Ectopic expression of C_4 photosynthetic pathway genes improves carbon assimilation and alleviate stress tolerance for future climate change

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Abstract Alteration in atmospheric carbon dioxide concentration and other environmental factors are the significant cues of global climate change. Environmental factors affect the most fundamental biological process including photosynthesis and different metabolic pathways. The feeding of the rapidly growing world population is another challenge which imposes pressure to improve productivity and quality of the existing crops. C_4 plants are considered the most productive, containing lower photorespiration, and higher water-use & N-assimilation efficiencies, compared to C_3 plants. Besides, the C_4 -photosynthetic genes not only play an important role in carbon assimilation but also modulate abiotic stresses. In this review, fundamental three metabolic processes (C_4 , C_3 , and CAM) of carbon dioxide assimilation, the evolution of C_4 -photosynthetic genes, effect of elevated CO_2 on photosynthesis, and overexpression of C_4 -photosynthetic genes for higher photosynthesis were discussed. Kranz-anatomy is considered an essential prerequisite for the terrestrial C_4 carbon assimilation, but single-celled C_4 plant species changed this well-established paradigm. C_4 plants are insensitive to an elevated CO_2 stress condition but performed better under stress conditions. Overexpression of essential C_4 -photosynthetic genes such as *PEPC*, *PPDK*, and *NADP-ME* in C_3 plants like *Arabidopsis*, tobacco, rice, wheat, and potato not only improved photosynthesis but also provided tolerance to various environmental stresses, especially drought. The review provides useful information for

sustainable productivity and yield under elevated CO_2 environment, which to be explored further for CO_2 assimilation and also abiotic stress tolerance. Additionally, it provides a better understanding to explore C_4 -photosynthetic gene(s) to cope with global warming and prospective adverse climatic changes.

Keywords Abiotic stress · C_3 · C_4 · CAM · Carbon assimilation · Global warming · Photosynthesis

Introduction

Earth is facing two major threats, first is global warming, which reaches an alarming stage, and the second is world-population that is also expanding rapidly. Global warming leads to climatic changes, an increase of the atmospheric CO_2 concentration and other greenhouse gases (GHGs) are a primary source (Nowak et al. 2004). In the atmosphere, CO_2 is the most prominent GHG which associated with an increase in atmospheric temperature. Furthermore, it is directly linked with the development and productivity of terrestrial plants system. Enhanced photosynthesis of crop plants leads to the CO_2 assimilation and also productivity, therefore considered the most promising approach to solve both problems. According to IPCC (2013) and NASA (2014), the pre-industrial level of global CO_2 concentration $285 \mu\text{mol mol}^{-1}$ which is now increased to the present level $400 \mu\text{mol mol}^{-1}$. About 1.35% increment is predicted annually for global CO_2 concentration, and about $936 \mu\text{mol mol}^{-1}$ CO_2 concentration is speculated with the rise of global atmospheric temperature of 6.4°C by the year 2100. These changes would severely affect the world flora and fauna including crop plant development and productivity. Researchers have a profound interest to

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mitigate the adverse effects of global climate changes by improving the atmospheric abundance of CO₂ and other GHGs. In the Kyoto protocol, enhanced biological carbon sequestration is one of the most essential, instantly valid, natural and cost-effective approach to alleviate the effects of increased CO₂ concentration from atmosphere (Ruan et al. 2012), which leads to improve the storage capacity of ecosystem through plant photosynthesis.

The photosynthesis, one of the fundamental biological process, follows three metabolic strategies to assimilate CO₂ from the environment, and plants are categorized into three groups; C₃, C₄, and CAM, accordingly. These plants have different photosynthesis carbon assimilation, morphological and physiological characteristics. Most of the terrestrial plants, including prominent crops such as rice, soybean, wheat and potato assimilate atmospheric CO₂ as a three-carbon compound, 3-phosphoglycerate (3-PGA), and follows Calvin–Benson cycle, also commonly known as C₃ photosynthesis pathway (Matsuoka et al. 2001). In C₃ photosynthesis metabolic process, ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) play a major role to assimilate atmospheric CO₂, and under a stressful condition, this enzyme has low affinity to differentiate O₂ from the CO₂. Resultant, O₂ competes with CO₂ at the active site of RuBisCO, which leads to about 50% loss of carbon from metabolic sites. Therefore, photosynthesis decreases and another metabolic process such as photorespiration increases because of oxygenase activity (Miyao 2003). During the evolution, hot environments were developed, and plants adapted to stress condition including inadequate water supply up to an extent (Edwards et al. 2001). Plants, evolved with C₄ carbon assimilation, release CO₂ at the proximity of RuBisCO, which promotes carboxylation, respectively over the oxygenation (Hatch 1987). This assimilation process prevents the possible loss of CO₂ which is commonly occurred through photorespiration and also improves the water and nitrogen-use efficiencies, compared to C₃ plants (Sage and Pearcy 1987). The Crassulacean Acid Metabolism (CAM) is evolved in some plants which are adapted to arid conditions (Winter and Holtum 2014). In these (CAM) plants, stomata remain closed during the day to diminish evapotranspiration but open in the night to assimilate atmospheric CO₂. Both, CAM and C₄ carbon assimilation process have evolved from C₃ ancestors, independently. In total, about 3% of flowering plants are C₄ (Sage et al. 2012) whereas about 6% represent CAM plants (Silvera et al. 2010).

Many essential crops (rice and wheat) possessing C₃ photosynthesis pathway compared to other crops (maize and sorghum) harboring C₄ pathway are relatively inefficient for CO₂ uptake and further assimilation. Consequently, they (C₃ crops) have a lower yield, water, and nitrogen-use efficiency. The genetic transformation and

recombinant DNA technology may provide a set of accessible and practical tools for enhancing carbon assimilation in crops by introgression C₄ characteristics into C₃ plants by genetic manipulation (Matsuoka et al. 2001). A number of endeavors have been made to over-express C₄-specific photosynthesis enzymes encoding genes such as phosphoenolpyruvate carboxylase (PEPC), pyruvate, phosphate dikinase (PPDK), NADP-malic enzyme (NADP-ME), nicotinamide adenine dinucleotide phosphate-malate dehydrogenase (NADP-MDH) or phosphoenolpyruvate carboxykinase (PCK) into C₃ plants using genetic engineering and transgenic technologies for increasing the efficiency of photosynthesis.

In this review, detailed characteristics of biological approaches for CO₂ assimilation in C₄ plants, types of C₄ pathways, and the molecular evolution of C₄ photosynthetic genes will be discussed followed by the effect of enhanced CO₂ concentration in C₄ plants. The primary focus of the review is the overexpression of C₄ photosynthetic gene(s) in C₃ plants using genetic engineering. The development of C₃ plants with C₄ features is an ambitious objective, and it would provide great revolutions for the crop improvement to feed a rapidly growing world population. Studies on the molecular characterization of C₄ assimilation and its introgression to C₃ plants will enhance our perception and provide novel information that can be explored further for developing crop plants with high photosynthesis traits and abiotic stress tolerance.

C₄ photosynthesis: an overview

The C₄ photosynthetic plants are considered one of the most efficient CO₂ sequester, productive and biomass producer on the planet. In the C₄ pathway, PEPC acts as an acceptor of atmospheric CO₂ in the cytosol of the mesophyll cells (O’Leary 1982), primarily fixes the carbon-dioxide in the bicarbonate form in mesophylls, and subsequent forms oxaloacetate (OAA) a four-carbon compound, which is converted to malate or aspartate. Aspartate then translocates to the specialized bundle sheath cells where CO₂ is decarboxylated by the decarboxylating enzymes (Furbank 2016) and re-released metabolic CO₂ re-fixes in the bundle sheath to a three-carbon compound 3-PGA by RuBisCO (Fig. 1) same as C₃ (Calvin–Benson) pathway (Hatch 1987). The spatial separation of mesophyll cells and bundle sheath cells has been supposed to be a precondition for an efficient CO₂ assimilation mechanism.

Photorespiration usually occurs in C₃ plants, consumes ATP and NADPH, and leads to loss of CO₂ from bundle sheath which decreases the efficiency of CO₂ sequestration (Leegood et al. 1995). The physical separation between RuBisCO and oxygen-generating light reaction decreases

Fig. 1 Schematic diagram of C₄ photosynthesis pathway. **a** Single-cell C₄ photosynthesis pathway and **b** Kranz-anatomy C₄ photosynthesis pathway (NADP-ME type). CA carbonic anhydrases; OAA oxaloacetic acid, Mal malate, Pyr pyruvate, Gly-3-P glycerol-3-phosphate

photorespiration and promotes CO₂ fixation. In the plant system, C₄ photosynthesis acts as a CO₂ pump which increases the intracellular availability of CO₂ to RuBisCO and thus inhibits the oxygenation reaction (Hatch 1987). This approach avoids significant losses of CO₂ by photorespiration, compared to C₃ plants.

