Developing C4 Rice: A Physiological Perspective in Relation to Climate Change

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Abstract

In developing countries in Asia, Africa and Latin America, rice cultivation is the principal activity and is also the source of income for more than 100 million households. In view of the yield plateauing, conversion of metabolic pathway of C3 to C4 photosynthesis is being considered as one of the possible routes to increase productivity of rice crop. Three distinct photosynthetic mechanisms were identified in higher plants viz, C3, C4 and CAM. C4 grasses dominate tropical and subtropical grasslands and savannas, and C3 grasses dominated the world's cooler temperate grassland regions. Genes encoding enzymes of the C4 cycle often belong to gene families having C4PEPC gene and other non-C4 is forms are known. C4 pathway is not yet reported in any Oryza species though rice is of tropical origin. Such a pathway may be present in the biologically diversified wild species of rice or in a near relative of rice. Transgenic rice exhibited reduced O_2 inhibition of photosynthesis, but this was most likely due to effects of phosphate recycling that affect photorespiration. Over expression of an unregulated phosphoenol pyruvate carboxykinase (PCK) from C4 plant, Urochloa panicoides, in the chloroplasts of rice leaf has also been done. Over expressing some of these C4-related genes in rice showed diverse effects, in upward and down regulation but with little impact on yield. Results suggest that the introduction of C4 photosynthesis enzymes into rice has a good potential to enhance its tolerance to stress, photosynthetic capacity, and yield, Hence engineering a C4 rice may be critical to meeting production goals within the next half-century. While our information of the constituent reactions of photosynthesis is well understood, their incorporation into the whole plant process is not. There is an imperative need for an increased understanding of the fundamental processes at the molecular level, and the assimilation of this knowledge to appreciate how a plant works. This understanding needs to include the multifaceted interactions amongst canopy architecture, the growth and development of a leaf, and the biochemistry of the photosynthetic apparatus in addition to just incorporating C4 enzymes in the rice plant. In this review article possibility of converting rice from C3 to C4 photosynthetic pathway are discussed.

Keywords: Rice, climate change, photosynthetic pathways, C3, C4, CAM, C4-related genes

Introduction

Human made climate change adversely affect world food grain production. Population of the world is expected to reach 9 billion by 2050 and food demand will increase. Besides food scarcity, malnourishment will reach alarming levels, especially amongst economically poor. The demand is expected to be at least 40% more than that of current production. Many scientists, economists and policy makers now agree that the world is facing a threat from climate warming. Though, the degree of the impact and its distribution is still debated, with reference to the vulnerable or non-vulnerable regions, the ultimate damage is in the guise of yield losses. With aid of technological interventions in computers and information technology, several models of climate changes and scenarios for each of the continent are studied and information for each of the continent and/or regions is available. Climate change, weather and microclimate are not the same though all of them are interrelated to each other. Weather is variable in a day is hot, cold or wet a place is at a particular time i.e., temperature, relative humidity or rainfall, which have limited or localized influence on crop yields. Climate change is the average weather of an area over period of time, not less than a decade that has a larger influence on various weather parameters which consistently exhibit a progressive increase or decrease or remain static. The effects of climate change are critical as the productivity of crop and food sustainability are adversely affected. Thus, the things that decide climate are relatively different from that of weather in the sense that, how far an area is from that of equator (latitude), sea and altitude and prevalent wind system. This means the position of the land and area is influenced by climate. Based on these, the world area has been delineated into different climatic zones, such as polar, tundra, temperate, tropical, desert and mountains. For instance, cereal crops like rice, wheat, maize are differentially influenced based on the type of abiotic stress and natural resources that are prevailing in these climatic zones which could range from 30% reduction of crop yields in Central and North Africa to 14% in sub-Saharan region for the same crop. Wheat production is predicted to be reduced to 50% in South Asia and to 6% in Latin America (http://siteresources.worldbank.org/Resources_Agriculture_Climate_change). All these predictions are necessitated based on the current available resources that are considered to be static. The natural vagaries of climate are unpredictable, as are the availability of natural resources at a given time. Therefore, crop yields may actually be far lower if the climate change continues at the same rate.

