylase; VPD, vapour pressure deficit; WUE, water-use efficiency.

Comparative Perspectives among Major C_4 Crops

The major C_4 crops share a CCM but differ in genome, ecology and breeding history, and their comparison is summarised in Table 1. Maize, sorghum and pearl millet are treated together in agroclimatological terms (Prasad et al., 2020), which gives a shared climatic frame for comparison. C_4 crops are generally more efficient than C_3 crops in their use of water and nitrogen (Yadav & Mishra, 2020), and maize and sorghum are considered tolerant of osmotic stress (Namata et al., 2025). The same review notes that CRISPR/Cas9 editing in these species is limited by delivery systems, editing efficiency, regulatory concerns and off-target effects, with prime editing and genotype-independent delivery as future priorities.

Sorghum has become a reference species for C_4 drought research. It has been presented as a monocot model for drought (Fontanet-Manzaneque et al., 2025; Figure 8), and its vulnerability and resilience under changing climatic conditions (Sharma & Joshi, 2022) and genomic designing for climate-smart production (Kuyyamudi Nanaiah & Rakshit, 2020) have been reviewed. A summary of sorghum improvement between 1980 and 2010 provides the historical baseline against which current genomic approaches can be judged (Reddy et al., 2012).

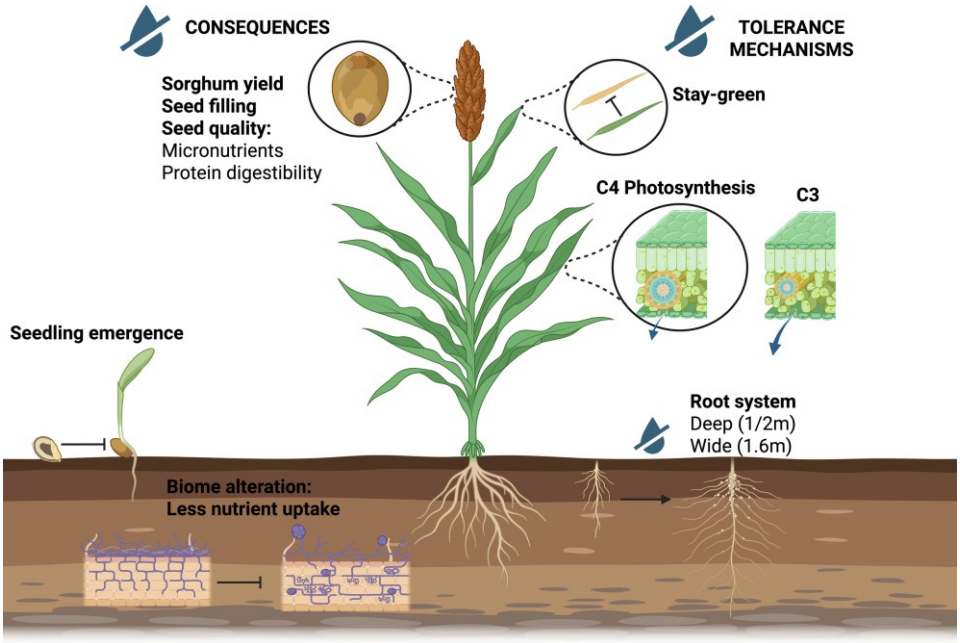


Figure 8. Effects of drought on sorghum and its tolerance mechanisms. Left (consequences): drought impairs seedling emergence, alters the root-associated biome and reduces nutrient uptake, and lowers yield, seed filling and seed quality (micronutrients, protein digestibility). Right (tolerance mechanisms):

the stay-green trait, C_4 photosynthesis (C_4 leaf anatomy contrasted with C_3) and a deep and wide root system. Reproduced from Fontanet-Manzaneque et al. (2025), *Frontiers in Plant Science*, Figure 2, under a CC BY 4.0 licence (https://creativecommons.org/licenses/by/4.0/); created in BioRender by J. B. Fontanet-Manzaneque (2025), https://BioRender.com/91z4p60; resized, with no other changes.

For pearl millet, Srivastava et al. (2022) stated that drought intensity is predicted to worsen with climate change and that the response of pearl millet to drought across growth stages has been studied comprehensively. They reported substantial progress in genomic resources used for diversity analysis, linkage mapping, marker–trait association and genomic selection, and noted that low-cost high-throughput genotyping platforms create opportunities for MAS in drought breeding programmes. Genomic designing of the crop for arid and semi-arid environments extends this view (Serba et al., 2020).

For bioenergy and sugar crops the harvested product is biomass or the sugar stored in it. High-biomass C_4 grasses have been considered as a way of filling the yield gap (Mullet, 2017), and molecular and agronomic approaches to the genetic determinants of biomass in C_4 crops have been reviewed for biofuel applications (Ain et al., 2022). The target for sugarcane and related grasses is therefore sustained carbon assimilation translated into biomass.

