



**The influence of
altering rice
photorespiration on
yield in future climate
conditions:
will adaptations
maintain worthwhile?**

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Abstract

Yield increases are needed to meet the increasing demand for food of the growing human population. One of the crops of which yield increases are needed is *Oryza sativa* (rice), whereas a large part of the human population relies on this crop for a large percentage of their caloric intake. The C3 photosynthetic metabolism of rice is considered inefficient due to high photorespiration rates, caused by the tropical climate in which rice crops are commonly cultivated. In photorespiration, the enzyme ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco) reacts with oxygen instead of carbon dioxide, which is used in the photosynthetic reaction, resulting in carbon losses through the release of CO₂. Research focusses both on reducing the photorespiration rate through altering the glycolate pathway in photorespiration, and converting rice into a plant with a C4 metabolism. Altering the glycolate pathway results in the release of CO₂ in the chloroplast, resulting in decreased rates of photorespiration and prevention of carbon losses. Furthermore, alternated pathways result in a decrease of energy losses through efficiency improvements of the cycle. In C4 plants, Rubisco is prevented from oxidation by a carbon-concentrating mechanism, causing extensive reduction of photorespiration. The process of C4 photosynthesis is more energy intensive compared to C3, but in the case of rice the energy losses due to photorespiration are higher. In current conditions, these adaptations would increase the yields of rice crops compared to the native C3 metabolism. However, climate projections indicate great increases in atmospheric CO₂ levels and temperature, which has been projected to influence these yield increasing strategies and the native photosynthetic metabolism. Projections based on the expected changes in temperature and atmospheric CO₂ increase indicate that the native C3 metabolism in rice will gain significantly in efficiency in future conditions, which is confirmed by the majority of experimental research. C4 rice yield is not expected to increase under future conditions, because photosynthesis in C4 plants is not affected significantly by these abiotic factors. The yield of C4 crops is even expected to decrease due to smaller crop duration time. There will still be potential for the altered glycolate pathway in photorespiration of rice, because the advantages of this pathway will remain. Focusing on the facilitation of alternated glycolate pathways in rice will therefore remain relevant, whereas the creation of C4 rice will not be very effective in realizing the aim of increasing yield.

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Introduction

The global human population will reach approximately 9.8 billion people in 2050, and thereafter will continue to grow towards 11.2 billion in the year 2100 (Food and Agriculture Organization of the United Nations, 2017). Given that there are currently approximately 7.7 billion people, the expected growth of the current population will be approximately 30% for 2050, and about 45% for the year 2100. This large increase of the human population will cause a growing demand for food in the years to come. The projection has been made that crop yields should increase with 50% to meet the needs of the human population in 2050 (Food and Agriculture Organization of the United Nations, 2017).

Current yield increases are not at a sufficient pace to meet future demands (Food and Agriculture Organization of the United Nations, 2017). One of the crops of which faster yield increases are needed is rice (*Oryza Sativa*). An increase in rice yield is of great importance to many, because rice accounts for more than 20% of caloric intake for over a third of the world's human population (Seck, Diagne, Mohanty & Wopereis, 2012). Whereas rice yields increased with approximately 3% in the period of 1975-1984, the yearly average yield increase was only 1.2% in the years 2005-2014 (Food and Agriculture Organization of the United Nations, 2017). At this pace, a 50% increase of this crop will not be achieved by far (Ray, Mueller, West & Foley, 2013).

Therefore, a different strategy for improving rice yields needs to be applied. One promising method which aims at the optimization of rice yields is the optimization of photosynthetic efficiency. The optimization of photosynthesis has the potential to facilitate a 50% yield increase, mainly by reducing major carbon losses occur in the current photosynthetic metabolism of this crop, caused by high photorespiration rates (Long, Zhu, Naidu & Ort, 2006).