Most of the C₄ plants are grasses, and the photosynthetic reactions are compartmentalized between two morphologically distinct cell types which are arranged in concentric circles veins or wreath-like structure (Nelson and Langdale 1992). This anatomical barrier, in which vascular bundle is surrounded by a thick layer of bundle sheath cells, which in turns surrounded by a thin layer of mesophyll cells is known as the Kranz-anatomy. It has dimorphic chloroplast: chloroplasts present in mesophyll cells are of the typical type whereas chloroplasts, an inhabitant of bundle sheath are without granum (agranulated). About 15 discrete categories of Kranz anatomy have been identified in different monocot and dicot lineages of C₄ photosynthesis (Dengler and Nelson 1999). Subsequently, based on the decarboxylating enzymes, C₄ photosynthesis has been classified into three subtypes (Table 1; Fig. 2). Generally, during the C₄ cycle, CO₂ is released either by a chloroplastic NADP-dependent malic enzyme (NADP-ME), mitochondrial NAD-dependent malic enzyme (NAD-ME) or cytosolic phosphoenolpyruvate carboxykinase (PEPCK) (Leegood 2002).

Earlier, it was stated that NADP-ME and NAD-ME subtypes are distinct biochemical pathways, work further co-operation of the PEPCK pathway. Many crop plants like maize, sugarcane, sorghum, and other biofuel crops belong to NADP-ME subtype, and switch-grass, pearl millet, *Amaranthaceae*, *Bristlegrasses* and *Portulaca oleracea* belong to NAD-ME subtype (Edwards and Walker 1983).

The NADP-ME and NAD-ME represent almost equal numbers of genera in the dicots, but NADP-ME dominates in monocot families (Sage et al. 2012). Most of the C₄ grasses have shown that the NADP-ME subtypes present predominantly in annual precipitation area whereas the NAD-ME subtype presents more in arid regions (Hattersley 1992). Three main paradigms have been suggested to describe the divergence of the evolutionary transformation between NADP-ME and NAD-ME from C₄ subtypes: the first one suggested that NAD-ME is the primary subtype of the ancestral C₄ type and NADP-ME was evolved from it (Washburn et al. 2015). The second model proposed that the NADP-ME and NAD-ME subtypes share some level of a C₄ ancestor after that predominated individually in different lineages. However, the third model suggested that both, NADP-ME, and NAD-ME subtypes evolved individually from C₃–C₄ intermediate and considered the most recent common ancestor (Washburn et al. 2015).

It is well established that CO₂ assimilation rate of the C₄ plant is much higher than C₃ plants along with water and nitrogen-use efficiencies (Ghannoum et al. 2010). However, the nitrogen content per unit leaf area is found lower in C₄ compared to C₃ plants, but the concentration of nitrogen per unit dry weight is almost similar. Both, C₃ and C₄ plants invest the same fraction of leaf nitrogen into photosynthetic components, C₄ plants dispense low nitrogen to RuBisCO and more to thylakoids components. In C₃ plants, about 30% of leaf nitrogen supplies to RuBisCO compared to 4–21% leaf-nitrogen distribution in C₄ plants (Sage et al. 1987). Consequentially, the C₄ photosynthesis required less nitrogen for CO₂ assimilation process, which significantly increases the metabolic CO₂ concentration in the chloroplast because of low gas permeability of bundle-sheath cells, followed by suppression of RuBisCO

Table 1 Subtypes of C_4 photosynthesis based on decarboxylation mechanism

Physiological characteristics	Subtypes		References
	NADP-ME	PEPCK	
Enzyme location	Chloroplast	Mitochondria	Von Caemmerer and Evans (2010), Pick et al. (2011)
Decarboxylase	NADP-ME	NAD-ME	Pick et al. (2011)
Evolution	Gene duplication from C_3 ancestor	Existing mitochondrial NAD-ME	Maier et al. (2011)
Physiology	Higher photosynthesis and nitrogen use efficiency	Higher nitrogen use efficiency	Pinto et al. (2016), Furbank (2016), Rao and Dixon (2016)
C_4 acid in bundle sheath cells (decarboxylated acid)	Malate	Aspartate	Pick et al. (2011)
C_3 acid returning to mesophyll cells	Pyruvate	Alanine	Pick et al. (2011)
C_4 plastid transporter (Pyruvate transport in chloroplasts of mesophyll cells)	Proton: pyruvate co-transporter	Sodium: pyruvate co-transporter	Weber and von Caemmerer (2010), Furumoto et al. (2011)
Examples	Maize, sugarcane, and sorghum	Switchgrass, pearl millet, and amaranth	Edwards and Walker (1983)

oxygenase activity and almost eliminating photorespiration. The advantages of higher water- and nitrogen-use efficiency of C_4 compared to C_3 photosynthesis is fully appreciated at high temperature and light, where oxygenase reaction increases. At higher temperature, C_4 photosynthesis is more efficient than C_3 . Therefore the worldwide distribution of C_4 grasses is positively correlated with the temperate growing season. Although some C_4 plants show cold adaptation, they still require warm daytime to exist in a cold environment. Thus, C_4 species are poorly distributed in cold climates compared to C_3 plants (Sage and McKown 2006). Overall, the current hypothesis is that at a higher temperature, C_4 photosynthesis is more efficient than C_3 photosynthesis.

Most C_4 photosynthesis plant has Kranz-anatomy in which mesophyll and bundle-sheath cells, provides the spatial separation of the two carboxylase reactions, which is crucial for the terrestrial C_4 photosynthesis. Eventually discovery of some terrestrial species within the Chenopodiaceae (now Amaranthaceae) family; *Suaeda aralocaspica*, *Suaeda monoica* and *Bienertia sinuspersici*, where C_4 photosynthesis operates within an individual cell, has changed the established paradigm that the Kranz-anatomy is essential for terrestrial C_4 carbon assimilation (Voznesenskaya et al. 2001). C_4 photosynthesis was determined by using gas exchange studies and carbon isotope perception of a single cell and Kranz-anatomy of C_4 plants (Leegood 2002). Further, it concluded that both types of anatomy showed similar CO_2 assimilation rate and same values of leakiness at high light (King et al. 2012). Nevertheless, an efficiency of single cell C_4 photosynthesis remains in question because they lack the cell wall and plasma membrane compartment for CO_2 diffusion which is found in Kranz-anatomy of C_4 plants. A comparative study of the photosynthesis efficiency of single-cell C_4 species (*B. sinuspersici* and *S. aralocaspica*) and a Kranz type C_4 species (*S. eltonica*) revealed that both (single-cell and Kranz type) C_4 plants have similar photosynthesis capacity (King et al. 2012). Single cell type of C_4 photosynthesis was also discovered in some aquatic plants *Sagittaria subulata* (Alismataceae), green algae, diatoms, and aquatic monocots (Hydrocharitaceae: *Egeria densa* and *Hydrilla verticillata*; Von Caemmerer et al. 2014; Reinfelder et al. 2000; Johnston et al. 2001). These terrestrial and aquatic single-celled systems, exhibit the remarkable plasticity inherent in the plant cell, they provide the exquisite control of subcellular mechanism that could be applied to achieve very complex biochemical features. The discovery of single-cell C_4 photosynthesis, would be an alternative approach to engineering C_3 plants to execute Kranz-type C_4 photosynthesis without having a dual-cell system.

Furthermore, to concentrate CO_2 , C_4 photosynthesis required at least two (NADP-ME and NAD-ME type) or

Fig. 2 Schematic diagram of three subtypes of C₄ photosynthesis. **a** NADP-ME subtype, **b** NAD-ME subtype, and **c** PEPCK subtype. *Ala-amino* alanine aminotransferase, *Asp-amino* aspartate aminotransferase

one (PEPCK type) extra ATP molecules per CO₂ fixation as compared to C₃ photosynthesis without the need of additional reduction equivalents. Consequently, the demands of ATP is relative to NADPH in C₄ photosynthesis are higher than in C₃ photosynthesis, because C₃ plants photosynthesis requires both ATP and NADPH (Edwards and Walker 1983). However, in C₄ plants, increase in ATP to NADPH ratio is compensated by improved cyclic electron flow nearby PSI, which delivers additional ATP without concurrently generating NADPH (Ishikawa et al. 2016).

Evolution of C₄ photosynthetic genes

The evolution of C₄ photosynthesis has been achieved through some divergences of pre-existing C₄ photosynthesis and represented by about 60 individual origins from 19 families of angiosperms (Bräutigam et al. 2017). A significant step of photosynthesis evolution is the up-regulation of a C₄ metabolic cycle, but the precise reason is remains unclear due to unavailability of methods for

measuring in situ C₄ cycles in C₃–C₄ intermediates species (Sage 2016). Environmental obligations influenced C₄ evolution which led by several alterations of the anatomic, biochemical and genetic pre-conditions. It is hypothesized that the C₄ plants evolved under high photorespiration rates, low atmospheric CO₂ concentration and limited water supply in a warm environment (Ehleringer and Monson 1993). However, C₄ photosynthesis described as an adaptation to hot and dry environments. A combination of high salinity, drought, and temperature (but low humidity) promotes photorespiration, and CO₂ deficiency (Ehleringer and Monson 1993). The high efficiency of C₄ photosynthesis under low atmospheric CO₂, high heat, drought, and saline environment suggested that abiotic stress conditions were the essential driving force of C₄ evolution.