The outlook for C_4 crops under future climate scenarios has been assessed (Watson-Lazowski & Ghannoum, 2021), and, beyond the interaction with drought, the responses of maize, sugarcane and sorghum to elevated [CO_2] combined with other stresses still need to be investigated (Silva et al., 2020). Ectopic expression of C_4 pathway genes in C_3 plants has been discussed as a means of improving carbon assimilation and stress tolerance under future climates (Yadav & Mishra, 2020). *Setaria viridis* was highlighted as a new C_4 model species that could accelerate discovery of the genes controlling C_4 photosynthesis (Sage & Zhu, 2011), which is the basis for the model-system role assigned to *S. viridis* in Table 1; the crop species, foxtail millet (*S. italica*), is included for its drought adaptation (Liu et al., 2026).

Table 1. Comparative summary of major C_4 crops and the *Setaria* model system

Crop	Major strength	Key stress	Breeding opportunity	Literature in this review
Maize	High productivity	Drought and heat	Genome editing (CRISPR/Cas9); limited transpiration as a selection trait	Correia et al. (2021); Namata et al. (2025)
Sorghum	Drought adaptation	Water deficit	Stay-green and photosynthetic traits	Fontanet-Manzaneque et al. (2025); Ortiz et al. (2017); Sharma & Joshi (2022)
Pearl millet	Heat and drought tolerance	Drought and heat	Genomic selection and MAS for drought tolerance	Serba et al. (2020); Srivastava et al. (2022)
Sugarcane	Biomass production	Drought	Genomics-assisted breeding; carbon partitioning to biomass	Calderan-Rodrigues et al. (2021); Mullet (2017); Tripathy (2022)
***Setaria* (*S. viridis*, model; *S. italica*, foxtail millet)**	Genetic and model system	Drought (foxtail millet)	Functional genomics	Ermakova et al. (2026); Liu et al. (2026); Sage & Zhu (2011)

Note. Entries summarise the evidence discussed in this review; the final column lists the sources on which each row is based.

From Photosynthetic Traits to Yield: Translating Molecular Gains into Crop Improvement

A higher assimilation rate does not automatically become more grain, sugar or biomass. Processes in sources and sinks need to be understood both as standalone mechanisms and at crop level, and there is often a chasm between the two; the largest yield increases are likely to come from integrating key processes within crop ideotypes with optimised phenology, in agricultural environments that are often more limited by water than by carbon (Paul et al., 2020). The emphasis of that work is on cereals, particularly wheat, but the logic applies to C_4 crops, where the CCM reduces photorespiratory carbon loss and attention shifts towards water and sink capacity.

Broader syntheses connect leaf-level processes to crop outcomes. They cover the leveraging of photosynthetic efficiency to improve yield (Miglani et al., 2021) and the nexus between photosynthesis and radiation-use efficiency under climate change (Sahoo et al., 2025). For C_4 species grown as biofuel feedstocks, biomass production and feedstock quality can be optimised from a lignocellulosic perspective (Byrt et al., 2011), so climate resilience in these crops is judged not only by grain or sugar yield but also by the quantity and quality of biomass.

Stress at critical developmental stages can limit yield through reproductive processes, because drought and heat affect physiological, developmental, growth and yield processes differently (Prasad et al., 2008). Heterosis, stress and the environment provide a possible road map towards general improvement of crop yield (Blum, 2013), a framework relevant to maize and sorghum hybrids, where yield stability depends on genotype-by-environment interaction.

Bridging leaf and field requires measurement at several scales. Molecular phenotyping tools have been applied to explore the carbon potential of sugarcane (Calderan-Rodrigues et al., 2021; Figure 9), and chlorophyll fluorescence combined with unmanned aerial vehicle (UAV) imaging has been used to characterise photosynthetic and canopy traits in soybean (Singh-Bakala et al., 2025), a C_3 example that illustrates how fluorescence and canopy imaging can be combined. Crop models provide the natural place to test whether a trait such as faster stomatal closure or higher Rubisco content would raise yield across seasons and sites (Boote et al., 2025).