Climates have changed naturally throughout the history. Human activities such as burning fossil fuels produce greenhouse gases that cause global warming. The effects include altering of ocean currents and ice sheets and rising of sea level and increasing frequency or abnormal occurrence of severe weather condition such as drought, cyclone or flood. These aberrations are abrupt rather than early or late arrival of monsoons during normal weather situation at a given location where the crop activities can be adjusted periodically based on the weather predictions. In these crop based models (Abrol and Gadgil 1999; Nareshkumar and Agarwal 2011), transpiration, a biophysical process contributes significantly for yield prediction of the locally dominant crop. But, yield predictions by global climate models (Cayan et al 2008), largely considers the heat balances based on the evaporation that leads to changes in the direction of clouds, rainfall, wind, and temperatures. Universally, it is established that yield losses do occur to varied intensities.

Contributing factors to climate change

The atmospheric concentration of carbon dioxide has increased approximately by 30% since the mid-18th century, and projections indicate that CO_2 could increase from current levels of approximately 360 ppm to between 540 and 970 ppm by the end of the 21st century (IPCC 2007). Atmospheric concentrations of other greenhouse gases like methane, tropospheric ozone, nitrous oxide, chloro-fluoro-carbons etc, have also increased as a result of anthropogenic activities. The climatic elements which affect plant growth and development viz, atmospheric CO_2 levels, temperature, precipitation, radiation, humidity and wind speed are likely to be altered with the increased emission of greenhouse gases. In India, the mean annual air temperature for the period 1901–1988, as represented by 73 stations, revealed warming by 0.4^0 C, which is comparable to the global trend of 0.5^0 C in the intervening period (http://www.ifpri.org; Subrahmanyam et al 2011).

Rice, C4 pathway and climate change

Rice cultivation is the principal activity and source of income for more than 100 million households in developing countries in Asia, Africa and Latin America. The cultivation of rice extends from drylands to wetlands and from the banks of the Amur River at 53° north latitude to central Argentina at 40° South latitude. Rice is also grown in cool climates at altitudes of over 2600 m above sea level in the mountains of Nepal, as well as in the hot deserts of Egypt (http://www.fao.org/climatechange/). Rice breeding has accomplished extraordinary achievements in the past half-a-century, due to two advances: rising harvest index in semi-dwarf rice varieties and developing heterotic hybrids. Mammoth challenges on yield pressure due to increases in global population together with reductions in arable land area worldwide and genetic diversity in rice cultivars are a few critical issues to meet with the food demand. In view of the yield plateauing, conversion of metabolic pathway of C3to C4 photosynthesis is being considered as one of the possible routes to increase productivity of rice crop. Three distinct photosynthetic mechanisms were identified in higher plants viz., C3, C4 and CAM (Tieszen 1983) (Table1). C3 plants use a Calvin Benson cycle characterized by the use of ribulose biphosphate carboxylase oxydase (RUBISCO) while C4 plants use a Hatch-Slack cycle by phosphoenolpyruvate carboxylase (PEPC). The third metabolism known as Crassulacean acid metabolism (CAM) has both C3 and C4-like characteristics depending on growth environment such as light intensity. C3 photosynthetic pathway is common in all the three groups, while CAM and C4 share a common characteristic of primary product of C4 acid but distinguishable by spatial and temporal means of biochemical activity. Presence of a common C3 photosynthetic pathway in these plants indicates the adaptation and evolution of the metabolic pathways as elucidated by using the modern tools of carbon discrimination and others. Besides a conversion of photosynthetic pathway from C3 to C4, climate changes necessitate investigations on other related pathways i.e. light reactions, synthetic processes such as biomass, water use efficiency, nutrition and hormone metabolisms. All activities of plants depend on their genetic background and environment under which they are grown. It is not known, if a change in photosynthetic process in rice alone is sufficient for resilience to climate change. Present CO_2 levels are sufficient enough to carry the carboxylation process of RUBISCO in rice.