The pre-existing analogous genes of ancestral C₃ photosynthesis may evolve the C₄ photosynthesis genes and modified the expression level to control the kinetic properties of respective enzymes. Functions of C₄ photosynthetic genes/enzymes are found exclusive to C₄ plants, they may also present in C₃ plants, but activities of the

corresponding enzymes are almost undetectable (Hatch 1987). C_4 photosynthetic genes and their homologs from C_3 plants are considered as C_4 -specific and C_4 -like genes, respectively (Miyao 2003). Besides these (C_4 -like or C_4 -specific) genes, both C_3 and C_4 plants possessed other homologs with a housekeeping function and designated as C_3 -like genes. The isoform of C_4 photosynthetic genes may also originate from a pre-existing, non-photosynthetic gene of C_3 ancestral species.

Effect of elevated CO_2 on C_4 photosynthesis

The elevated CO_2 concentration in the atmosphere has both positive and negative impacts and, the study of the plant response to predicted climatic changes is of the immense importance (Fig. 3). Elevated CO_2 was demonstrated to mitigate the detrimental effects of abiotic stress conditions on plants (AbdElgawad et al. 2015). Plant-response to elevated CO_2 depends on the metabolic stages of a plant and response may vary species to species or plant to plants.

Furthermore, C_3 and C_4 plants behave differently under the elevated CO_2 environment (Srinivasarao et al. 2016). The ratio of oxygenation and carboxylation of RuBP depends on the relative concentration of CO_2 and O_2 at the site of RuBisCO, and a high CO_2 increases carbon assimilation by reducing photorespiration. Earlier, it was proven that doubling of current atmospheric CO_2 concentration will induce the C_4 plant growth by 10–20% whereas about 40–50% of C_3 plants (Bowes 1993). C_3 plants have potential to regulate the CO_2 pump during carbon assimilation under an elevated CO_2 condition, and therefore, respond positively due to the substrate specificity of RuBisCO in current atmospheric CO_2 concentration (Leakey et al. 2009).

C_4 photosynthesis is considered near-saturated at current atmospheric CO_2 , and a further increase of CO_2 may not be productive. However, some C_4 plants showed positive growth response under elevated CO_2 condition (Yadav et al. 2018). It was found that some photosynthetic enzymes, RuBisCO, PPDK and NADP-MDH activities were up-regulated in sugarcane under the elevated CO_2

Fig. 3 Physiological and biochemical response of plants under an elevated CO_2 environment condition

plants, whereas PEPC and NADP-ME enzyme activities were unaffected. Elevated CO₂ up-regulated the essential enzymes of photosynthetic and sucrose metabolism, reduced stomatal conductance and transpiration, and finally improved leaf water use efficiency with better plant water status (Jiang et al. 2016). Elevated CO₂ also significantly mitigates the negative impact of drought and heat condition such as photosynthesis inhibition, biomass reduction, H₂O₂ production, chlorophyll fluorescence declination, and protein oxidation (AbdElgawad et al. 2015). Similarly, elevated CO₂ may alleviate the impairment of oxidative stress from various abiotic stresses including drought, heat, salinity, and ozone, by amelioration the antioxidant defenses systems of enzymatic and non-enzymatic, including ascorbate peroxidase (APX), superoxide dismutase (SOD), peroxidase (POX), glutathione reductase (GR), glutathione peroxidase (GPX), catalase (CAT), dehydroascorbate reductase (DHAR), and non-enzymatic compounds such as glutathione (GSH), ascorbate (ASC), alkaloids and phenolic compounds (Gill and Tuteja 2010).

Elevated CO₂ may also enhance plant diversity and productivity by decreasing stomatal conductance, transpiration rate, stomatal density and consequently increasing water use efficiency, photosynthetic pigments, ratio of root/shoot biomass and soil water availability (Jiang et al. 2016). A comparative study, performed with C₃ (sunflower) and C₄ (maize) crops under elevated CO₂ and different stress conditions revealed interesting results (Vanaja et al. 2011). Under elevated CO₂ condition, sunflower (a C₃ plant) showed enhanced growth under both normal and drought stress conditions, while maize (a C₄ plant) showed better growth response under drought stress condition only. The stomatal conductance, leaf water potential and transpiration decrease in both (C₃ and C₄) crops under drought stress. However, an ameliorative effect was noticed in elevated CO₂ condition which promotes higher photosynthesis in sunflower under drought stress treatment compared to drought stress in control (normal CO₂) condition. Similarly, a comparative study of two different kinds of grass and legumes exposed to drought, elevated temperature and elevated CO₂ showed that drought inhibits plant growth, photosynthesis assimilation rate, stomatal conductance, but induces osmolytes followed by antioxidants in all species, whereas elevated CO₂ ameliorated the detrimental effects of stresses (AbdElgawad et al. 2015). The enhanced CO₂ concentration improved salt resistant in C₃ species *Chenopodium quinoa* as well as in the C₄ species *Atriplex nummularia* (Geissler et al. 2015). Overall, it is hypothesized that C₃ plants perform better and produce more biomass (Hussin et al. 2017), than C₄ plants under elevated CO₂ stress condition. However, under the same elevated CO₂ condition, an increase in the growth, biomass, and yield of C₄ plants was

found under drought stress conditions because of higher carbon assimilation and improved photosynthesis efficiency.

Overexpression of C₄ photosynthetic genes

The productivity and biomass yield of crop plants are significantly correlated with the carbon assimilation of a particular plant and about 90–95% of dry biomass from plant photosynthesis. However, many researches confirmed that the high photosynthesis is essential for high biomass production. As already discussed, C₄ plants thoroughly reduce the deleterious effects of photorespiration by concentrating CO₂ in vicinity of RuBisCO of a chloroplast. However, bundle-sheath cells act as a barrier or protecting layer to avoid the leakage of CO₂, which leads to improvements in radiation use efficiency and productivity of plants (Mitchell and Sheehy 2006). Based on the advantages of C₄ carbon assimilation over the limited factors of C₃ plants, introgression of C₄ photosynthetic enzymes in C₃ plants are a reliable, cost-effective and prospective approach to enhance the productivity and biomass to improve the photosynthesis of C₃ plants and also biological CO₂ sequestration (Edwards et al. 2001; Leegood 2002). The C₄ photosynthetic genes encoding for enzymes PEPC, PPDK, or NADP-ME were overexpressed in tobacco (Laporte et al. 2002; Müller et al. 2018), potato (Ishimaru et al. 1998; Häusler et al. 2001), rice (Taniguchi et al. 2008; Gu et al. 2013; Shen et al. 2015), wheat (Kershanskaya and Teixeira da Silva 2010; Peng et al. 2018;) and *Arabidopsis* (Fahnenstich et al. 2007; Kandoi et al. 2016).

Overexpression of C₄ PEPC enzyme-encoding gene in C₃ plants

Phosphoenolpyruvate carboxylase (PEPC: EC 4.1.1.31) catalyzes the primary atmospheric CO₂ fixation step by C₄ carbon assimilation. Besides, its non-photosynthetic isoforms are also involved in carbon–nitrogen interactions, seed germination, and maturation, stomatal opening and regulation of pH in cytosol, additionally play an important role in stress tolerance (Cheng et al. 2016). It is well known that the *PEPC* gene up-regulated under drought and salinity stress conditions in C₃ and C₄ plants (Zhou et al. 2011; Kandoi et al. 2016). Overexpression of C₄ *PEPC* gene in C₃ plants showed an improve photosynthesis and also enhance drought stress tolerance (Zhou et al. 2011) (Table 2). Overexpression of the *PEPC* gene, isolated from maize and transformed to a model plant tobacco showed about twofold higher PEPC activity in leaves in transgenic lines compared to wild-type, without any significant

Table 2 Overexpression of C_4 phosphoenolpyruvate carboxylase enzyme encoding *PEPC* gene in C_3 plants