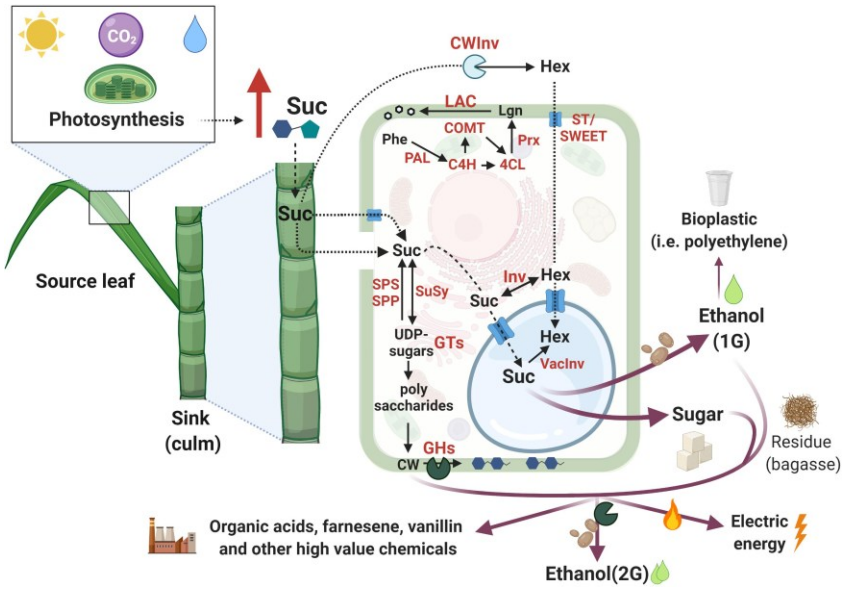


Figure 9. Carbon partitioning in sugarcane from photosynthesis in the source leaf to sucrose (Suc) storage in the culm (sink) and to added-value products: sugar, first- and second-generation ethanol (1G,

2G), bioplastic, high-value chemicals and electricity from the bagasse residue. The red arrow indicates increased content and red letters indicate proteins involved in the reactions shown (e.g. SPS, sucrose phosphate synthase; SuSy, sucrose synthase; CWInv, cell-wall invertase; Inv, invertase; VacInv, vacuolar invertase; ST/SWEET, sugar transporters). Hex, hexoses. Pathways are simplified and images are not to scale. Reproduced from Calderan-Rodrigues et al. (2021), *Frontiers in Plant Science*, Figure 2 (created with BioRender), under a CC BY 4.0 licence (https://creativecommons.org/licenses/by/4.0/); resized, with no other changes.

Natural variation is a further resource for photosynthetic improvement in next-generation breeding (Zhou et al., 2026). In foxtail millet (*Setaria italica*), integrated drought resilience has been traced from molecular regulation and multi-omics to climate-resilient breeding (Liu et al., 2026), although most drought-responsive genes in this species still await functional validation.

Three practical points follow for breeders. Photosynthetic targets should be tied to a defined yield-limiting stage, because a trait that improves vegetative growth may have little effect if grain set is the constraint. Selection should use stress and non-stress environments, because the same allele can have different effects in each. Models and canopy-scale phenotyping should be used before large-scale deployment, to avoid advancing leaf-level gains that do not scale.

Knowledge Gaps and Future Directions

The regulators of the C_4 syndrome remain poorly defined. Knowledge of the genes controlling Kranz anatomy has long lagged behind knowledge of C_4 enzymes (Sage & Zhu, 2011), although candidate anatomy loci are now emerging from association studies (Alenazi et al., 2024). Most links between photosynthetic traits and genes come from association mapping that has not yet been validated functionally. Evidence is also uneven across crops: maize and sorghum have the largest sets of photosynthesis-specific studies, whereas several of the measurement approaches cited here were developed in C_3 species, so C_4-specific validation of these methods is still needed.

Combined and sequential stresses are under-represented. Heat with drought and drought followed by rewatering have been examined mainly in maize among the studies reviewed here, and although elevated [CO_2] has been combined with drought in field trials of maize and sorghum (Manderscheid et al., 2014; Wall et al., 2001), fewer studies have combined it with heat (Al-Salman et al., 2023), so generalisation to pearl millet, sugarcane and *Setaria* is limited. Translation from leaf to field is rarely tested, and there are few examples in which genetic modification has improved intrinsic yield in the field and progressed to commercial application in a major crop (Paul et al., 2020). Crop models can extrapolate leaf-level effects (Boote et al., 2025), provided they are parameterised with photosynthetic trait data from C_4 species. Delivery and regulation also limit genome editing, because transformation efficiency, genotype dependence and regulatory frameworks remain barriers in maize and sorghum (Fontanet-Manzaneque et al., 2025; Namata et al., 2025).

The most useful next steps are likely to be multi-environment field trials that combine gas exchange, fluorescence and canopy imaging with yield, so that trait value can be tested directly, together with functional validation of GWAS candidates by editing in sorghum, maize or *Setaria*. Simultaneous editing of genes underlying co-limiting steps, supported by multiplex approaches, and the integration of trait data into crop models for scenario testing would extend this work. Natural variation (Zhou et al., 2026) and phenomics integrated with multi-omics (Liang et al., 2025) address complementary parts of this route, and combining them offers a way to scale up validation across genotypes and environments.

Conclusion

C_4 crops combine high productivity with high intrinsic water-use efficiency, yet the sources reviewed here show that this advantage can be eroded by heat, drought, high VPD and changing CO_2, and that the pathway still has identifiable bottlenecks. Evidence from maize, sorghum and finger millet indicates that useful variation exists in gas exchange, electron transport, photoprotection and recovery traits, and that association mapping can link these traits to genomic regions. Molecular breeding, genome editing and synthetic biology provide the means to deploy this variation, but most candidate loci still need functional validation, and gains measured at leaf level must be tested at canopy and field scale. A pipeline that moves from stress-informed phenotyping to QTL or GWAS, candidate genes, selection or editing, and multi-environment testing offers a credible route to stable yields under climate change. Coordinated modification of co-limiting steps, together with attention to photoprotection and sink capacity, is likely to matter more than any single intervention.