In photorespiration, the enzyme ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco) reacts with oxygen instead of carbon dioxide. During this reaction CO₂ is released, resulting in carbon losses (Bauwe, Hagemann & Fernie, 2010). The rate of photorespiration increases at high temperatures (Brooks & Farquhar, 1985; Ku & Edwards, 1977). Rice is commonly grown in tropical climates and therefore photorespiration rates of rice leaves are usually very high. Research has been focussing on strategies that could facilitate a decreased amount of photorespiration. Two of the main methods that are considered promising, which will be discussed in this paper, are engineering the C4 metabolism in rice, and the application of an alternative pathway (AP) in the photorespiration metabolism of rice.

In C4 plants, photorespiration is suppressed by increasing the CO₂ concentration in the surrounding of Rubisco, resulting in very low photorespiration rates. C4 photosynthesis is less energy efficient compared to C3 photosynthesis, but the losses due to the extensive photorespiration in rice are significantly higher. Therefore, the engineering of C4 rice is considered a reasonable method of increasing rice yield (von Caemmerer, Quick & Furbank, 2012; Sage & Zhu, 2011).

Another method that could be applied to rice is an AP of glycolate molecules in photorespiration, with a focus on the reduction of carbon losses by releasing CO₂ in the chloroplast during the process of photorespiration. This method has been proven useful in *Arabidopsis thaliana* and *Nicotiana tabacum* (Tobacco) (Maier et al., 2012; Peterhansel et al., 2010; South, Cavanagh, Liu & Ort, 2019). Adopting these strategies in rice could greatly decrease photorespiration rates, and thereby make photosynthesis more efficient.

Even though these methods seem very promising, the influence of changes in the abiotic environment might alter the extent of success. In addition to temperature, the atmospheric CO₂ concentration is known to strongly influence photorespiration rates. High atmospheric CO₂ levels are able to increase the rate of photosynthesis and thereby decrease photorespiration to great extent (Brooks & Farquhar, 1985; Ku & Edwards, 1977). Projections indicate a rise in both atmospheric CO₂ concentrations and temperature, which will tremendously, but with opposite effects, influence C3 photosynthesis, and therefore photosynthesis of rice crops (Food and Agriculture Organization of the United Nations, 2017; Pachauri, Mayer & Intergovernmental Panel on Climate Change, 2015).

The effects that climate change will have on the current C3 metabolism, and the engineered pathways has to be assessed in order to identify whether the engineering of new photosynthetic pathways in rice will be able to increase rice yield in future environmental conditions. Therefore, this paper aims at answering the following research question: **How will the projected increase of**

temperature and atmospheric CO₂ concentration affect rice yield by influencing the current C3 photosynthetic metabolism, rice with an engineered C4 metabolism, and rice with alternative glycolate pathways?

This question will be answered using literature. First, the lack of efficiency of the C3 metabolism in rice in current environmental conditions will be explained in detail. Thereafter, the mechanisms of the alternatives to the native metabolism and its proposed advantages will be discussed. Subsequently, the influence of changing environmental conditions on the discussed photorespiration pathways will be looked at, using both model predictions and data from experimental studies. Based on the previous findings, a conclusion to the research question will be drawn, focusing on whether engineering of rice photosynthesis will be beneficial in future conditions.

Efficiency of C3 photosynthesis in rice

As was already mentioned in the introduction, rice photosynthesis is considered inefficient because of high photorespiration rates. It is estimated that rice yield could increase by approximately 50% when different pathways are introduced in this crop (Mitchell & Sheehy, 2006). A brief explanation on the current C3 metabolism of rice will describe the basis of these claims.

At the beginning of the Calvin cycle in C3 plants, two molecules of 3-phosphoglycerate (3-PGA) are formed through the addition of carbon dioxide (CO₂) to ribulose-1,5-bisphosphate (RuBP). This reaction is catalyzed by the enzyme Rubisco. The remainder of the C3 photosynthesis results in the formation of glyceraldehyde 3-phosphate (G3P), of which five out of six of the produced G3P molecules are used for the regeneration of RuBP, whereas one is used for energetic purposes. During this carboxylation reaction, oxygen (O₂) is released (Berg, Tymoczko & Stryer, 2002).