Host plant (C_4)	Recipient plant (C_3)	Results	References
Maize	Tobacco	Twofold higher PEPC activity but no physiological changes in the photosynthesis rate or CO_2 assimilation	Hudspeth et al. (1992)
Maize	Rice	Two–three-fold higher PEPC activity under CO_2 atmospheric condition	Ku et al. (2000)
Maize	Rice	Two–three-fold higher PEPC activity in the leaves of some transgenic lines	Agarie et al. (2002)
Maize	Rice	Slightly lower CO_2 assimilation rate (7.3%)	Fukayama et al. (2003)
Maize	Rice	14–60 fold higher PEPC activity	Suzuki et al. (2006)
Maize	Indica rice	Enhanced photosynthesis under high-temperature	Bandyopadhyay et al. (2007)
Maize	Rice	Higher (22%) grain production	Jiao (2008)
Maize	Wheat	Higher (25–50%) grain yield	Kershanskaya and Teixeira da Silva (2010)
Maize	Rice	Enhanced drought tolerance	Zhou et al. (2011)
Sugarcane	Indica rice	Higher grain filling and total grain numbers but the grain filling percent and 1000-grain weights remained unchanged	Lian et al. (2014)
Maize	Rice	Improved drought tolerance	Shen et al. (2015)
Maize	Arabidopsis	About Seven–ten-fold increase in PEPC activity, higher chlorophyll content, enhanced electron transport rate (ETR), lower nonphotochemical quenching of chlorophyll a fluorescence, and a higher performance index	Kandoi et al. (2016)
Maize	Rice	About 12% increase in grain yield	Sen et al. (2017)
Maize	Wheat	Enhanced growth, and yield under low N condition	Peng et al. (2018)

deviation in the photosynthetic rate of transgenic plants (Hudspeth et al. 1992). However, overexpressing the maize C_4 *PEPC* transgenic rice exhibited reduced O_2 inhibition of photosynthesis and photosynthetic rate comparable to wild-type (Ku et al. 2000). Similarly, an increase of the PEPC enzyme activity and the photosynthetic rate was detected under high temperature in rice plant by improving stomatal conductance and elevated internal CO_2 concentration (Suzuki et al. 2006; Bandyopadhyay et al. 2007). Similar results were observed with transgenic wheat and rice overexpressing maize and sugarcane C_4 -specific *PEPC* gene, respectively (Lian et al. 2014; Shen et al. 2015). The overexpression of *Zea mays* *PEPC* gene in *Arabidopsis thaliana*, and wheat plants showed high PEPC activity and improved photosynthesis, growth, and yield under salinity and low nitrogen conditions by modulating carbon metabolism and nitrogen assimilation (Kandoi et al. 2016; Peng et al. 2018). Earlier, it was noticed that overexpression of maize *PEPC* gene in rice reduces the carbon assimilation rate (7.3%) but improves photorespiration in transgenic plants (Fukayama et al. 2003).

Some researchers demonstrated that the overexpression of a single C_4 photosynthesis enzyme can significantly change the carbon assimilation in C_3 plants. However, researcher also overexpressed multiple C_4 photosynthesis enzymes, including combinations of NADP-ME and PEPC

in C_3 plants for enhanced photosynthesis (Häusler et al. 2001) as well as PEPC and PPDK (Matsuoka et al. 2000; Jiao 2008). Four enzymes; maize C_4 -specific PPDK and PEPC, rice C_3 -like NADP-ME and sorghum C_4 -specific NADP-MDH were overexpressed in the mesophyll cells of rice plants individually or in combination (Taniguchi et al. 2008). However, individual overexpression of C_4 -specific genes did not alter the CO_2 assimilation.

Overexpression of C_4 PPDK enzyme-encoding gene in C_3 plants

PPDK (EC 2.7.9.1) is one of the rate-limiting enzyme of C_4 photosynthesis, which is abundantly present in the chloroplast of mesophyll cells (Hatch 1987). It catalyzes the reversible Pi- and ATP-dependent interconversion of pyruvate to PEP (phosphoenolpyruvate), the first acceptor of atmospheric CO_2 in C_4 plants. PPDK enzyme is also used by some bacteria and unicellular parasitic protists, while they are absent in insects or vertebrates and also a remarkable objective for the antiparasitic and antimicrobial drug besides C_4 -specific herbicides (Minges and Groth 2017). PPDK enzyme could also be involved in some abiotic stress caused by drought (Wang et al. 2018) and biotic stress induced by viral infection (Lu et al. 2019). It is already described that the relations of PPDK to stress, in

Table 3 Overexpression of C_4 pyruvate phosphate dikinase enzyme encoding *PPDK* gene in C_3 plants

Host plant (C_4)	Recipient plant (C_3)	Results	References
Maize	Potato	Four–five-fold higher enzyme activity	Ishimaru et al. (1998)
<i>Mesembryanthemum</i>	Tobacco	Higher seed production per capsule	Sheriff et al. (1998)
Maize	Rice	Higher photosynthetic rates (up to 35%)	Ku et al. (2000)
Maize	Rice	40-fold higher PPDK activity in leaves	Fukayama et al. (2001)
Maize	Rice	High nitrogen content in leaves	Zhang et al. (2007)
<i>Echinochloa</i>	Rice	Higher enzyme activity	Wang and Li (2008)
Maize	Arabidopsis	Higher photosynthesis rate (123%)	Wang et al. (2012)
Maize	Rice	Higher photosynthesis with enhanced drought tolerance	Gu et al. (2013)
Maize	Rice	Higher grain yield (12%), increased leaf blade size, root biomass, and plant height with anatomical changes such as greater leaf vein number, bundle sheath cells, and bulliform cells	Sen et al. (2017)

stressed environment like abscisic acid, drought stress caused by polyethylene glycol, salts, submergence, low-oxygen, and cold stress, evidently induce the activity of PPDK enzyme (Wang and Li 2008). PPDK enzyme-encoding gene from C_4 plants has been successfully introduced and expressed in C_3 plants (Table 3). Transgenic potato lines overexpressing *ZmPPDK* showed higher enzyme activity, but negligible effects were noticed in photosynthetic characteristics compared to control plants (Ishimaru et al. 1998). Similarly, *Echinochloa PPDK* gene, introduced in H65 rice plant, showed higher enzyme activity without any changes in carbon assimilation (Wang and Li 2008). Whereas, overexpression of maize chloroplastic *PPDK* gene showed high photosynthesis in transgenic rice than wild-type plants due to an elevated internal CO_2 concentration and improved stomatal conductance (Ku et al. 2000; Gu et al. 2013). Higher photosynthesis was also noticed in a model plant *Arabidopsis thaliana* overexpressing *C₄-PPDK* gene (Wang et al. 2012).

Overexpression of C_4 NADP-ME enzyme-encoding gene in C_3 plants

A subtype of C_4 plants harbors NADP-ME in the chloroplast of bundle sheath cells (Furbank 2016). During the decarboxylation, NADP-ME helps to enrich the CO_2 around RuBisCO and slow down photorespiration to improve the net photosynthesis. The activity of NADP-ME

showed contrary correlated with the photorespiration and also regulate stomatal opening (Müller et al. 2018). However, introgression of *NADP-ME* in C_3 plants might be lowered photorespiration and improved CO_2 assimilation in C_3 plants (Table 4). Overexpression of *NADP-ME* gene cloned from sorghum in rice showed elevated activity about 1–7 folds. Though high photoinhibition was observed in transgenic rice under high light intensity but no significant change was observed in the carbon assimilation rate (Chi et al. 2004). Similarly, maize *NADP-ME* gene, overexpressed in transgenic tobacco lines, showed abnormal stomatal behavior due to alteration of malate metabolism in guard cells (Laporte et al. 2002). Some negative results such as immature chlorophyll and abnormal chloroplast with agranal thylakoid membranes were observed in transgenic rice lines harboring maize NADP-ME (Takeuchi et al. 2000). Overexpression of *ZmNADP-ME*, transformed to the guard and vascular companion cells of the tobacco plant, led to enhance net carbon assimilation rate, higher biomass, more water use efficiency, earlier flowering, and shorter life cycle (Müller et al. 2018).

Overexpressing C_4 photosynthetic gene(s) improves abiotic stress tolerance

Photosynthesis is a metabolic process which is highly susceptible to environmental stresses. Overexpression of an individual gene encoding C_4 photosynthetic enzyme could

Table 4 Overexpression of C₄ NADP-ME enzyme encoding gene in C₃ plants

Host plant (C ₄)	Recipient plant (C ₃)	Results	References
Maize	Rice	20–70 fold higher activity causing aberrant chloroplast with agranal thylakoid membranes	Takeuchi et al. (2000)
Flaveria	Potato	No changes in enzyme activity	Häusler et al. (2001)
Maize	Rice	Enhanced photoinhibition, led to bleaching of leaf color and retarded growth under natural light	Tsuchida et al. (2001)
Maize	Tobacco	Altered stomatal behavior and water relations	Laporte et al. (2002)
Sorghum	Rice	No significant changes in carbon assimilation, though photoinhibition increased under high light intensity	Chi et al. (2004)
Maize	Arabidopsis	Did not show any differences in morphology and development when grown in long days. However, dark-induced senescence progressed more rapidly in transgenic plants	Fahnenstich et al. (2007)
Maize	Tobacco	Increased CO ₂ fixation, enhanced water use efficiency, earlier flowering and shorter life cycle	Müller et al. (2018)

modify the carbon assimilation in C₃ plants but up to an extent, therefore, a number of genes encoding different C₄ pathway enzymes were attempted towards improvement of net photosynthetic efficiency along with abiotic stress tolerance (Matsuoka et al. 2001; Häusler et al. 2001; Gu et al. 2013; Qi et al. 2017). It is a well-known fact that adverse climatic conditions (which will increase soon) including high-temperature, drought, salinity, and cold affect the crop productivity. There is an imperative need to improve plant defense system to combat with abiotic stresses, and improved photosynthesis may serve the purpose, additionally will lead to CO₂ assimilation and production of higher biomass.