References

Achon, I., & Enciso-Maldonado, G. A. (2026). Integrating molecular insights on stress responses and agronomic traits for breeding in forage grasses. *Crop & Pasture Science, 77*(1), Article CP25162. https://doi.org/10.1071/CP25162

Ain, N. U., Haider, F. U., Fatima, M., Habiba, Zhou, Y., & Ming, R. (2022). Genetic determinants of biomass in C_4 crops: Molecular and agronomic approaches to increase biomass for biofuels. *Frontiers in Plant Science, 13*, Article 839588. https://doi.org/10.3389/fpls.2022.839588

Alenazi, A. S., Pereira, L., Christin, P.-A., Osborne, C. P., & Dunning, L. T. (2024). Identifying genomic regions associated with C_4 photosynthetic activity and leaf anatomy in *Alloteropsis semialata*. *New Phytologist, 243*(5), 1698–1710. https://doi.org/10.1111/nph.19933

Al-Salman, Y., Ghannoum, O., & Cano, F. J. (2023). Elevated [CO_2] negatively impacts C_4 photosynthesis under heat and water stress without penalizing biomass. *Journal of Experimental Botany, 74*(9), 2875–2890. https://doi.org/10.1093/jxb/erad063

Barupal, M., & Kumar, A. (2024). CRISPR/Cas systems for enhancing photosynthesis: Climate resilience and food production. In A. Kumar, S. Arora, S. Ogita, Y.-Y. Yau, & K. Mukherjee (Eds.), *Gene editing in plants: CRISPR-Cas and its applications* (pp. 477–519). Springer Nature Singapore. https://doi.org/10.1007/978-981-99-8529-6_18

Batista-Silva, W., da Fonseca-Pereira, P., Martins, A. O., Zsögön, A., Nunes-Nesi, A., & Araújo, W. L. (2020). Engineering improved photosynthesis in the era of synthetic biology. *Plant Communications, 1*(2), Article 100032. https://doi.org/10.1016/j.xplc.2020.100032

Blum, A. (2013). Heterosis, stress, and the environment: A possible road map towards the general improvement of crop yield. *Journal of Experimental Botany, 64*(16), 4829–4837. https://doi.org/10.1093/jxb/ert289

Boote, K. J., Hoogenboom, G., Porter, C. H., Pavan, W., Shelia, V., Singh, U., White, J. W., Asseng, S., Lizaso, J. I., Moreno, L. P., Cuadra, S., Kimball, B., Hopf, A., Oliveira, F. A. A., Ferreira, T. B., Boriolo Dias, H., Alderman, P., Batchelor, W. D., Pequeno, D., . . . Tsuji, G. Y. (2025). The Decision Support System for Agrotechnology Transfer (DSSAT) crop modeling ecosystem: Evolution and advances. In G. Hoogenboom (Ed.), *Current crop models: State-of-the-art and future developments* (pp. 3–64). Burleigh Dodds Science Publishing. https://doi.org/10.19103/AS.2025.0155.01

Boyles, R. E., Cooper, E. A., Myers, M. T., Brenton, Z., Rauh, B. L., Morris, G. P., & Kresovich, S. (2016). Genome-wide association studies of grain yield components in diverse sorghum germplasm. *The Plant Genome, 9*(2), Article plantgenome2015.09.0091. https://doi.org/10.3835/plantgenome2015.09.0091

Bukhat, S., Rasul, S., Athar, H.-u.-R., & Manzoor, H. (2025). Genome editing in improving photosynthesis in crop plants. In J. M. Al-Khayri, M. N. Sattar, S. K. Sopory, & S. M. Jain (Eds.), *Genome editing for crop improvement: Theory and methodology* (pp. 217–246). CABI. https://doi.org/10.1079/9781800622517.0016

Byrt, C. S., Grof, C. P. L., & Furbank, R. T. (2011). C_4 plants as biofuel feedstocks: Optimising biomass production and feedstock quality from a lignocellulosic perspective. *Journal of Integrative Plant Biology, 53*(2), 120–135. https://doi.org/10.1111/j.1744-7909.2010.01023.x

Calderan-Rodrigues, M. J., de Barros Dantas, L. L., Cheavegatti Gianotto, A., & Caldana, C. (2021). Applying molecular phenotyping tools to explore sugarcane carbon potential. *Frontiers in Plant Science, 12*, Article 637166. https://doi.org/10.3389/fpls.2021.637166

Correia, P. M. P., da Silva, A. B., Vaz, M., Carmo-Silva, E., & Marques da Silva, J. (2021). Efficient regulation of CO_2 assimilation enables greater resilience to high temperature and drought in maize. *Frontiers in Plant Science, 12*, Article 675546. https://doi.org/10.3389/fpls.2021.675546