Rubisco is both a carboxylase and an oxygenase. Contrary to the previously mentioned carboxylase reaction, O₂ is used whereas CO₂ is formed during the oxygenation reaction of Rubisco, generally known as photorespiration. Instead of two 3-PGA molecules, the oxygenation reaction of Rubisco produces one 3-PGA molecule and one 2-phosphoglycolate (2PG) molecule. 2PG accumulation is toxic to the cell, because of inhibition of the enzyme triose phosphate isomerase, which is involved in the regeneration of RuBP (Anderson, 1971). Therefore, the metabolization of 2PG is an important process. During photorespiration, 2PG is converted into 3PGA, which thereafter can be used in the Calvin cycle (Sharkey, 1985). During this reaction, 25% of the fixed carbon is lost through the release of CO₂. The loss of carbon affects the ability of plants to regenerate RuBP, causing a reduction in photosynthetic capacity. This photosynthetic capacity is additionally reduced by time-loss which occurs during photorespiration, because the rate of the carboxyl reaction of Rubisco is four times that of the oxygenase reaction under normal atmospheric conditions at a temperature of 25 degrees Celsius. Furthermore, toxic by-products are produced, which need to be removed at the cost of energy and reducing capacity (Bauwe et al., 2010; Sharkey, 1985).

The rate of photorespiration increases with temperature. There are two main reasons why temperature affects the rate of photorespiration. Firstly, the increase of temperature causes a decrease in specificity of Rubisco to CO₂ relative to O₂, which results in increased rates of the oxygenation reaction of Rubisco (Brooks & Farquhar, 1985). Secondly, CO₂ solubility decreases more rapidly with increasing temperatures relative to oxygen, causing increased oxygenation of Rubisco, because of higher oxygen levels in the mesophyll (Ku & Edwards, 1977). Since rice is grown at high temperatures, photorespiration is relatively high, and therefore C3 photosynthesis is considered an inefficient metabolism for this crop. Another factor which influences photorespiration rates significantly is drought, which is often associated with high temperature. Drought causes plants to close their stomata, which subsequently leads to a decrease of CO₂ and an increase of oxygen in leaves (Cornic & Briantais, 1991).

The atmospheric CO₂ concentration greatly affects the photorespiration rate. High concentrations of CO₂ in the atmosphere increase the rate of the carboxylation reaction in C3 plants, because it causes an increase in the CO₂ concentration in the mesophyll (Sharkey, 1988). Contrary to temperature, CO₂ concentrations are relatively similar for each location on earth. The forecasted increase of atmospheric CO₂ is thought to increase the yield of C3 plants whereas the yield of C4

plants will remain approximately constant (Collatz, Berry & Clark, 1998). This implies that the forecasted increase of CO₂ will increase the efficiency of the current rice photosynthetic metabolism (Baker & Allen, 1993).

It is clear that the photorespiration rates in current conditions cause the C₃ metabolism in rice to be inefficient. The rising atmospheric CO₂ concentrations might reduce this inefficiency by decreasing the amount of photorespiration. As global temperature is also increasing, the influence of these changes combined has to be assessed in order to establish whether the C₃ metabolism in rice will remain inefficient in future conditions.

Alternatives to the C₃ photosynthetic metabolism

C₄ rice

A lot of research regarding increasing efficiency of rice crops focusses on the creation of transgenic C₄ rice (Lin, Coe, Quick & Bandyopadhyay, 2019). Photorespiration rates in rice with a C₄ metabolism would be much lower than in the current C₃ rice crops in the environment in which rice is cultivated. Additional to these yield gains, it has been argued that C₄ plants have higher water- and nitrogen use efficiency (Ghannoum, Evans & von Caemmerer, 2010).