Table 1. Features of C3, C4 and CAM Characteristics of higher plants (Modified from Tieszen 1983)

Character	C3	C4	CAM
Leaf Thickness	Thin to Medium	Thin to Medium	Succulent
Anatomy	Mesophyll Diffuse	BS Krantz	Mesophyll
Inter veinal distance	Large 5 Chlorenchyma	Small (<4 cells)	
Chloroplasts	Mono morphicgranal	Dimorphic	Monomorphic
PrimaryCO_2 enzyme	RUBISCO	PEPC	PEPC
Secondary	None	RUBISCO	RUBISCO
Primary substrate	RUBP	PEP and RUBP	MDH
CO_2 product	3 PGA	OAA and others	OAA and others
ATP/NADPH	1.3:2	1.5:2	1.5:2
Transpiration rate	High in saturated atmosphere	Low: Unsaturated atmosphere	Saturated -1/4th
Light compensation	5 W.M-2	Full intensity	High intensity
Photorespiration	Present and high	Little	Little
O_2 inhibition	Present	little	
CO_2compensation point	50 ppm (15-150 ppm)	<5 ppm	
CO_2 evolution in light	High	Low	
Maximum photosynthetic rate	15-35 mg CO_2 /dm^2/h	~35-40	20-25
Stomatal conductance	Similar	Similar	Similar
Mesophyll conductance	Low	High	High
Water use efficiency	Low	High	Very high
Nitrogen use efficiency	Low	High	Not Known
Quantum of yield	High at low temperature	Independent	Independent
Canopy conversion efficiency	3-3.4%	3-4.5%	
Optimum temperature	15-25	25-35	25-35
Km (Michaelis- Menton Constant) for CO_2,	13-26 µicro m	28-60 µicro m	
Enzyme size	Large	Small	
Translocation rates	Slow	Rapid	
Starch accumulation	Normal in mesophyll	Normal in PEP	
C Discrimination	-28 to -23%	-12.5 to -9%	
Climatic adaptation	Temperate	Tropical	Arid tropical
Carbonic anhydrase	High in mesophyll	High location uncertain	

The influence of any further increase CO_2 levels on the efficiency of RUBISCO due to competitive inhibition is also not known. There is a need to understand on the required modifications in the dynamic physiological processes, stomatal resistance and gaseous fluxes in view of climate change, particularly in the context of a conversion of C3 to C4 rice.

Despite the controversy regarding the contributions on photosynthesis (El–Sharkawy 2009), the progress achieved in elucidating “The Benson-Calvin-Bassham cycle” i.e. commonly known as C3 cycle led to evolution of C4 syndrome (Karpilov 1960; Hatch and Slack 1966). Biotechnological tools came handy for resurgence of interest in photosynthesis, due to certain advantages believed to be associated with C4 systems. The relation between the partial pressure of atmospheric carbon dioxide (pCO_2) and Paleogene climate was poorly resolved. Pagani et al (2005) used stable carbon isotopic values of di-unsaturated alkenones extracted from deep sea cores to reconstruct pCO_2 from the middle Eocene to the late Oligocene (~45 to 25 million years ago). Results demonstrated that pCO_2 ranged from 1000 to 1500 parts per million by volume in the middle to late Eocene, then decreased in several steps during the Oligocene, and reached modern levels by the latest

Oligocene. The fall in pCO_2 likely allowed for a critical expansion of ice sheets on Antarctica and promoted conditions that forced the onset of terrestrial C4 photosynthesis. The relation between the partial pressure of atmospheric carbon dioxide (pCO_2) and Paleogene climate is poorly resolved. Declining CO_2 levels and temperature factors facilitated evolution of C4 (Ehleringer et al 1997). Evolved first in the tropics moved to north when CO_2 levels dropped to 250 ppm in the Miocene. However, the phylogeny failed alongside climate data for each of the species originated but was correlated with marked reduction in annual rainfall and were exhaustively reviewed (Langdale 2011: Hibberd et al 2008).