Croce, R., Carmo-Silva, E., Cho, Y. B., Ermakova, M., Harbinson, J., Lawson, T., McCormick, A. J., Niyogi, K. K., Ort, D. R., Patel-Tupper, D., Pesaresi, P., Raines, C., Weber, A. P. M., & Zhu, X.-G. (2024). Perspectives on improving photosynthesis to increase crop yield. *The Plant Cell, 36*(10), 3944–3973. https://doi.org/10.1093/plcell/koae132

Driever, S. M., & Kromdijk, J. (2013). Will C_3 crops enhanced with the C_4 CO_2-concentrating mechanism live up to their full potential (yield)? *Journal of Experimental Botany, 64*(13), 3925–3935. https://doi.org/10.1093/jxb/ert103

Eid, A., Mohan, C., Sanchez, S., Wang, D., & Altpeter, F. (2021). Multiallelic, targeted mutagenesis of magnesium chelatase with CRISPR/Cas9 provides a rapidly scorable phenotype in highly polyploid sugarcane. *Frontiers in Genome Editing, 3*, Article 654996. https://doi.org/10.3389/fgeed.2021.654996

Ermakova, M., Sharwood, R. E., & Furbank, R. T. (2026). Tuning the C_4 supercharger: Improving photosynthesis and yield in C_4 crops. *New Phytologist, 249*(1), 76–92. https://doi.org/10.1111/nph.70692

FAO. (2024). *World food and agriculture – Statistical yearbook 2024*. https://doi.org/10.4060/cd2971en

Fontanet-Manzaneque, J. B., Hernández, D. M., Giordano, A., & Caño-Delgado, A. I. (2025). Sorghum as a monocot model for drought research. *Frontiers in Plant Science, 16*, Article 1665967. https://doi.org/10.3389/fpls.2025.1665967

Gao, D. T., Shi, C. Y., Li, Q. L., Wei, Z. F., Liu, L., & Feng, J. R. (2021). Drought tolerance monitoring of apple rootstock M.9-T337 based on infrared and fluorescence imaging. *Photosynthetica, 59*(Special Issue), 458–467. https://doi.org/10.32615/ps.2021.035

Ghannoum, O., Al-Salman, Y. M., & Cano, F. J. (2025). Opportunities for improving intrinsic water use efficiency in C_4 plants under climate change. *New Phytologist, 248*(6), 2656–2673. https://doi.org/10.1111/nph.70660

Guan, X.-K., Song, L., Wang, T.-C., Turner, N. C., & Li, F.-M. (2015). Effect of drought on the gas exchange, chlorophyll fluorescence and yield of six different-era spring wheat cultivars. *Journal of Agronomy and Crop Science, 201*(4), 253–266. https://doi.org/10.1111/jac.12103

Gupta, R., Sahani, I., Kushwaha, V. K., Khan, B. R., Singh, K., & Yadav, D. (2025). Stomatal conductance and electron transport responses of finger millet (*Eleusine coracana*) genotypes to atmospheric carbon dioxide, vapor pressure deficit, photosynthetically active radiation, and temperature. *Science of the Total Environment, 1003*, Article 180661. https://doi.org/10.1016/j.scitotenv.2025.180661

Habde, S., Nandigam, V. K., Raigar, O. P., Majhi, P. K., Amandeep, Gudi, S., & Singh, G. (2026). Breeding by design in rice: Multi trait plant type modifications for sustainable agriculture. *Rice Science*. Advance online publication. https://doi.org/10.1016/j.rsci.2026.06.004

Habermann, E., Dias de Oliveira, E. A., Contin, D. R., Delvecchio, G., Viciedo, D. O., de Moraes, M. A., de Mello Prado, R., de Pinho Costa, K. A., Braga, M. R., & Martinez, C. A. (2019). Warming and water deficit impact leaf photosynthesis and decrease forage quality and digestibility of a C_4 tropical grass. *Physiologia Plantarum, 165*(2), 383–402. https://doi.org/10.1111/ppl.12891

Hussain, B., Raza, Q., Atif, R. M., & Ahmad, M. Q. (2022). New breeding techniques (NBTs) and biotechnology for boosting rice grain yield to feed 5 billion in 2050. In N. Sarwar, Atique-ur-Rehman, S. Ahmad, & M. Hasanuzzaman (Eds.), *Modern techniques of rice crop production* (pp. 681–700). Springer Singapore. https://doi.org/10.1007/978-981-16-4955-4_33

Khan, T. A., Mundra, S., & Gururani, M. A. (2025). Advancements in photosynthetic efficiency: Pathways, regulation, and biotechnological applications for enhancing crop productivity. *Plant Signaling & Behavior, 20*(1), Article 2596483. https://doi.org/10.1080/15592324.2025.2596483