The C₄ metabolism prevents photorespiration by increasing the CO₂ concentration near the Rubisco molecules and thereby suppressing the oxygenation reaction of Rubisco. In C₄ plants, CO₂ is fixed in the mesophyll cells by PEP-carboxylase, where Rubisco is excluded. The fixed carbon in the form of malate is transferred to the bundle sheath (BS) cells, in which Rubisco is present. Malate is converted to pyruvate in the BS cells, during which CO₂ is released. Rubisco reacts with the formed CO₂, after which the Calvin cycle continues as in normal C₃ photosynthesis. The pyruvate molecule is used for the regeneration of PEP-carboxylase in the mesophyll (Sage & Monson, 1999; 1998; Kellogg, 2013). The C₄ metabolism is able to reduce photorespiration by over 80%, resulting in increased carbon assimilation (Sage & Zhu, 2011).

The development of C₄ rice is a very difficult process, which has not yet succeeded. The C₄ metabolism evolved from the C₃ metabolism, thought to have been triggered by low CO₂ atmospheric concentrations and/or increased temperature (Christin & Osborne, 2013). Reconstruction of the evolutionary steps that created the C₄ metabolism is an important method in finding the mechanisms necessary to induce C₄ in rice plants (von Caemmerer *et al.*, 2012; Hibberd, Sheehy & Langdale, 2008; Lin *et al.*, 2019). The fact that C₄ has evolved over 66 times is considered a cause for optimism regarding the feasibility of this project (Hibberd *et al.*, 2008). Comparing transcriptomes of related C₃ and C₄ species at different stages of development will prospectively facilitate the identification of key regulatory genes necessary for C₄ photosynthesis (Gowik & Westhoff, 2011; Wang, Vlad & Langdale, 2016).

Evolutionary research suggests that the structure of rice leaves might have limited the evolution of C₄ in rice (Christin & Osborne, 2013). The leaves of C₄ plants are, with a few exceptions, organized in a way specific for this metabolism, called the Kranz anatomy. Characteristics of this anatomy are enlarged bundle sheet (BS) cells surrounding the veins, embedded in the mesophyll tissue (fig. 1) (Sage, R. F. & Monson, R. K. (1999)). This anatomy is needed for the functioning of the carbon concentrating mechanism, which makes C₄ plants more efficient under carbon limiting circumstances. The BS cells of rice are much smaller and have a smaller amount of chloroplasts than C₄ plants with Kranz anatomy. Furthermore, rice has a larger amount of mesophyll cells between vascular bundles (fig. 1) (von Caemmerer *et al.*, 2012). Attempts

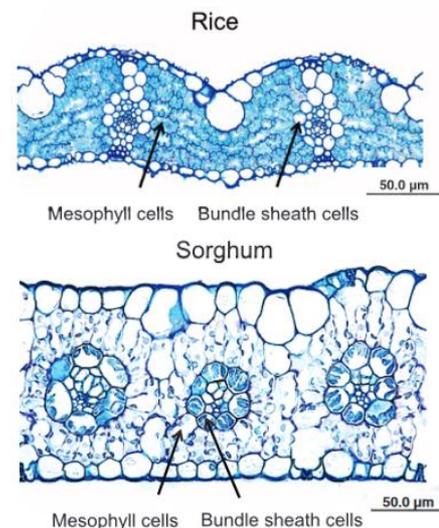


Figure 1: Differences in leaf anatomy between C₃ and C₄ plants, exemplified by a rice (C₃) without Kranz anatomy and a Sorghum (C₄) leaf with Kranz anatomy. The figure originates from Caemmerer *et al.*,

to develop C4 leaves in rice involve the down-regulation of Rubisco in mesophyll cells and the screening for and analysis of rice mutants with high vein density and other C4 features (Kajala *et al.*, 2011). A functioning Kranz anatomy in rice leaves has not yet been achieved.