Other reports suggest that, drought is not a factor but has been co evolved between Miocene to Pliocene periods during which time, grass grazing animals dominated (Osborne 2011). The results of long term experiments of CO_2 on C3 predicted and favoured productivity but in combination with temperature favoured C4 plants (Morgan etal 2011). Paradoxically the advantages of Pn are reduced in C4 under drought (Lara et al 2008; Taylor et al 2011) but, in maize C4 is reported to be advantageous (Leakey et al 2004).

C4 grasses dominate tropical and subtropical grasslands and savannas, and C3 grasses dominated the world's cooler temperate grassland regions (Edwards and Smith 2010). Genes encoding enzymes of the C4 cycle often belong to gene families having C4 phosphoenol pyruvate carboxylase (PEPC) gene and other non-C4 isoforms are known. Apart from the single cell systems, the two cell angiosperms 62 C4 taxa, 36 eudicots, 6 hedges, 18 grasses and 2 aquatic lineages in the genera *Hydrilla* and *Egeria* (Sage et al 2011). Christin et al (2010) proposed that C4 pathway originated from C3 independently several times. Of these, 58 lineages have Krantz anatomy. In total there are 7500 C4 species of which 4500 are grasses (Sage et al 2011). Independent origin of the C4 pathway within a number of angiosperm families imply that its evolution might not be as intricate as assumed, possibly signifying that there may have been genetic predisposition in some C3 plants to C4 evolution.

Single celled aquatic systems such as *Hydrilla vercillata*, *Egeria densa* and *Elodea canadensis* marine *Udotea flabellum,* exhibit C4 pathway under certain environmental conditions. Organized network of microtubules and actin filaments are required for the formation of chlorenchyma cells in aquatic single celled C4 species (Chuong et al 2006). In *Suaeda* species, the distal and proximal (single cell) dimorphism is apparent which at initial stages does not appear. Thus, ecological drivers such as declining CO_2, increased temperatures and periods of drought have been associated with C4 evolution. In different genera, the morphological, physiological and molecular changes must necessarily be associated with biochemical changes of other metabolic pathways such as nitrogen, water relations of plants and also hormonal pathways which regulate the synthetic processes.

Hydrilla, *Elodea* or *Egeria* plants are scattered in winter while in summer dense mats are formed reducing CO_2 availability. At this point of time, they behave like C4 species. Phosphoenol pyruvate (PEP) is cytosolic and no structural change is observed in these single celled systems. In *Moricandia*, the cell thickness is similar to C4 plants. *Eloecharis* under submerged situation acts like C3 and when it is aerial acts as C4 and is regulated by abscissic acid (ABA). Elevated CO_2 leads to decreased N content in N limited environment and its effects are not consistent with nitrate or ammonium. Photosynthesis acclimation is more in N limited plants. As a consequence stimulation of plant growth is decreased. In marine macro algae *Udotea flabellum*, diatom *Thalassiosira weissflogil* and chaenopods *Binertia* and *Suaeda*-single cells, and in several angiosperms, C4 variants as three sub-types viz, NADP-malic enzyme (ME), NAD- ME and asapartate (ASP) are observed where malate/aspartate is transported out of the mesophyll cells to BS for CO_2 fixation. The pyruvate generated is transferred back to mesophyll by pyruvate ortho phosphate dikinase (PPdk) and or PEP to oxaloacetic acid (OAA) formation via Carbonic anhydrase (CA) in bundle sheath (BS) and transported to cytoplasm of mesophyll.