Kim, K.-H., Lim, S. H., Lim, S. D., Ha, J., & Lee, B.-M. (2026). Climate-resilient soybean: Integrated breeding strategies for mitigating drought and heat stress. *Agriculture, 16*(4), Article 445. https://doi.org/10.3390/agriculture16040445

Kumar, A., Muthuramalingam, P., Verma, L., Kumar, R., Kumar, N., Misra, J., Ravi, K., Shin, H., & Ramesh, M. (2026). Modern genomic and omics-based technologies for millet breeding and genetic improvement. *Frontiers in Plant Science, 17*, Article 1782766. https://doi.org/10.3389/fpls.2026.1782766

Kumar, A., Pandey, S. S., Kumar, D., & Tripathi, B. N. (2023). Genetic manipulation of photosynthesis to enhance crop productivity under changing environmental conditions. *Photosynthesis Research, 155*(1), 1–21. https://doi.org/10.1007/s11120-022-00977-w

Kumar, U., Dwivedi, D., & Das, U. (2025). Advancements in CRISPR-mediated multiplex genome editing: Transforming plant breeding for crop improvement and polygenic trait engineering. *Biotechnology Journal, 20*(11), Article e70148. https://doi.org/10.1002/biot.70148

Kuyyamudi Nanaiah, G., & Rakshit, S. (2020). Genomic designing for climate smart sorghum. In C. Kole (Ed.), *Genomic designing of climate-smart cereal crops* (pp. 171–219). Springer. https://doi.org/10.1007/978-3-319-93381-8_5

Leakey, A. D. B., Ferguson, J. N., Pignon, C. P., Wu, A., Jin, Z., Hammer, G. L., & Lobell, D. B. (2019). Water use efficiency as a constraint and target for improving the resilience and productivity of C_3 and C_4 crops. *Annual Review of Plant Biology, 70*(1), 781–808. https://doi.org/10.1146/annurev-arplant-042817-040305

Leakey, A. D. B., Uribelarrea, M., Ainsworth, E. A., Naidu, S. L., Rogers, A., Ort, D. R., & Long, S. P. (2006). Photosynthesis, productivity, and yield of maize are not affected by open-air elevation of CO_2 concentration in the absence of drought. *Plant Physiology, 140*(2), 779–790. https://doi.org/10.1104/pp.105.073957

Li, X., Yang, S., Zhang, J., & Wan, S. (2026). Photo-oxidative stress in plants: ROS signaling, damage propagation, and systems-level resilience. *Antioxidants, 15*(3), Article 371. https://doi.org/10.3390/antiox15030371

Liang, X., Yu, S., Ju, Y., Wang, Y., & Yin, D. (2025). Multi-scale remote-sensing phenomics integrated with multi-omics: Advances in crop drought–heat stress tolerance mechanisms and perspectives for climate-smart agriculture. *Plants, 14*(18), Article 2829. https://doi.org/10.3390/plants14182829

Liu, G., Li, S., He, Q., Zhang, C., Zhang, J., & Tang, Z. (2026). Integrated drought resilience in foxtail millet: From molecular regulation and multi-omics to climate-resilient breeding. *Water, 18*(15), Article 1823. https://doi.org/10.3390/w18151823

Manderscheid, R., Erbs, M., & Weigel, H.-J. (2014). Interactive effects of free-air CO_2 enrichment and drought stress on maize growth. *European Journal of Agronomy, 52*, 11–21. https://doi.org/10.1016/j.eja.2011.12.007

Mersha, K. Y., Muktar, M. S., Negawo, A. T., Mulat, M. W., & Abebe, F. M. (2026). Accelerating climate resilient agriculture through the integration of molecular breeding with genome editing and omics approaches. *Discover Agriculture, 4*(1), Article 167. https://doi.org/10.1007/s44279-026-00615-2

Miglani, G. S., Kaur, R., Sharma, P., & Gupta, N. (2021). Leveraging photosynthetic efficiency toward improving crop yields. *Journal of Crop Improvement, 35*(3), 361–402. https://doi.org/10.1080/15427528.2020.1824168

Morales, F., Ancín, M., Fakhet, D., González-Torralba, J., Gámez, A. L., Seminario, A., Soba, D., Ben Mariem, S., Garriga, M., & Aranjuelo, I. (2020). Photosynthetic metabolism under stressful growth conditions as a bases for crop breeding and yield improvement. *Plants, 9*(1), Article 88. https://doi.org/10.3390/plants9010088

Mullet, J. E. (2017). High-biomass C_4 grasses—Filling the yield gap. *Plant Science, 261*, 10–17. https://doi.org/10.1016/j.plantsci.2017.05.003

Namata, M. J., Xu, J., Habyarimana, E., Palakolanu, S. R., Wang, L., & Li, J. (2025). Genome editing in maize and sorghum: A comprehensive review of CRISPR/Cas9 and emerging technologies. *The Plant Genome, 18*(2), Article e70038. https://doi.org/10.1002/tpg2.70038

