

Original Research Article

Mitigating Photorespiration and Enhancing Yield by developing C₄ Rice (*Oryza sativa* L.)

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ABSTRACT

In order to provide adequate nutrition into the future, rice yields need to be increased by 60%, a change that may be achieved by introduction of the C₄ photosynthetic cycle into rice. The international C₄ Rice Consortium was founded in order to test the feasibility of installing the C₄ engine into rice. This can be achieved by two way metabolic C₄ engineering and identification of determinants of leaf anatomy by screens them for higher vein density. The aim of the metabolic C₄ engineering approach is to generate a two-celled C₄ shuttle in rice by expressing the classical enzymes of the NADP-ME C₄ cycle in a cell-appropriate manner. The aim is also to restrict RuBisCO and glycine decarboxylase expression to the bundle sheath (BS) cells of rice in a C₄-like fashion by specifically down-regulating their expression in rice mesophyll (M) cells. In addition to the changes in biochemistry, two-celled C₄ species show a convergence in leaf anatomy that include increased vein density and reduced numbers of M cells between veins. Rice yields need to increase, as rice is a C₃ plant which displays high photorespiration. To reduce the losses by photorespiration it is required to improve net photosynthesis via an increased photosynthetic efficiency could provide significant gains in terms of grain yield. In this project our aim is to identify the vein density of different rice-genotype as higher vein density is associated with increased rate of photosynthesis and decreased rate of photorespiration.

Keywords

C₄ rice,
Photosynthesis,
Photorespiration

Introduction

Rice (*Oryza sativa* L.) is the staple food of over half of the world's population, and rice consuming population is increasing at the rate of 1.098% per annum. The yield potential of rice needs to be increased by at least 50% by 2050 to support rapidly increasing human population. Increasing photosynthetic efficiency is seen as a key factor in meeting this challenge and helping

to reduce the yield gap (Sheehy *et al.*, 2007). Escalating population means more demand for food, water and land at a time, when the natural resource base for agriculture is being degraded because large areas of farmland are being diverted from food production to industrialization and bio-fuel production. An unpredictable climate change is threatening to further reduce agriculturally viable land due to more instances of drought and flood. As a growing population and global climate

change place increasing pressure on the world's food supply, it is essential that we continue to improve crop performance in terms of grain productivity to keep pace with population growth. The increase in crop productivity conferred by the plant types created during green revolution period supported the population boom following the two world wars. Since then, despite the use of improved varieties and advanced technologies, the yield potential of present day rice cultivars has improved just a little indicating that these varieties have hit a yield ceiling (Sheehy *et al.*, 2008). To provide adequate food and nutrition to the global population that is expected to reach 9 billion by 2050. Rice yields need to increase by at least 60% (FAO 2009). Most of the plants use the C₃ pathway of photosynthesis that is inefficient in CO₂ fixation. However, some plants use a supercharged photosynthetic mechanism called C₄ photosynthesis. The C₄ pathway is used by the most productive vegetation and crop on earth. In addition to faster photosynthesis, C₄ plants demand less nitrogen. Overall our aim is to introduce the characteristic of C₄ into C₃ crop. This would increase yield, reduce land area needed for cultivation, decrease irrigation, and limit fertilizer applications. If current C₃ crops could be converted to use C₄ photosynthesis, large economic and environmental benefits would ensure from both there increased productivity and the reduced inputs associated with increase in photosynthesis, and so its manipulation remains an unexplored target for crop improvement both for food and biomass (Saga *et al.*, 2009). Recently an attempt is underway to increase the rice yield potential by engineering an efficient C₄ type photosynthesis into rice. As rice plant is operating the C₃ photosynthetic pathway and it is commonly grown in tropical conditions of high light intensity and temperature, rice

(*Oryza sativa*) displays high photorespiration. To reduce the losses by photorespiration it is required to improve net photosynthesis via an increased photosynthetic efficiency could provide significant gains in terms of grain yield. This requires an understanding of C₃ and C₄ leaf development and establishing whether there is sufficient plasticity in the rice genome to produce plants with C₄-like properties (Kajala *et al.*, 2011) or by improving existing C₃ photosynthesis by means of increasing leaf thickness, vein density. In conclusion, the future success of photosynthetic improvement in rice will rely on the screen of much larger numbers of rice genotype and C₄ plants in order to identify the genes determining key conserved morphological features such as interveinal cell number, cell size and the degree to which rice mesophyll cells are lobed.