Many C₃ crops such as wheat have lower photosynthetic efficiency at the optimal growth temperature, whereas C₄ crop such as sorghum and maize have higher biomass production, greater photosynthesis and improved abiotic stress tolerance compared to C₃ plants (Shen et al. 2015). The maize *PEPC* gene transformed into transgenic wheat conferred drought tolerance and increase grain yield. Lines with improved drought tolerance showed extensive root system as well as higher photosynthetic capability during stress compared to control (wild-type) plants and control (unstressed) condition (Qin et al. 2016). Transgenic wheat overexpressing *ZmPEPC* gene showed significantly higher photosynthetic rate under high temperature (Qi et al. 2017). Transformation of gene encoding C₄ PEPC enzyme is considered a promising strategy for improving rice crop production under drought stress (Zhou et al. 2011; Zai-Song et al. 2012; Gu et al. 2013; Shen et al. 2015). These study suggested that the overexpression of PEPC enzyme not only improve the photosynthetic rate but also enhanced involved in drought resistance in transgenic plants (Zhou

et al. 2011). Under drought stress condition, overexpressed *PEPC* gene in transgenic rice did not show any change under high PAR higher than 1200 $\mu\text{mol m}^{-2} \text{s}^{-1}$, while, untransformed wild-type rice plant showed a dramatic decrease in the net photosynthetic rate (Zai-Song et al. 2012).

Challenges and limitations

There are lots of inconsistencies in the performance of the transgenic C₃ plants expressing C₄ enzymes. One of the enormous challenges is the complexity of C₄ photosynthesis pathway and some inherent incompatibility between C₃ species and C₄ photosynthesis. Although, factors including, phylogenetic distance (Häusler et al. 2002), gene silencing (Chandler and Vaucheret 2001), splicing (Goodall and Filipowicz 1991), integration pattern (Gelvin 1998), rearrangement of transgene during transformation protocol (Karami 2008) which create inconsistency in the performance and efficacy of overexpressed enzyme. Inappropriate initiation and termination of transcription, along with incorrect splicing can also take place when enzymes from monocots are overexpressed into dicots (Goodall and Filipowicz 1991). A similar observation was also seen when the C₄-specific *PEPC* gene from maize was not significantly expressed in leaves of tobacco, because of incorrect transcription initiation (Hudspeth et al. 1992). Matsuoka et al. 2001, also suggested that the introgression of a recombinant gene comprising of cDNA for a C₄ gene, attached to an active promoter alone or with enhancer sequences, could enhance the activity of C₄ enzymes in C₃ plants.

Conclusion and prospects

Global warming is one of the primary constraints for the production of biomass and yield of a plant. Researchers have been concerned about improving the productivity and quality of crop plants. C_4 plants contain lower photorespiration, higher water, and N assimilation efficiencies compared to C_3 plants. Different C_4 pathway enzymes encoding genes were overexpressed into C_3 plants (*Arabidopsis*, tobacco, rice, and potato) to improve the carbon assimilation. RuBisCO enzyme is ubiquitous, and it will not be limiting in future climatic conditions in C_3 plants, but other photosynthetic enzymes are prone to restrict photosynthesis performance under adverse conditions. Simultaneously, C_4 plants are insensitive to enhance CO_2 condition, although, enzyme activity will affect only in a stressed condition. In addition, the C_4 photosynthetic genes not only play an important role in carbon assimilation but also induce drought stress tolerance. C_4 photosynthetic enzymes are of researcher's choice for improving the carbon assimilation and stress tolerance in C_3 plants through genetic transformation. Although, this approach is reasonable regarding output because these C_4 enzymes are also expressed a very low level in leaves and non-photosynthetic tissues of C_3 plants. Furthermore, overexpression of some single-cell C_4 pathway has the potential to support C_3 photosynthesis under drought stress. In C_4 photosynthesis, PEPC, PPDK, and NADP-ME enzymes are essential for carbon assimilation, and their non-photosynthetic isoforms play an important role in carbon fixing, nitrogen assimilation and pH regulation under various abiotic stress conditions. However, the influence of this employment on the photosynthesis and biomass production of the plants in elevated CO_2 stress condition has not yet been tested.

Overexpression of C_4 photosynthesis genes in C_3 plants approaches has intended at improving the highest potential activity of the particular enzymes in C_3 plants. However, it is also obligatory to select many transgenic lines to acquire the preferred expression level of C_4 enzyme and its position in C_3 plants. In addition, it is also necessary to engineer C_4 photosynthetic enzymes with respective promoters to be restored acclimatized in a specific environment of C_3 plants to carry out C_4 mechanism. Thus, it is further investigated that the overexpression of individual C_4 gene encoding enzyme not sufficient or more than one genes are required to achieve an overall improvement in photosynthesis and yield of C_3 plants. Therefore, some previous results indicated that a single C_4 enzyme did not increase photosynthesis in rice, by inhibited photosynthesis through reduced Rubisco activity and stimulation of respiration in the light. In addition, it is crucial for manipulations of carbon assimilation in C_3 plants to find out an alternative

approach to reduce photorespiration and comprehend the complexity of evolutionary anatomical development of different cell types in C_4 plants. It is obvious that engineering of C_4 requires introgression of additional genes in C_3 plants (Yadav and Mishra 2019). It is also hypothesised that some genes that suppress C_4 cycle should be removed from the recipient (C_3) plant. Genome editing by CRISPR/Cas9 would be a very useful technology to knock-out such repressing C_3 genes (Schuler et al. 2016; Patel and Mishra 2019).

Furthermore, for the improvement of carbon assimilation by photosynthesis and abiotic stress tolerance in C_3 plants, scientists have also explored halophytes for arid, semi-arid and marginal lands where other plants are not able to survive. Although, they also provide better nutritional quality food and biofuel, having good water saver, reduce CO_2 emission with higher photosynthetic rate and biomass producer. Halophytes were explored comprehensively for the genes (Jha et al. 2011; Chaturvedi et al. 2012, 2014; Joshi et al. 2013; Singh et al. 2014a; Udawat et al. 2014, 2016; Tiwari et al. 2015) and promoters (Tiwari et al. 2014, 2016, 2019; Singh et al. 2016) that enhanced photosynthesis (Patel et al. 2015; Jha et al. 2019) and also provide abiotic stress tolerance (Singh et al. 2014b; Pandey et al. 2016; Udawat et al. 2017; Mishra and Tanna 2017). Many significant abiotic stress tolerant plants with prospective interest for agriculture and environmental administration have already been identified, such as *Suaeda monoica* (Yadav et al. 2018), *Atriplex halimus* (Lutts et al. 2004), and *Salicornia bigelovii* (Zeraf et al. 2010). However, most of the halophytes plants have not been identified nor compared to conventional crop with known genetic information for genetic transformation. In future, halophytes would be used as an alternative approach to improvement of carbon assimilation and stress tolerance of C_3 crop plants using genetic engineering.

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References

- AbdElgawad H, Farfan-Vignolo ER, De Vos D, Asard H (2015) Elevated CO_2 mitigates drought and temperature-induced oxidative stress differently in grasses and legumes. *Plant Sci* 231:1–10
- Agarie S, Miura A, Sumikura R, Tsukamoto S, Nose A, Arima S, Miyao-Tokutomi M (2002) Overexpression of C_4 PEPC caused O_2 -insensitive photosynthesis in transgenic rice plants. *Plant Sci* 162(2):257–265
- Bandyopadhyay A, Datta K, Zhang J, Yang W, Raychaudhuri S, Miyao M, Datta SK (2007) Enhanced photosynthesis rate in genetically engineered *indica* rice expressing *PEPC* gene cloned from maize. *Plant Sci* 172(6):1204–1209