There are plants that are able to perform single-cell C4 photosynthesis. Options have been explored regarding the application of single-cell C4 to rice, but were examined as theoretically inefficient. This is due to the fact that high BS resistance to CO₂ diffusion is essential to single-cell C4, and rice BS cells show low resistance to CO₂ diffusion. In the case of low resistance, the CO₂ concentrating mechanism does not work efficiently in single-cell C4 photosynthesis (von Caemmerer, 2003). Therefore, efficient C4 rice plants need to acquire Kranz anatomy to gain photosynthetic efficiency.

Despite the fact that C4 rice has not been developed successfully, researchers still consider the engineering of the C4 metabolism in rice as an achievable project which will result in high yield gains in the near future (von Caemmerer *et al.*, 2012).

Glycolate pathway

Another approach to increasing rice efficiency is making adjustments to the current C3 metabolism in order to make it more efficient. Promising adjustments have been made by altering the glycolate pathway in photorespiration in other crops. These alterations, which might be applied to rice crops, will now be described.

The glycolate pathway of the photorespiration process starts with the 2PG molecule, which forms during the oxygenation reaction of Rubisco. The 2PG molecule is hydrolysed to form glycolate. Glycolate thereafter moves into the cell peroxisomes, where it is oxidized to form glyoxylate. The glyoxylate molecule subsequently enters the mitochondria. In the mitochondria, two glycine molecules are used to form serine. During the serine formation, NADH, ammonium (NH₃) and CO₂ are produced. Serine is exported to the peroxisome, where it is converted into hydroxy-pyruvate, which thereafter is converted into glycerate. The glycerate molecule is subsequently transferred to the chloroplast, where it can be used in the Calvin cycle (fig. 2) (Bauwe *et al.*, 2010; Berg *et al.*, 2002).

Multiple adjustments to the glycolate pathway have been made, aiming at reduced energy losses through lowering both the energy costs of the glycolate pathway and increasing the CO₂ concentration in the chloroplast in order to reduce the rate of photorespiration. Three pathways have been tested on *Nicotiana tabacum*, of which two have also been tested in *Arabidopsis thaliana*. The pathways were named AP1 (alternative pathway 1), AP2 and AP3 in *N. tabacum* (South *et al.*, 2019) and these names will also be used in this report.

AP1 is based on the photorespiration pathway found in the bacterial cells of *Escherichia coli*. Glycolate is converted into glyoxylate by the enzyme glycolate dehydrogenase (GDH). Thereafter, two glycolate molecules form tartronic semi-aldehyde through being ligated by byglyoxylate carboligase (GCL). In the latter reaction, CO₂ is released into the chloroplast. Tartronic-semialdehyde reductase (TSR) subsequently converts tartronic semi-aldehyde into glycerate, which thereafter can be used in the Calvin cycle (fig. 2) (Kebeish *et al.*, 2007).