There is need to develop rice plants with improved photosynthetic capacity and efficiency in order to enhance potential grain yield. The rice plant carries out C₃ photosynthetic carbon fixation consequently rice productivity suffers because of the enhanced levels of photorespiration, which reduces photosynthetic capacity by around 30-35% when the ambient temperature is 30-35⁰C (Nakhoda *et al.*, 2012). Alterations in internal leaf morphology may be needed to underpin some of these improvements one target is the production of kranz-like anatomy, commonly considered to be required to achieve the desired levels of photosynthesis seen in C₄ crops. Introducing C₄ characteristics into rice to overcome the limitations imposed by photorespiration is a highly challenging project. But if some important characteristic of C₄ plant selected in rice plant, one of which is high vein density. Kranz anatomy typically has two or three mesophyll cells interspersing adjacent vein. As a first step to determining the

potential for alterations in vein patterning and mesophyll cells in rice leaves (McKown and Dengler, 2009). There would be variation in vein arrangement and sequential distribution of major and minor veins across the leaf width, although there would be significant correlation between the total number of veins present and the width of the leaf. High vein density trait would be linked to a narrow leaf width trait. The more genetically robust narrow leaf width trait was proposed to be used as a reliable phenotypic marker for finding high vein density variant in rice in future screens (Smillie *et al.*, 2012). There is need to find out the impact of vein density on photosynthesis of different rice variety. However over the latter half of the last decade there has been resurgence regarding high yield requirements and hopes are being laid on C₄ systems. By 2050 the global population will reach 9 billion and yield enhancements of the order of 50-60% are required. Since photosynthesis underpins agricultural productivity one of the alternatives to break the yield barriers could be the introduction of naturally selected better variant of photosynthesis i.e. C₄ pathway into contemporary rice cultivars. C₄ pathway with its enhanced carbon tunneling and concomitant increase in nitrogen and water use efficiencies may provide the scale of yield enhancement required (Mishra *et al.*, 2017).

Review

Rice (*Oryza sativa* L.) is the single most important food crop, being the primary food source for more than one third of the world's population, and grown in 11% of the world's cultivated area (Khush, 1993). Present rice (paddy) yields average about 5.0 t/ha in the intensive rice systems, which is less than 60% of the climate-adjusted yield potential of existing high-yielding varieties in Asia,

more than two billion people are getting 60-70 per cent of their energy requirement from rice and its derived products. In India, rice occupies an area of 44 million hectare with an average production of 90 million tones with productivity of 2.0 tons per hectare. Demand for rice is growing every year and it is estimated that in 2025 the requirement would be 140 million tons. To sustain present food self-sufficiency and to meet future food requirements, India has to increase its rice productivity by 3 per cent per annum (Thiyagarajan and Selvaraju, 2001). Around 480 million metric tons of milled rice is produced worldwide annually. Record increases in rice production occurred during the last three decades of the twentieth century. The Green Revolution from 1940s to 1960s led to an increase in agriculture production among the developing countries, due to advancement in research and technology. A significant increase in yield levels was observed in many Asian countries from the pre-Green Revolution times. Asian countries have the largest share in global rice production. In Asia, the demand for rice is expected to increase by 70% over the next three decades, driven primarily by population growth. Rice is currently grown in over a hundred countries producing more than 715 million tons of paddy rice annually. Fifteen countries account for 90% of the world's rice harvest. China and India alone account for 50% of the rice production. Together with Indonesia, Bangladesh, Vietnam, Myanmar, Thailand, the Philippines, Japan, Pakistan, Cambodia, the Republic of Korea, Nepal, and Sri Lanka, Asian countries account for 90% of the world's total rice production. Other major non-Asian rice producing countries include Brazil, the United States, Egypt, Madagascar, and Nigeria, accounting for 5% of the rice produced globally. In Africa, rice is the fastest growing food staple. Total cereal production in Africa has risen steadily

from 9.3% in 1961 to 15.2% in 2007. However, only 54% of the rice consumption is supplied locally (Muthayya *et al.*, 2014). According to very recent reports, United States Department of Agriculture (USDA) estimates that the World Rice Production 2016/2017 will be 481.08 million metric tons. Rice Production last year was 470.82 million tons. This year's 481.08 estimated million tons could represent an increase of 10.26 million tons or a 2.18% in rice production around the globe (Anonymous, 2016).

To boost food production for a rapidly growing global population, crop yields must significantly increase. One of the avenues being recently explored is the improvement of photosynthetic capacity by installing the C₄ photosynthetic pathway into C₃ crops to drastically increase their yield. Crops with an enhanced photosynthetic mechanism would better utilize the solar radiation that can be translated into yield. This subsequently will help in producing more grain yield, reduce water loss and increase nitrogen use efficiency especially in hot and dry environments (Karki *et al.*, 2013). In C₃ species such as rice, carbon fixation occurs in a cyclical process known as the Calvin cycle. The primary fixation of carbon dioxide in the Calvin cycle occurs when CO₂ is conjugated with the five carbon sugar ribulose biphosphate (RUBP), forming a highly unstable six carbon intermediate, which undergoes almost immediate spontaneous decomposition to form two molecules of phosphoglycerate, which in turn are converted to glycerate 3-phosphate (GALP). GALP is reduced using NADH and ATP to regenerate RUBP, and thus the Calvin cycle continues. This conjugation of CO₂ and RUBP is catalysed by the enzyme Rubisco (ribulose 1, 5 biphosphate carboxylase / oxygenase), and it is the inefficiency of this enzyme that has

promoted the evolution of C₄ photosynthesis within plants. Rubisco is one of the great enigmas of evolutionary biology. Firstly, it is a very slow acting enzyme, which means that Rubisco is present in extremely high concentrations in the chloroplast stroma at approximately 1.68g m⁻² leaf tissue (Jin *et al.*, 2006). Secondly, Rubisco also has the ability to fix oxygen, as well as carbon dioxide; thus Rubisco is regarded as an oxygenase as well as a carboxylase. Oxygenase activity is favoured over carboxylase activity at higher temperatures and oxygen concentrations. The fixation of oxygen results in the production of phosphoglycolate, an apparently functionless substance that is entered into the photorespiration cycle to recover some of the carbon wasted within the molecule (Muhaidat *et al.*, 2007).