- Bowes G (1993) Facing the inevitable: plants and increasing atmospheric CO₂. *Annu Rev Plant Biol* 44:309–332
- Bräutigam A, Schlüter U, Eisenhut M, Gowik U (2017) On the evolutionary origin of CAM photosynthesis. *Plant Physiol* 174(2):473–477
- Chandler VL, Vaucheret H (2001) Gene activation and gene silencing. *Plant Physiol* 125:145–148
- Chaturvedi AK, Mishra A, Tiwari V, Jha B (2012) Cloning and transcript analysis of type 2 metallothionein gene (*SbMT-2*) from extreme halophyte *Salicornia brachiata* and its heterologous expression in *E. coli*. *Gene* 499:280–287. <https://doi.org/10.1016/j.gene.2012.03.001>
- Chaturvedi AK, Patel MK, Mishra A, Tiwari V, Jha B (2014) The *SbMT-2* gene from a halophyte confers abiotic stress tolerance and modulates ROS scavenging in transgenic tobacco. *PLoS ONE* 9:e111379. <https://doi.org/10.1371/journal.pone.0111379>
- Cheng G, Wang L, Lan H (2016) Cloning of PEPC-1 from a C₄ halophyte *Suaeda aralocaspica* without Kranz anatomy and its recombinant enzymatic activity in responses to abiotic stresses. *Enzym Microb Technol* 83:57–67
- Chi W, Zhou JS, Zhang F, Wu NH (2004) Photosynthetic features of transgenic rice expressing sorghum C₄ type NADP-ME. *Acta Bot Sin* 46(7):873–882
- Dengler NG, Nelson T (1999) Leaf structure and development in C₄ plants. In: Sage RF, Monson RK (eds) C₄ plant biology. San Diego, pp 133–172
- Edwards G, Walker D (1983) C₃, C₄: mechanisms, cellular and environmental regulation, of photosynthesis. University of California Press, Berkeley
- Edwards GE, Furbank RT, Hatch MD, Osmond CB (2001) What does it take to be C₄? Lessons from the evolution of C₄ photosynthesis. *Plant Physiol* 125(1):46–49
- Ehleringer JR, Monson RK (1993) Evolutionary and ecological aspects of photosynthetic pathway variation. *Annu Rev Ecol Evol Syst* 24(1):411–439
- Fahnenstich H, Saigo M, Niessen M, Zanor MI, Andreo CS, Fernie AR, Maurino VG (2007) Alteration of organic acid metabolism in Arabidopsis overexpressing the maize C₄ NADP-malic enzyme causes accelerated senescence during extended darkness. *Plant Physiol* 145(3):640–652
- Fukayama H, Tsuchida H, Agarie S, Nomura M, Onodera H, Ono K, Makino A (2001) Significant accumulation of C₄-specific pyruvate, orthophosphate dikinase in a C₃ plant, rice. *Plant Pathol* 127(3):1136–1146
- Fukayama H, Hatch MD, Tamai T, Tsuchida H, Sudoh S, Furbank RT, Miyao M (2003) Activity regulation and physiological impacts of maize C₄-specific phosphoenolpyruvate carboxylase overproduced in transgenic rice plants. *Photosynth Res* 77(2–3):227–239
- Furbank RT (2016) Walking the C₄ pathway: past, present, and future. *J Exp Bot* 67(14):4057–4066
- Furumoto T, Yamaguchi T, Ohshima-Ichie Y, Nakamura M, Tsuchida-Iwata Y, Shimamura M, Bräutigam A (2011) A plastidial sodium-dependent pyruvate transporter. *Nature* 476(7361):472
- Geissler N, Hussin S, Mervat MM, El-Far Koyro HW (2015) Elevated atmospheric CO₂ concentration leads to different salt resistance mechanisms in a C₃ (*Chenopodium quinoa*) and a C₄ (*Atriplex nummularia*) halophyte. *Environ Exp Bot* 118:67–77
- Gelvin SB (1998) The introduction and expression of transgenes in plants. *Curr Opin Biotechnol* 9:227–232
- Ghannoum O, Phillips NG, Sears MA, Logan BA, Lewis JD, Conroy JP, Tissue DT (2010) Photosynthetic responses of two eucalypts to industrial-age changes in atmospheric (CO₂) and temperature. *Plant Cell Environ* 33(10):1671–1681
- Gill SS, Tuteja N (2010) Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiol Biochem* 48:909–930
- Goodall GJ, Filipowicz W (1991) Different effects of intron nucleotide composition and secondary structure on pre-mRNA splicing in monocot and dicot plants. *EMBO J* 10:2625–2644
- Gu JF, Qiu M, Yang JC (2013) Enhanced tolerance to drought in transgenic rice plants overexpressing C₄ photosynthesis enzymes. *Crop J* 1(2):105–114
- Hatch MD (1987) C₄ photosynthesis: a unique blend of modified biochemistry, anatomy and ultrastructure. *Biochim Biophys Acta Bioenerg* 895(2):81–106
- Hattersley PW (1992) C₄ photosynthetic pathway variation in grasses (Poaceae): its significance for arid and semi-arid lands. In: Chapman GP (ed) Desertified grasslands: their biology and management. Linnean Society Symposium Series. Academic Press, London, pp 181–212
- Häusler RE, Rademacher T, Li J, Lipka V, Fischer KL, Schubert S, Hirsch HJ (2001) Single and double overexpression of C₄-cycle genes had differential effects on the pattern of endogenous enzymes, attenuation of photorespiration and on contents of UV protectants in transgenic potato and tobacco plants. *J Exp Bot* 52(362):1785–1803
- Häusler RE, Hirsch HJ, Kreuzaler F, Peterhänsel C (2002) Overexpression of C₄-cycle enzymes in transgenic C₃ plants: a biotechnological approach to improve C₃-photosynthesis. *J Exp Bot* 53:591–607
- Hudspeth RL, Grula JW, Dai Z, Edwards GE, Ku MS (1992) Expression of maize phosphoenolpyruvate carboxylase in transgenic tobacco: effects on biochemistry and physiology. *Plant Physiol* 98(2):458–464
- Hussin S, Geissler N, El-Far MM, Koyro HW (2017) Effects of salinity and short-term elevated atmospheric CO₂ on the chemical equilibrium between CO₂ fixation and photosynthetic electron transport of *Stevia rebaudiana* Bertoni. *Plant Physiol Biochem* 118:178–186
- IPCC, Stocker TF, Qin D, Plattner GK, Tignor M, Allen SK, Boschung J, Midgley PM (2013) Summary for policymakers in climate change: the physical science basis, contribution of working group I to the fifth assessment report of the intergovernmental panel on climate change. Cambridge University Press, Cambridge, p 1535
- Ishikawa N, Takabayashi A, Noguchi K, Tazoe Y, Yamamoto H, von Caemmerer S, Endo T (2016) NDH-mediated cyclic electron flow around photosystem I is crucial for C₄ photosynthesis. *Plant Cell Physiol* 57(10):2020–2028
- Ishimaru K, Ohkawa Y, Ishige T, Tobias DJ, Ohsugi R (1998) Elevated pyruvate, orthophosphate dikinase (PPDK) activity alters carbon metabolism in C₃ transgenic potatoes with a C₄ maize PPDK gene. *Physiol Plant* 103(3):340–346
- Jha B, Sharma A, Mishra A (2011) Expression of *SbGSTU* (tau class glutathione S-transferase) gene isolated from *Salicornia brachiata* in tobacco for salt tolerance. *Mol Biol Rep* 38:4823–4832. <https://doi.org/10.1007/s11033-010-0625-x>
- Jha RK, Patel J, Mishra A, Jha B (2019) Introgression of halophytic salt stress-responsive genes for developing stress tolerance in crop plants. In: Hasanuzzaman M, Shabala S, Fujita M (eds) Halophytes and climate change: adaptive mechanisms and potential uses. CABI, Wallingford, pp 288–299. <https://doi.org/10.1079/9781786394330.0275>
- Jiang Y, Xu Z, Zhou G, Liu T (2016) Elevated CO₂ can modify the response to a water status gradient in a steppe grass: from cell organelles to photosynthetic capacity to plant growth. *BMC Plant Biol* 16(1):157
- Jiao DM (2008) Redesigning C₄ rice from limited C₄ photosynthesis. In: Charting new pathways to C₄ rice, pp 145–162