Nazari, M., Kordrostami, M., Ghasemi-Soloklui, A. A., Eaton-Rye, J. J., Pashkovskiy, P., Kuznetsov, V., & Allakhverdiev, S. I. (2024). Enhancing photosynthesis and plant productivity through genetic modification. *Cells, 13*(16), Article 1319. https://doi.org/10.3390/cells13161319

Ortiz, D., Hu, J., & Salas Fernandez, M. G. (2017). Genetic architecture of photosynthesis in *Sorghum bicolor* under non-stress and cold stress conditions. *Journal of Experimental Botany, 68*(16), 4545–4557. https://doi.org/10.1093/jxb/erx276

Paul, M. J., Watson, A., & Griffiths, C. A. (2020). Linking fundamental science to crop improvement through understanding source and sink traits and their integration for yield enhancement. *Journal of Experimental Botany, 71*(7), 2270–2280. https://doi.org/10.1093/jxb/erz480

Peng, S., Jiang, M., & Li, X. (2026). Systems-level integration of stress signaling, multi-omics, and predictive breeding for abiotic stress tolerance in *Brassica* crops. *Horticulturae, 12*(8), Article 1033. https://doi.org/10.3390/horticulturae12081033

Prasad, P. V. V., Djanaguiraman, M., Stewart, Z. P., & Ciampitti, I. A. (2020). Agroclimatology of maize, sorghum, and pearl millet. In J. L. Hatfield, M. V. K. Sivakumar, & J. H. Prueger (Eds.), *Agroclimatology: Linking agriculture to climate* (Agronomy Monograph 60, pp. 201–241). American Society of Agronomy; Crop Science Society of America; Soil Science Society of America. https://doi.org/10.2134/agronmonogr60.2016.0005

Prasad, P. V. V., Staggenborg, S. A., & Ristic, Z. (2008). Impacts of drought and/or heat stress on physiological, developmental, growth, and yield processes of crop plants. In L. R. Ahuja, V. R. Reddy, S. A. Saseendran, & Q. Yu (Eds.), *Response of crops to limited water: Understanding and modeling water stress effects on plant growth processes* (Advances in Agricultural Systems Modeling 1, pp. 301–355). American Society of Agronomy; Crop Science Society of America; Soil Science Society of America. https://doi.org/10.2134/advagricsystmodel1.c11

Qi, M., Liu, X., Li, Y., Song, H., Yin, Z., Zhang, F., He, Q., Xu, Z., & Zhou, G. (2021). Photosynthetic resistance and resilience under drought, flooding and rewatering in maize plants. *Photosynthesis Research, 148*(1–2), 1–15. https://doi.org/10.1007/s11120-021-00825-3

Ramazan, S., Bhat, H. A., Zargar, M. A., Ahmad, P., & John, R. (2021). Combined gas exchange characteristics, chlorophyll fluorescence and response curves as selection traits for temperature tolerance in maize genotypes. *Photosynthesis Research, 150*(1–3), 213–225. https://doi.org/10.1007/s11120-021-00829-z

Rayaprolu, L., Jayasankar, K., Aarts, M. G. M., & Harbinson, J. (2025). Photosynthetic variation for climate-resilient crops: Photosynthetic responses to fluctuating light and chilling in tomato. *Physiologia Plantarum, 177*(3), Article e70241. https://doi.org/10.1111/ppl.70241

Reddy, B. V. S., Ashok Kumar, A., Sharma, H. C., Srinivasa Rao, P., Blümmel, M., Ravinder Reddy, Ch., Sharma, R., Deshpande, S. P., Mazumdar, S. D., & Dinakaran, E. (2012). Sorghum improvement (1980–2010): Status and way forward. *Journal of SAT Agricultural Research, 10*, 1–14. http://oar.icrisat.org/id/eprint/6245

Sage, R. F., & Zhu, X.-G. (2011). Exploiting the engine of C_4 photosynthesis. *Journal of Experimental Botany, 62*(9), 2989–3000. https://doi.org/10.1093/jxb/err179

Sahoo, R., Samanta, D., Sow, S., Ranjan, S., Nath, D., Sadhu, S., Kumar, N., Rana, L., Kumar, A., Roy, D. K., Dharminder, Kumar, R., Raigar, B. L., Das, S., & Panday, R. (2025). Nexus between photosynthesis and radiation use efficiency towards achieving sustainability in the era of climate change: An overview. *Discover Environment, 3*(1), Article 67. https://doi.org/10.1007/s44274-025-00252-4

Sales, C. R. G., Wang, Y., Evers, J. B., & Kromdijk, J. (2021). Improving C_4 photosynthesis to increase productivity under optimal and suboptimal conditions. *Journal of Experimental Botany, 72*(17), 5942–5960. https://doi.org/10.1093/jxb/erab327