C3-C4 intermediates

In *Flaveria,* C3, C3-C4 intermediate, C4 like and C4 species have all been identified (Ku et al 1983). Other intermediates such as *Moricandia arvensis* are altogether a different family with no known C4 species. In *Eloecharis vivipara* (Ueno 1998) and in *Flaveria*, brownie induction of C4 developmental transition occurs due to abscissic acid, submergence to aerial position or light intensity. In maize too such cues are seen wherein radiation plays a pivotal role in formation especially in cells that are within two cell radius of a vein (Langdale and Nelson 1991). Among the various physiological processes of C3 and C4 groups, carbon nitrogen balance in association with water relation of rice is the most critical issue which has evoked interest with modern biotechnological tools employed aimed to understand the complexity of the C3 C4 metabolism (Kajala et al 2011). C4 pathway is not yet reported in any *Oryza* species though rice is of tropical origin. Such a pathway may be present in the biologically diversified wild species of rice or in a near relative of rice.

In C4 species, it is well known that enrichment of CO_2 at the site of RUBPC minimizes oxygenation and enhances N use efficiency thereby photorespiration. Possibly, simple transitions require modifications associated with manifestations at various levels, architecture, Calvin-Hatch cycles, protein translation, photorespiration, cell-to-cell connections and organelle environments within a cell. Approximate gain of 40% photosynthetic outputs and higher water use efficiency (WUE) at the cost of photorespiration makes C4 more efficient. But C4 plants demand for development of specialized leaf anatomy, compartmentalization and transport processes that require sophistication from protein functions and gene expression.

Phenotype, morphological and anatomical modifications

Physically, there appears to be no difference in leaf structures such as parallel venation but for the thickness. Leaf thickness could be manipulated with external inputs and environment, Nitrogen is known to enhance the leaf thickness and the also the chlorophyll content. However, at cell and organelle levels, it has been reported that peroxisomes, mitochondria and chloroplasts are in neighbourhood in the mesophyll cells (MC) of C4 plants. But, in bundle sheath cells (BS) cells, such organization is not established. This seems to facilitate the transport of products to and fro into different regions for further processing in the mesophyll cells. Such organelle arrangement may be important to isolate the various by-products that originate from both photosynthesis and photorespiratory cycles. Consistently more closely spaced veins are observed in C4 with a ratio of 1:1 between BS and MS (Muhaidat et al 2007). These veins are separated by 4 (2 from each side) in C4 species as opposed to 20 in C3 species. Induction of procambium at more regular intervals for vein formation appears to be a prerequisite for Krantz anatomy. Likely role of auxin in establishing the vascular system is indicated. Systems analysis of maize leaf development provided candidates for regulation (Pick et al 2011).

C3 and C4 species of *Atriplex* were hybridized, the independent inheritance of genes conferring C4 characteristics such as Krantz anatomy, elevated PEPC and low CO_2 compensation point indicated that plant breeding was unlikely to be a successful route. Hydraulic integrity needs to be enhanced which is high in BS and particularly in hot and arid environments. This is a pre-adaptive feature for climate. Phosphoenol pyruvate carboxylase (PEPCase) release CO_2 not bicarbonate and carbonic anhydrase presence in mesophyll cells in these plants is yet to be established. To introduce C4 pathway into C3 plant, an active Pn driven CO_2 concentrating mechanism, CO_2 capturing system, supply of energy for CO_2 reduction, an intermediate pool of captured CO_2, a mechanism to release CO_2 from intermediate pool, a compartment in which to concentrate CO_2 from RUBISCO, a means to reduce leakage of CO_2 from the site of elevation, and modification of kinetic properties of RUBISCO are required (Leegood 2002).

Biochemistry

In C3 plants, the C4 pathway plays house-keeping roles (Aubry et al 2011). Ex; CA and PEPCase and is used to provide C skeletons to TCA cycle and for ammonium assimilation (Masumoto et al 2010). PEP CK mobilizes sugars from lipids in seeds during germination (Leegood and ap Rees 1978). Provision of PEP for Shikkimate pathway, and metabolism of nitrogenous compounds, PPDK generated PEP has also been shown to contribute to seed metabolism. Thus, C4 biochemistry evolved through modifications of existing functions rather than de novo. Mutant of photorespiration for glycolate oxidase in maize was lethal at ambient CO_2 since glycolate build is toxic and this mutant survives under high CO_2. Glycolate decarboxylase is the first step of catalyzed for release of CO_2 in to BS cells which may be first step in C4 for CO_2 enrichment.