- Johnston AM, Raven JA, Beardall J, Leegood RC (2001) Carbon fixation: Photosynthesis in a marine diatom. *Nature* 412(6842):40
- Joshi M, Jha A, Mishra A, Jha B (2013) Developing transgenic *Jatropha* using the *SbNHX1* gene from an extreme halophyte for cultivation in saline wasteland. *PLoS ONE* 8:e71136. <https://doi.org/10.1371/journal.pone.0071136>
- Kandoi D, Mohanty S, Tripathy BC (2016) Towards efficient photosynthesis: overexpression of *Zea mays* phosphoenolpyruvate carboxylase in *Arabidopsis thaliana*. *Photosynth Res* 130(1–3):47–72
- Karami O (2008) Factors affecting *Agrobacterium*-mediated transformation of plants. *Transgenic Plant J* 3:127–137
- Kersanskaya OI, Teixeira da Silva JA (2010) Photosynthetic basis for wheat crop improvement: genetic modification of photosynthesis. *Khazakistan plant science and biotechnology. Asian-Australas J Biosci Biotechnol* 4:27–34
- King JL, Edwards GE, Cousins AB (2012) The efficiency of the CO₂-concentrating mechanism during single-cell C₄ photosynthesis. *Plant Cell Environ* 35(3):513–523
- Ku MSB, Cho D, Ranade U, Hsu TP, Li X, Jiao DM, Ehleringer J, Miyao M, Matsuoka M (2000) Photosynthetic performance of transgenic rice plants overexpressing maize C₄ photosynthesis enzymes. In: Sheehy JE, Mitchell PL, Hardy B (eds) Redesigning rice photosynthesis to increase yield. Elsevier, Amsterdam, pp 193–204
- Laporte MM, Shen B, Tarczynski MC (2002) Engineering for drought avoidance: expression of maize NADP-malic enzyme in tobacco results in altered stomatal function. *J Exp Bot* 53(369):699–705
- Leakey AD, Ainsworth EA, Bernacchi CJ, Rogers A, Long SP, Ort DR (2009) Elevated CO₂ effects on plant carbon, nitrogen, and water relations: six important lessons from FACE. *J Exp Bot* 60(10):2859–2876
- Leegood RC (2002) C₄ photosynthesis: principles of CO₂ concentration and prospects for its introduction into C₃ plants. *J Exp Bot* 53(369):581–590
- Leegood RC, Lea PJ, Adcock MD, Häusler RE (1995) The regulation and control of photorespiration. *J Exp Bot* 46:1397–1414
- Lian L, Wang X, Zhu Y, He W, Cai Q, Xie H, Zhang J (2014) Physiological and photosynthetic characteristics of *indica* Hang2 expressing the sugarcane *PEPC* gene. *Mol Biol Rep* 41(4):2189–2197
- Lu ZS, Chen QS, Zheng QX, Shen JJ, Luo ZP, Fan K, Liu PP (2019) Proteomic and phosphoproteomic analysis in tobacco mosaic virus-infected tobacco (*Nicotiana tabacum*). *Biomolecules* 9(2):39
- Lutts S, Lefèvre I, Delpérée C, Kivits S, Dechamps C, Robledo A, Correal E (2004) Heavy metal accumulation by the halophyte species Mediterranean saltbush. *J Environ Qual* 33:1271–1279
- Maier A, Zell MB, Maurino VG (2011) Malate decarboxylases: evolution and roles of NAD (P)-ME isoforms in species performing C₄ and C₃ photosynthesis. *J Exp Bot* 62(9):3061–3069
- Matsuoka M, Fukayama H, Tsuchida H, Nomura M, Agarie S, Ku MSB, Miyao M (2000) How to express some C₄ photosynthesis genes at high levels in rice. *Stud Plant Sci* 7:167–175
- Matsuoka M, Furbank RT, Fukayama H, Miyao M (2001) Molecular engineering of C₄ photosynthesis. *Annu Rev Plant Biol* 52(1):297–314
- Minges A, Groth G (2017) Small-molecule inhibition of pyruvate phosphate dikinase targeting the nucleotide binding site. *PLoS ONE* 12(7):e0181139
- Mishra A, Tanna B (2017) Halophytes: potential resources for salt stress tolerance genes and promoters. *Front Plant Sci* 8:829. <https://doi.org/10.3389/fpls.2017.00829>
- Mitchell PL, Sheehy JE (2006) Super charging rice photosynthesis to increase yield. *New Phytol* 171(4):688–693
- Miyao M (2003) Molecular evolution and genetic engineering of C₄ photosynthetic enzymes. *J Exp Bot* 53(381):179–189
- Müller GL, Lara MV, Oitaven P, Andreo CS, Maurino VG, Drincovich MF (2018) Improved water use efficiency and shorter life cycle of *Nicotiana tabacum* due to modification of guard and vascular companion cells. *Sci Rep* 8(1):4380
- NASA (2014) Global climate change: vital signs of the planet. <https://climate.nasa.gov/400ppmquotes>. Accessed 4 Dec 2014
- Nelson T, Langdale JA (1992) Developmental genetics of C₄ photosynthesis. *Annu Rev Plant Biol* 43(1):25–47
- Nowak RS, Ellsworth DS, Smith SD (2004) Functional responses of plants to elevated atmospheric CO₂-do photosynthetic and productivity data from FACE experiments support early predictions. *New Phytol* 162(2):253–280
- O’Leary MH (1982) Phosphoenolpyruvate carboxylase: an enzymologist’s view. *Annu Rev Plant Physiol* 33(1):297–315
- Pandey S, Patel MK, Mishra A, Jha B (2016) In planta transformed cumin (*Cuminum cyminum* L.) plants, overexpressing the *SbNHX1* gene showed enhanced salt endurance. *PLoS ONE* 11:e0159349. <https://doi.org/10.1371/journal.pone.0159349>
- Patel J, Mishra A (2019) Genome editing: advances and prospects. In: Khurana S, Gaur R (eds) Plant biotechnology: progress in genomic era. Springer, Singapore, pp. 147–174. https://doi.org/10.1007/978-981-13-8499-8_7
- Patel MK, Joshi M, Mishra A, Jha B (2015) Ectopic expression of *SbNHX1* gene in transgenic castor (*Ricinus communis* L.) enhances salt stress by modulating physiological process. *Plant Cell Tiss Organ Cult* 122:477–490. <https://doi.org/10.1007/s11240-015-0785-4>
- Peng C, Xu W, Hu L, Li Y, Qi X, Wang H, Zhao M (2018) Effects of the maize C₄ phosphoenolpyruvate carboxylase (*ZmPEPC*) gene on nitrogen assimilation in transgenic wheat. *Plant Growth Regul* 84(1):191–205
- Pick TR, Bräutigam A, Schlüter U, Denton AK, Colmsee C, Scholz U, Weber AP (2011) Systems analysis of a maize leaf developmental gradient redefines the current C₄ model and provides candidates for regulation. *Plant Cell* 23(12):4208–4220
- Pinto H, Powell JR, Sharwood RE, Tissue DT, Ghannoum O (2016) Variations in nitrogen use efficiency reflect the biochemical subtype while variations in water use efficiency reflect the evolutionary lineage of C₄ grasses at inter-glacial CO₂. *Plant Cell Environ* 39(3):514–526
- Qi X, Xu W, Zhang J, Guo R, Zhao M, Hu L, Li Y (2017) Physiological characteristics and metabolomics of transgenic wheat containing the maize C₄ phosphoenolpyruvate carboxylase (*PEPC*) gene under high temperature stress. *Protoplasma* 254(2):1017–1030
- Qin N, Xu W, Hu L, Li Y, Wang H, Qi X, Hua X (2016) Drought tolerance and proteomics studies of transgenic wheat containing the maize C₄ phosphoenolpyruvate carboxylase (*PEPC*) gene. *Protoplasma* 253(6):1503–1512
- Rao X, Dixon RA (2016) The differences between NAD-ME and NADP-ME subtypes of C₄ photosynthesis: more than decarboxylating enzymes. *Front Plant Sci* 7:1525
- Reinfelder JR, Kraepiel AM, Morel FM (2000) Unicellular C₄ photosynthesis in a marine diatom. *Nature* 407(6807):996–999
- Ruan CJ, Shao HB, Teixeira da Silva JA (2012) A critical review on the improvement of photosynthetic carbon assimilation in C₃ plants using genetic engineering. *Crit Rev Biotechnol* 32(1):1–21
- Sage RF (2016) Tracking the evolutionary rise of C₄ metabolism. *J Exp Bot* 67(10):2919–2922
- Sage RF, McKown AD (2006) Is C₄ photosynthesis less phenotypically plastic than C₃ photosynthesis? *J Exp Bot* 57:303–317