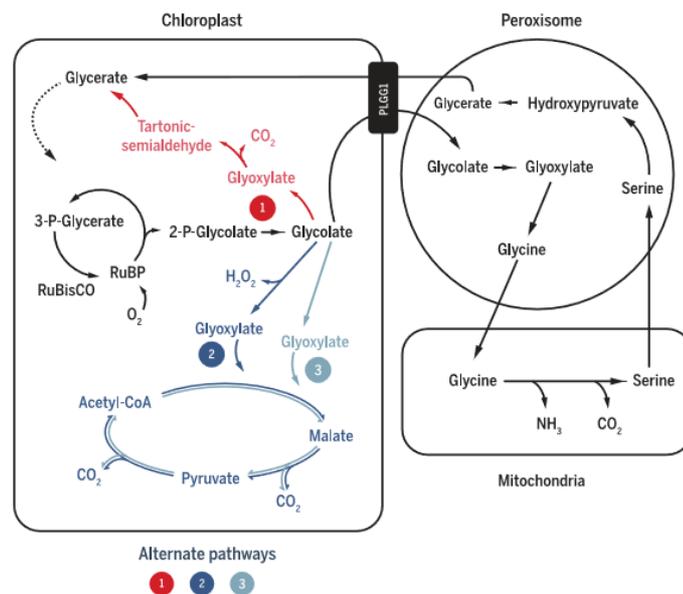


Figure 2: The native photorespiration pathway (black), AP1(red), AP2(dark-blue) and AP3(light-blue). Explanation and genes used for introduction of the APs can be found in the text. The figure originates from South *et al.*, 2019.

AP1 has been tested in both *A. thaliana* and *N. tabacum*. Five genes of *Escherichia coli*, coding for 3 of the GDH sub-units, GCL and TSR, were transplanted into both plant types. In the *N. tabacum* study, glycolate transport from the chloroplast was blocked for half of the treated plants, using an RNAi module to reduce the expression of the chloroplast glycolate-glycerate transporter PLGG1. RNAi was also used in the treatment of the other APs of *N. tabacum*. AP1 in *A. thaliana* showed enhanced biomass production, reduced photorespiration rates and enhanced CO₂ release in chloroplast tissue (Kebeish *et al.*, 2007). This pathway was successful in tobacco as well for plants without the RNAi module, with an overall increased biomass by 13% (South *et al.*, 2019).

Another method of targeting the glycolate pathway in photorespiration is through the introduction of the glycolate oxidative cycle (AP2) or the comparable glycolate dehydrogenase cycle (AP3) into chloroplasts. Both these APs start with converting glycolate into glyoxylate, comparable to the previously explained pathway. The difference between AP2 and AP3 is that during the formation of glyoxylate in the AP2 pathway, H₂O₂ is released (fig 2). This is due to the fact that in AP2 glycolate oxidase for the conversion of glycolate into glyoxylate, which releases H₂O₂ as a by-product, was introduced. AP2 uses a catalase (CAT) from *E.coli* to break down H₂O₂ in water and oxygen. The AP3 pathway uses a glycolate dehydrogenase enzyme, which does not produce H₂O₂ during the formation of glyoxylate. Therefore, the gene coding for CAT is not present in AP3 (Maier *et al.*, 2012; South *et al.*, 2019).

After the first step, AP2 and AP3 use the same pathway: the glyoxylate molecule enters a cycle in which it is subsequently converted into malate with the help of Acetyl-coA and malate synthase (MS). Malate is turned into pyruvate, and during this reaction both a CO₂ and an NADPH molecule are released from the cycle. The pyruvate molecule is used to form Acetyl-coA, which thereafter is needed to close the cycle. During the formation of Acetyl-coA another CO₂ and an NADPH molecule are released (fig.2) (Maier *et al.*, 2012; South *et al.*, 2019).

AP2 showed a slightly increased biomass relative to the wild-type in *A. thaliana*. The suggestion was made that the increase in biomass might have been reduced by the accumulation of H₂O₂ (Maier *et al.*, 2012). AP2 did not result in increased growth in *N. tabacum*. Contrary to AP2, AP3 showed an increase of 18% in a greenhouse experiment compared to the wildtype without the RNAi module in *N. tabacum*, and an even larger increase of 24% when the RNAi module was introduced. Biomass increased over 40% in replicated field trials for AP3 lines with the RNAi module (South *et al.*, 2019).

The increased biomass of plants containing an AP can be attributed to lowered photorespiration rates, lower energy costs of the APs compared to the conventional photorespiratory pathway, and the prevention of production of by-products that have to be detoxified or re-fixed (Maier *et al.*, 2012; Peterhansel *et al.*, 2010; South *et al.*, 2019). A downside to AP2 and AP3 is that there is no formation of 3PGA which could be re-used in the Calvin cycle, which affects the regeneration of RuBP, and therefore might lower the catalytic rate of photosynthesis (Maier *et al.*, 2012).