Metabolite transfer

BS cell wall prevents CO_2 leakage is thought, however NAD-ME (malic enzyme) type have no suberized cells walls and therefore, positional roles of chloroplasts and mitochondria comes to play in these exceptional circumstances. In C3 plants one transport process has to occur across chloroplasts envelope for every three CO_2 molecules assimilated into triose phosphate. By contrast 30 transport steps are required per triose phosphate (TP) generated in NADP-ME C4 plants. The difference has implications in terms of energetic costs of Photosynthesis, the establishment of plasmodesmata connections and the proteins that are to be modified in C4 evolution. Two transporter viz, TP and PEP/phosphate translocator, candidates for M cell malate/ oxaloacetate (OAA) antiporter and for a sodium –dependent pyruvate transporter have been identified though several of the metabolites are transported between the BS and MS cells. Quantitative and cell specific proteomic data and functional assays for identifying these are only a matter of time as reviewed (Langdale 2011).

The key feature of NADP-ME subtype is movement of malate and pyruvate between mesophyll and BS cells and decarboxylation of malate in BS in BS chloroplasts. The second variants NAD-ME PEP CK- sub types move aspartate or alanine to form malate or OAA for undergoing decarboxylation in BS cell cytoplasm. These two are characteristically require more transport steps and therefore more energy required.

Other physiological processes

The evolutionary significance of photosynthetic processes is related to the radiation use efficiencies in C3 and C4 plant species. The C3 plant rice has been reported to be low in radiation use efficiency (Raghuveerrao et al 2012) ranging between 2-5% while maize a C4 species had greater than 5% RUE. Thus, a relationship between the biomass in relation to RUE is existed. It should be noted that, species comparison of different genetic back grounds could also be determining the physiological efficiencies of plants rather individual processes. This was mostly due to complexities associated with other metabolic processes such as nitrogen investment in the various enzymes, sulphur and water use efficiencies as well (Friso et al 2010). Nitrogen required is 8% in C4 while it is 20% in C3 (rubisco) leading to much higher proportion of N required per CO_2 fixed. Decreased stomatal conductance increased WUE are known to be relatively higher in C4 plants compared to that of C3 plants particularly at higher CO_2 levels. Relatively higher physiological efficiencies of C4 plants have been proposed to be associated with biomass production as well as yield. However, it seems that, various physiological pathways are directly related to evolutionary significance rather than the biomass production and yield. So far, evidences linking the biomass production and yield were not shown to be related to C4 process since, most of the C3-C4 intermediate species such as *Flaveria, Panicum* species, or even the single celled algal species or expression of some of the genetically modified transgenics were reported to have either higher biomass or yield. Thus, the excitement did not reflect in terms of yield correlations with biomass.

Some advantages of C4 biochemistry

Two distinctive cell types, mesophyll and bundle-sheath: Firstly, CO_2 assimilation is carried out in mesophyll cells. The chief carboxylating enzyme, phosphoenol pyruvate carboxylase (PEPC), in concert with carbonic anhydrase (CA) is vital to create rapid equilibrium between $CO_2$and HCO_3^-.This step is responsible for the hydration and fixation ofCO_2 to produce the C4 acid, oxaloacetate. In NADP-ME-type C4 species, oxaloacetate is then converted to another C4 acid - malate, catalyzed by malate dehydrogenase (MDH). Malate then diffuses into chloroplasts in the proximal bundle-sheath cells, where CO_2 is released to yield pyruvate by the decarboxylating NADP-ME. The released CO_2 concentrates around the secondary carboxylase-Rubisco, and is re-assimilated by it through the Calvin cycle which is a C3 pathway. Pyruvate is transferred back into mesophyll cells and catalyzed by pyruvate orthophosphate dikinase (PPDK) to regenerate the primary CO_2 acceptor, PEP. Phosphorylation of a conserved serine residue close to the amino-terminal end of the PEPC polypeptideis important to its activity by reducing sensitivity to the feedback inhibitor malate and a catalyst named PEPC kinase (PPCK). This process which is called in a nutshell the “C4 photosynthesis” results in more efficient carbon assimilation at high temperatures because its combination of morphological and biochemical features reduce photorespiration, a loss of CO_2 that occurs during C3 photosynthesis at high temperatures. PPDK regulatory protein (PPDKRP), a bifunctional serine/threonine kinase-phosphatase, catalyzes both the ADP-dependent inactivation and the Pi dependent activation of PPDK (Hibberd 2009; Tiwari et al 2005; Yokota and Shigeoka 2008).