Salesse-Smith, C. E., Adar, N., Kannan, B., Nguyen, T., Wei, W., Guo, M., Ge, Z., Altpeter, F., Clemente, T. E., & Long, S. P. (2025). Adapting C_4 photosynthesis to atmospheric change and increasing productivity by elevating Rubisco content in sorghum and sugarcane. *Proceedings of the National Academy of Sciences, 122*(8), Article e2419943122. https://doi.org/10.1073/pnas.2419943122

Salesse-Smith, C. E., Wang, Y., & Long, S. P. (2025). Increasing Rubisco as a simple means to enhance photosynthesis and productivity now without lowering nitrogen use efficiency. *New Phytologist, 245*(3), 951–965. https://doi.org/10.1111/nph.20298

Serba, D. D., Yadav, R. S., Varshney, R. K., Gupta, S. K., Mahalingam, G., Srivastava, R. K., Gupta, R., Perumal, R., & Tesso, T. T. (2020). Genomic designing of pearl millet: A resilient crop for arid and semi-arid environments. In C. Kole (Ed.), *Genomic designing of climate-smart cereal crops* (pp. 221–286). Springer. https://doi.org/10.1007/978-3-319-93381-8_6

Sharma, R., & Joshi, M. (2022). Vulnerability and resilience of sorghum to changing climatic conditions: Lessons from the past and hope for the future. In S. Roy, P. Mathur, A. P. Chakraborty, & S. P. Saha (Eds.), *Plant stress: Challenges and management in the new decade* (pp. 169–181). Springer. https://doi.org/10.1007/978-3-030-95365-2_11

Silva, R. G. da, Alves, R. de C., & Zingaretti, S. M. (2020). Increased [CO_2] causes changes in physiological and genetic responses in C_4 crops: A brief review. *Plants, 9*(11), Article 1567. https://doi.org/10.3390/plants9111567

Simpson, C. J. C., Reeves, G., Tripathi, A., Singh, P., & Hibberd, J. M. (2022). Using breeding and quantitative genetics to understand the C_4 pathway. *Journal of Experimental Botany, 73*(10), 3072–3084. https://doi.org/10.1093/jxb/erab486

Singh-Bakala, H., Ravelombola, F., Washburn, J. D., Shannon, G., Zhang, R., & Lin, F. (2025). Photosynthetic and canopy trait characterization in soybean (*Glycine max* L.) using chlorophyll fluorescence and UAV imaging. *Agriculture, 15*(24), Article 2576. https://doi.org/10.3390/agriculture15242576

Srivastava, R. K., Yadav, O. P., Kaliamoorthy, S., Gupta, S. K., Serba, D. D., Choudhary, S., Govindaraj, M., Kholová, J., Murugesan, T., Satyavathi, C. T., Gumma, M. K., Singh, R. B., Bollam, S., Gupta, R., & Varshney, R. K. (2022). Breeding drought-tolerant pearl millet using conventional and genomic approaches: Achievements and prospects. *Frontiers in Plant Science, 13*, Article 781524. https://doi.org/10.3389/fpls.2022.781524

Tripathy, S. K. (2022). Genomics-assisted precision breeding for drought tolerance in sugarcane. In R. K. Gaur (Ed.), *Omics approaches for sugarcane crop improvement* (pp. 131–162). CRC Press. https://doi.org/10.1201/9781003292425-7

van Bezouw, R. F. H. M., Keurentjes, J. J. B., Harbinson, J., & Aarts, M. G. M. (2019). Converging phenomics and genomics to study natural variation in plant photosynthetic efficiency. *The Plant Journal, 97*(1), 112–133. https://doi.org/10.1111/tpj.14190

Wall, G. W., Brooks, T. J., Adam, N. R., Cousins, A. B., Kimball, B. A., Pinter, P. J., LaMorte, R. L., Triggs, J., Ottman, M. J., Leavitt, S. W., Matthias, A. D., Williams, D. G., & Webber, A. N. (2001). Elevated atmospheric CO_2 improved *Sorghum* plant water status by ameliorating the adverse effects of drought. *New Phytologist, 152*(2), 231–248. https://doi.org/10.1046/j.0028-646x.2001.00260.x

Watson-Lazowski, A., & Ghannoum, O. (2021). The outlook for C_4 crops in future climate scenarios. In K. M. Becklin, J. K. Ward, & D. A. Way (Eds.), *Photosynthesis, respiration, and climate change* (pp. 251–281). Springer. https://doi.org/10.1007/978-3-030-64926-5_9

Yadav, S., & Mishra, A. (2020). Ectopic expression of C_4 photosynthetic pathway genes improves carbon assimilation and alleviate stress tolerance for future climate change. *Physiology and Molecular Biology of Plants, 26*(2), 195–209. https://doi.org/10.1007/s12298-019-00751-8

Zhou, Y., Li, X., Wei, S., Soualiou, S., Struik, P. C., Yin, X., & Zhou, W. (2026). Harnessing natural variation for photosynthetic improvement in next-generation crop breeding. *Journal of Integrative Plant Biology, 68*(8), 2608–2623. https://doi.org/10.1111/jipb.70317