- Sage RF, Pearcy RW (1987) The nitrogen use efficiency of C_3 and C_4 plants: I. leaf nitrogen, growth, and biomass partitioning in *Chenopodium album* (L.) and *Amaranthus retroflexus* (L.). *Plant Physiol* 84(3):954–958
- Sage RF, Pearcy RW, Seemann JR (1987) The nitrogen use efficiency of C_3 and C_4 plants: III. Leaf nitrogen effects on the activity of carboxylating enzymes in *Chenopodium album* (L.) and *Amaranthus retroflexus* (L.). *Plant Physiol* 85(2):355–359
- Sage RF, Sage TL, Kocacinar F (2012) Photorespiration and the evolution of C_4 photosynthesis. Kocacinar, photorespiration and the evolution of C_4 photosynthesis. *Annu Rev Plant Biol* 63:19–47
- Schuler ML, Mantegazza O, Weber AP (2016) Engineering C_4 photosynthesis into C_3 chassis in the synthetic biology age. *Plant J* 87(1):51–65
- Sen P, Ghosh S, Sarkar SN, Chanda P, Mukherjee AS, Datta K, Datta K (2017) Pyramiding of three C_4 specific genes towards yield enhancement in rice. *Plant Cell Tissue Organ Cult* 128(1):145–160
- Shen WJ, Chen GX, Xu JG, Jiang Y, Liu L, Gao ZP, Lv CF (2015) Overexpression of maize phosphoenolpyruvate carboxylase improves drought tolerance in rice by stabilization the function and structure of thylakoid membrane. *Photosynthetica* 53(3):436–446
- Sheriff A, Meyer H, Riedel E, Schmitt JM, Lapke C (1998) The influence of plant pyruvate, orthophosphate dikinase on a C_3 plant with respect to the intracellular location of the enzyme. *Plant Sci* 136(1):43–57
- Silvera K, Neubig KM, Whitten WM, Williams NH, Winter K, Cushman JC (2010) Evolution along the crassulacean acid metabolism continuum. *Funct Plant Biol* 37:995–1010
- Singh N, Mishra A, Jha B (2014a) Over-expression of the peroxisomal ascorbate peroxidase (*SbpAPX*) gene cloned from halophyte *Salicornia brachiata* confers salt and drought stress tolerance in transgenic tobacco. *Mar Biotechnol* 16:321–332. <https://doi.org/10.1007/s10126-013-9548-6>
- Singh N, Mishra A, Jha B (2014b) Ectopic over-expression of peroxisomal ascorbate peroxidase (*SbpAPX*) gene confers salt stress tolerance in transgenic peanut (*Arachis hypogaea*). *Gene* 547:119–125. <https://doi.org/10.1016/j.gene.2014.06.037>
- Singh VK, Mishra A, Haque I, Jha B (2016) A novel transcription factor-like gene *SbSDR1* acts as a molecular switch and confers salt and osmotic endurance to transgenic tobacco. *Sci Rep* 6:31686. <https://doi.org/10.1038/srep31686>
- Srinivasarao C, Kundu S, Shanker AK, Naik RP, Vanaja M, Venkanna K, Rao VUM (2016) Continuous cropping under elevated CO_2 : differential effects on C_4 and C_3 crops, soil properties and carbon dynamics in semi-arid alfisols. *Agr Ecosyst Environ* 218:73–86
- Suzuki S, Murai N, Kasaoka K, Hiyoshi T, Imaseki H, Burnell JN, Arai M (2006) Carbon metabolism in transgenic rice plants that express phosphoenolpyruvate carboxylase and/or phosphoenolpyruvate carboxykinase. *Plant Sci* 170:1010–1019
- Takeuchi Y, Akagi H, Kamasawa N, Osumi M, Honda H (2000) Aberrant chloroplasts in transgenic rice plants expressing a high level of maize NADP-dependent malic enzyme. *Planta* 211(2):265–274
- Taniguchi Y, Ohkawa H, Masumoto C, Fukuda T, Tamai T, Lee K, Miyao M (2008) Overproduction of C_4 photosynthetic enzymes in transgenic rice plants: an approach to introduce the C_4 -like photosynthetic pathway into rice. *J Exp Bot* 59(7):1799–1809
- Tiwari V, Chaturvedi AK, Mishra A, Jha B (2014) The transcriptional regulatory mechanism of the peroxisomal ascorbate peroxidase (*pAPX*) gene cloned from an extreme halophyte *Salicornia brachiata*. *Plant Cell Physiol* 55:1471–1774. <https://doi.org/10.1093/pcp/pct172>
- Tiwari V, Chaturvedi AK, Mishra A, Jha B (2015) Introgression of the *SbASR-1* gene cloned from a halophyte *Salicornia brachiata* enhances salinity and drought endurance in transgenic groundnut (*Arachis hypogaea*) and acts as a transcription factor. *PLoS ONE* 10:e0135541. <https://doi.org/10.1371/journal.pone.0131567>
- Tiwari V, Patel MK, Chaturvedi AK, Mishra A, Jha B (2016) Functional characterization of the tau class Glutathione-S-Transferases gene (*SbGSTU*) promoter of *Salicornia brachiata* under salinity and osmotic Stress. *PLoS ONE* 11:e0148494. <https://doi.org/10.1371/journal.pone.0148494>
- Tiwari V, Patel MK, Chaturvedi AK, Mishra A, Jha B (2019) Cloning and functional characterization of the Na^+/H^+ antiporter (*NHX1*) gene promoter from an extreme halophyte *Salicornia brachiata*. *Gene* 683:233–242. <https://doi.org/10.1016/j.gene.2018.10.039>
- Tsuchida H, Tamai T, Fukayama H, Agarie S, Nomura M, Onodera H, Toki S (2001) High level expression of C_4 -specific NADP-malic enzyme in leaves and impairment of photoautotrophic growth in a C_3 plant, rice. *Plant Cell Physiol* 42(2):138–145
- Udawat P, Mishra A, Jha B (2014) Heterologous expression of an uncharacterized universal stress protein gene (*SbUSP*) from the extreme halophyte, *Salicornia brachiata*, which confers salt and osmotic tolerance to *E. coli*. *Gene* 536:163–170. <https://doi.org/10.1016/j.gene.2013.11.020>
- Udawat P, Jha RK, Sinha D, Mishra A, Jha B (2016) Overexpression of a cytosolic abiotic stress responsive universal stress protein (*SbUSP*) mitigates salt and osmotic stress in transgenic tobacco plants. *Front Plant Sci* 7:518. <https://doi.org/10.3389/fpls.2016.00518>
- Udawat P, Jha RK, Mishra A, Jha B (2017) Overexpression of a plasma membrane-localized *SbSRP*-like protein enhances salinity and osmotic stress tolerance in transgenic tobacco. *Front Plant Sci* 8:582. <https://doi.org/10.3389/fpls.2017.00582>
- Vanaja M, Yadav SK, Archana G, Jyothi Lakshmi N, Ram Reddy PR, Vagheera P, Abdul Razak SK, Maheswari M, Venkateswarlu B (2011) Response of C_4 (maize) and C_3 (sunflower) crop plants to drought stress and enhanced carbon dioxide concentration. *Plant Soil Environ* 57(5):207–215
- Von Caemmerer S, Evans JR (2010) Enhancing C_3 photosynthesis. *Plant Physiol* 154(2):589–592
- Von Caemmerer S, Edwards GE, Koteyeva N, Cousins AB (2014) Single cell C_4 photosynthesis in aquatic and terrestrial plants: a gas exchange perspective. *Aquat Bot* 118:71–80
- Voznesenskaya EV, Franceschi VR, Kierats O, Freitag H, Edwards GE (2001) Kranz anatomy is not essential for terrestrial C_4 plant photosynthesis. *Nature* 414:543
- Wang J, Li R (2008) Integration of C_4 -specific *ppdk* gene of *Echinochloa* to C_3 upland rice and its photosynthesis characteristics analysis. *Afr J Biotechnol* 7(6):783–787
- Wang YM, Xu WG, Hu L, Zhang L, Li Y, Du XH (2012) Expression of maize gene encoding C_4 -pyruvate orthophosphate dikinase (*PPDK*) and C_4 -phosphoenolpyruvate carboxylase (*PEPC*) in transgenic *Arabidopsis*. *Plant Mol Biol Rep* 30(6):1367–1374
- Wang H, Liu C, Ma PA, Lu C, Li K, Wang W (2018) Functional characterization of cytosolic pyruvate phosphate dikinase gene (*MecyPPDK*) and Promoter (*MecyPPDKP*) of cassava in response to abiotic stress in transgenic tobacco. *Crop Sci* 58:2002–2009
- Washburn JD, Schnable JC, Davidse G, Pires JC (2015) Phylogeny and photosynthesis of the grass tribe Paniceae. *Am J Bot* 102:1493–1505
- Weber AP, Von Caemmerer S (2010) Plastid transport and metabolism of C_3 and C_4 plants comparative analysis and possible biotechnological exploitation. *Curr Opin Plant Biol* 13(3):256–264

- Winter K, Holtum JA (2014) Facultative crassulacean acid metabolism (CAM) plants: powerful tools for unravelling the functional elements of CAM photosynthesis. *J Exp Bot* 65(13):3425–3441
- Yadav S, Mishra A (2019) Introgression of C₄ pathway gene(s) in C₃ plants to improve photosynthetic carbon assimilation for crop improvement: a biotechnological approach. In: Ahmad P, Ahanger MA, Alyemeni MN, Alam P (eds) *Photosynthesis, productivity, and environmental stress*. Wiley, Hoboken, pp. 267–282. <https://doi.org/10.1002/9781119501800.ch13>
- Yadav S, Mishra A, Jha B (2018) Elevated CO₂ leads to carbon sequestration by modulating C₄ photosynthesis pathway enzyme (PPDK) in *Suaeda monoica* and *S. fruticosa*. *J Photochem Photobiol* 178:310–315. <https://doi.org/10.1016/j.jphotobiol.2017.11.022>
- Zai-Song DING, Bao-Yuan ZHOU, Xue-Fang SUN, Ming ZHAO (2012) High light tolerance is enhanced by overexpressed PEPC in rice under drought stress. *Acta Agron Sin* 38(2):285–292
- Zerai DB, Glenn EP, Chattervedi R, Lu Z, Mamood AN, Nelson SG, Ray DT (2010) Potential for improvement of *Salicornia bigelovii* through selective breeding. *Ecol Eng* 36:730–739
- Zhang J, Datta SK, Wang G, Xie H (2007) Integration of C₄-specific PPDK gene of maize to C₃ rice and its characteristics in relation to photosynthesis. *Front Agric China* 1(3):243–249
- Zhou BY, Ding ZS, Zhao M (2011) Alleviation of drought stress inhibition on photosynthesis by overexpression of PEPC in rice. *Acta Agron Sin* 37(1):112

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