Water equilibrated at normal atmospheric pressure dissolves 11-mM CO_2, which forms 110-mM HCO_3^- at pH 7.2 and 25^0C. While RuBisCO fixes CO_2, phosphoenol pyruvate carboxylase (PEPC) uses HCO_3^- as the substrate. This distinguishing feature bestows a remarkable benefit to C4 plants. Since the Km for HCO_3^- of maize PEPC is as low as 20 mM, this enzyme can show evidence of sub-maximal activity in the mesophyll cytosol. The active C4 operation as an auxiliary metabolic CO_2-pumping system confers significantly better nitrogen investment and water-use efficiencies to C4 plants compared with C3 plants. If this CO_2-pumping system could be introduced into C3 plants, the transgenic plants would be expected to show highly improved photosynthetic performance and productivity.

Current status of engineering C4 pathway in rice

Classical Kranz demands an examination of photosynthetic characteristics in C3 plants with altered leaf morphology (Hibberd et al 2008). Introducing a single enzyme such as PEP carboxylase and others (Table 2) in rice had 12% more expression of total leaf-soluble protein but altered stomatal conductance than *per se* Pn rate. (Bandyopadhyay et al 2007; Chen et al 2009). Nevertheless, some confirmation suggests that the manipulations have led to the desired redirection of fluxes. Although overexpressing these C4-related genes in rice showed diverse effects, in upward and down regulation with little impacts on yield. For instance, ME resulted in stunting, leaf chlorophyll bleaching, and enhanced photo-inhibition of photosynthesis (Takeuchi et al 2000; Tsuchida et al 2001) Combinations of multiple C4-related genes. Transgenic rice exhibited reduced O_2 inhibition of photosynthesis, but this was most likely due to effects of phosphate recycling that affect photorespiration.

Table 2. C4 Gene incorporations in rice

Gene source	Gene	Gene effect	Reference
Maize increased net	*Phospho Pyruvate Dikinas (PPDK)*	Photosynthesis and decreased photorespiration in transgenic plants	Ji et al (2004)
Maize	*NADP-Malic Enzyme*	Photorespiration rate decreased and net photosynthetic rate increased in transgenic plants	Ji et al (2004)
Maize	*Phosphoenol pyruvate carboxylase (PEPC)* + *PPDK*	Increased net photosynthesis and decreased photorespiration in transgenic plants	Ji et al (2004)
Maize	*PEPC*	Photorespiration rate decreased and net photosynthetic rate increased in transgenic plants	Ji et al (2004)
Urochloa panicoides	*PCK (pyruvate carboxy kinase)*	Threefold greater sucrose synthesis was observed in transgenic plants than in control plants	Suzuki et al (2000)
Maize	*PEPC*	Transgenic plants exhibited a higher photosynthetic capacity (up to 35%) than untransformed plants. The increased photosynthetic capacity in these plants was mainly associated with an enhanced stomatal conductance and a higher internal CO_2 concentration	Ku et al (2007)
Maize	*PPDK*	A higher photosynthetic capacity (up to 35%) than untransformed plants	Ku et al (2007)
Maize	*PEPC*	Transgenic C4 plants were 30–35% more efficient in photosynthesis	Ku et al (1999)
Maize	*PEPC*	Photosynthetic capacity was increased greatly (50%) under high CO_2 supply. In CO_2-free air, CO_2 release in the leaf was less. In addition, transgenic rice was more tolerant to photoinhibition	Jiao et al (2005)
Maize	*NADP-ME +* *PPDK*	Photosynthetic rate was increased by 50%	Jiao et al (2002)