As a C₃ plant grown under tropical conditions, Asian rice has significant level of photorespiration due to the high temperatures at which it grows. Given current atmospheric CO₂ concentrations, at a temperature of 30°C, approximately 33% of the total level of Rubisco within a photosynthesising rice plant may be functioning as an oxygenase, thus representing a significant loss of CO₂ fixation to the plant and major limiting factor of photosynthesis (Evans and von Caemmerer, 2000). Fortunately, the evolution of C₄ photosynthesis provides a biological precedent that implies RUE of C₃ crops can be increased. C₄ species such as maize, sorghum, and sugarcane have 50% higher RUEs than those of C₃ crops such as rice, wheat, and potato (Kiniry *et al.*, 1989; Sage, 2004). Introducing the C₄ photosynthetic cycle into rice could allow a 50% increase in the RUE and crop yield (Mitchell and Sheehy, 2006; Hibberd *et al.*, 2008), as well as potentially improve nitrogen and water use efficiencies (Sage

and Percy, 1987; Makino *et al.*, 2003; Sage, 2004). In order to test the feasibility of introducing a two-celled C_4 cycle into rice, an international C_4 Rice Consortium led by the International Rice Research Institute (IRRI, Philippines) with 24 participating research groups was set up in 2008. Within this Consortium, activities range from systems biology, physiological phenotyping, and deep sequencing, through to metabolic engineering and screening of rice mutants for characteristics of Kranz anatomy. Overall, these complementary approaches should increase our understanding of the C_4 pathway and facilitate attempts to engineer it into C_3 species. It is clear that alternative approaches such as modifying existing (Lefebvre *et al.*, 2005). This correlates to an estimated reduction in the productivity of C_3 crops by over 30%. As Rubisco appears to be a major limiting factor to photosynthesis in C_3 plants grown under tropical conditions, and there is no known alternative. To this enzyme in the fixation of CO_2 in the Calvin cycle the options for the improvement of photosynthesis appear somewhat limited (Sage, 2004). Increasing the level of Rubisco in leaves could provide a method for increasing photosynthetic capacity; however this would not represent any gain in efficiency so improvements would still be severely limited by photorespiration (Mitchell and Sheehy, 2006). Leaf vein density (LVD), defined as the total length of veins per unit area, has been linked to rates of photosynthesis plant and leaf hydraulic conductance, leaf size and conductance and leaf allometry. Vein density affects the distance that water has to travel through the mesophyll space, thereby providing a mechanism to influence whole-leaf physiological rates. Long distances (low vein density) are associated with longer travel times and thus slower physiological rates; conversely, shorter distances (high vein density) are associated with faster rates

(Brodribb *et al.*, 2007). Leaf veins are an important aspect of leaf structure and responsible for both the mechanical support of leaves and the long-distance transport of water, nutrients, and photo assimilates. The molecular mechanisms by which vascular tissues acquire their identities are yet largely unknown, and there is high interest in analyzing and evaluating traits of veins or leaf venation networks and their genetic regulation (McKown and Dengler, 2009). The impact of vein density on photosynthesis is a major investigated topic. During the last decade, a positive correlation between leaf venation and photosynthesis has been observed. An optimization of photosynthetic rates was shown to occur by spatial coordination between leaf vein and stomatal densities. Additionally, there is interest in the impact of vein density on interveinal distances and the effect of climate, habitat, or growth form on vein density or vein width with respect to leaf hydraulic conductance (Sack and Scoffoni, 2013; Scoffoni *et al.*, 2015). Researchers are particularly interested in the evolution from C_3 to C_4 plants, which requires higher vein density and led to selecting for variation of vein density within species leaf venation studies analyzing traits of veins and venation networks are generally performed on microscopic images of leaves that are properly cleared after harvest (Field and Brodribb, 2013; Xiong *et al.*, 2015). It has been suggested that an increase in vein density contributed to the phylogenetic radiation and rise to ecological dominance of the angiosperms. Since the functioning of C_4 pathway requires cooperation between both cell types, the M and BS cells are arranged in form of concentric rings (wreath, Kranz anatomy) to maximize contact. For the same purpose BS cells have centrifugally arranged chloroplasts towards M cells and an extensive plasmodesmal network connects the two cell types to

facilitate easy flow of metabolites. BS cells are present in C₃ plants also but there they are involved in non-photosynthetic functions like solute transport and carbohydrate metabolism. Another peculiar feature is that veins act as organizing centres of the C₄ system. Difference in venation pattern is a characteristic variable between C₃ and C₄ leaf anatomy (Mishra *et al.*, 2017).

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