Overexpression of an unregulated phosphoenol pyruvate carboxykinase (PEPCK) from C4 plant, *Urochloa panicoides*, in the chloroplasts of rice leaf has also been done. In $^{14}CO_2$-labeling experiment, up to 20% of the radioactivity was incorporated into C4 acids (malate, oxaloacetate, and aspartate) in leaves of transgenic plants. PEPC transgenic rice plants were capable of keeping a higher photosynthetic rate, a higher photosynthetic quantum yield by PSII, and a higher capacity to dissipate excess energy photochemically and non-photochemically than untransformed plants under photoinhibitory and photooxidative conditions. Moreover, the grain yield of transgenic rice plants with PEPC and PPDK were higher than those of untransformed plants. These results suggest that the introduction of C4 photosynthesis enzymes into rice has a good potential to enhance its tolerance to stress, photosynthetic capacity, and yield (Zhang et al 2009).

Build up of NADPH caused distended and swollen thylakoid membranes and low chlorophyll concentrations in PEPC/ PCK-expressing transgenic rice plants with low photosynthetic CO_2 assimilation rates (Suzuki et al 2006). Photosynthetic rate and its relationship with the transgenic rice expressing C4 enzymes are of prime importance for yield realization. Significant increase in the photosynthesis rate in the transgenics at high temperature have been observed but without any improvement of yield (Bandyopadhyay et al 2007). Thus, it raises further questions whether the other C4 genes encoding the enzymes PPDK, NADP-ME and PEPCK should be introduced in one genome in addition to the PEPC either by transgene-breeding or by multigene transformation to get overall increase in yield in the natural field conditions.

Future outlook

C4 rice research is very laborious due to huge distances of anatomy and genetics between C3 and C4 plants. However, it is still valuable as an attempt to change the present status that rice yield has been hovering for a long period initial studies should be designed to investigate the mechanistic interface between leaf morphology and metabolism in C3 and C4 leaves (Zhang et al 2007). Developing climate resilient rice genotype or C4 pathway in rice by introduction of genes is still a long way. Although C4 rice may confer some advantage, predominantly in drought-prone upland settings, an imperative alternative to C4 rice is a C3 rice variety that is better-designed for a high CO_2 environment. It would possibly be easier to obtain a high-CO_2-adapted C3 rice variety than C4 rice, as this would involve simple changes in regulatory points, rather than engineering key changes in leaf anatomy and physiology. Considerable dissimilarity already exists in the response of rice to elevated CO_2 and many of these traits could be co-opted to engineer superior cultivars for future high CO_2 atmospheres. The genetics of CO_2 responsiveness is rapidly being explained, as long as a blueprint for the types of changes that can be exploited to design rice plant that is optimized for high CO_2. Currently, CO_2 levels are rising at slightly less than 2 ppm per year. If we take for granted they will rise over the next 50 years by an average of 3 ppm per year, the atmospheric CO_2 level will be 520 ppm in 2050. This may not be high enough to allow for C3 plants to yield more than C4 plants, even with C3 plants that are adapted to higher CO_2. Hence engineering C4 rice may be critical to meeting production goals within the next half-century. While our information of the constituent reactions of photosynthesis is well understood, their incorporation into the whole plant process is not. There is an imperative need for an increased understanding of the fundamental processes at the molecular level, and the assimilation of this knowledge to appreciate how a plant works. This understanding needs to include the multifaceted interactions amongst canopy architecture, the growth and development of a leaf, and the biochemistry of the photosynthetic apparatus in addition to just incorporating C4 enzymes in the rice plant.

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