Photorespiration is very similar between plant species, making the application of the APs to rice a feasible option. Especially AP1 and AP3 were able to increase yields and can therefore be considered as a very promising option for increasing rice production in the near future.

The effect of environmental changes on adapted photosynthesis

Photorespiration rates of C3 plants greatly depend on temperature and atmospheric CO₂ concentrations. Contrary to C3 crops, C4 plants are less dependent on these abiotic factors (Collatz *et al.*, 1998). The influence of climate change will therefore differ for C3 and C4 crops. This part of the report will focus on determining what the impact of changing abiotic factors will be for both C3 species and C4 species. This information will provide knowledge on what photosynthetic metabolism will be most beneficial to rice in future environmental conditions. The effects of changing conditions on the alternative glycolate pathways will also be taken into account, albeit in a less great extent due to the fact that there is little information on the influence of abiotic factors on this metabolism.

To determine which photosynthetic metabolism will work best in future circumstances, predictions on the changes of both atmospheric CO₂ concentration and temperature need to be taken

into account, since these are the two major abiotic factors to influence the efficiency of C3. Models based on the amount of radiative forcing were used by the Intergovernmental Panel on Climate change (IPCC) to create projections. The projections of the RCP4.0 (Representative Concentration Pathway) and RCP6.5 are based on the assumption that radiative forcing will be 4.0 and 6.5 W/m² in 2100. Both these scenarios consider applications of technology in reducing the amount of GHG emissions. The RCP2.6 model assumes the optimum of global annual greenhouse gas (GHG) emissions have their peak at 2010-2020 and thereafter will decline substantially, which will require a major decline in GHG emissions. Contrary to RCP2.6, RCP8.5 assumes continuous a continuous rise of GHG emissions (Pachauri *et al.*, 2015; van Vuuren *et al.*, 2011).

In this paper, the range of atmospheric CO₂ concentrations and temperature rise of RCP4.0 and RCP6.5 will be used as a framework, because these scenarios are intermediates of the extreme RCP2.6 and RCP8.5 scenarios (van Vuuren *et al.*, 2011). Based on RCP4.0 and RCP6.5, atmospheric CO₂ will be within the range of 538 ppm (parts per million) – 670 ppm in the year 2100. Temperature relative to 1986-2005 will have increased within the range of 1.1°C to 3.1°C in the year 2100 (Intergovernmental Panel on Climate Change, 2015).

Models have been created to determine the cross-over temperature for which C3 species would be favoured over C4 species in yield, in the presence of a range of atmospheric CO₂ levels (Ehleringer, Cerling & Helliker, 1997). According to this model, the cross-over temperature at an atmospheric CO₂ level of approximately 600 ppm, the projected intermediate level of RCP4.0 and RCP6.5 in the year 2100, has a range of approximately 32.0-36.5 °C for monocots (fig. 3). In Indonesia, one of the three main rice producing countries, the average daytime temperature is 30.2° (Harris, Jones, Osborn & Lister, 2014). The temperature increase predicted by RCP4.0 and RCP6.5 will cause the Indonesian temperature to be within the cross-over temperature or below, resulting in approximately similar yields or even higher yields of C3 rice crops compared to the crops with a C4 metabolism.

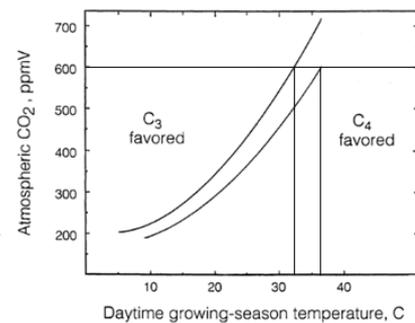


Figure 3 Modelled crossover temperatures of quantum yield for monocots as a function of atmospheric CO₂ concentrations. The figure originates from Ehleringer *et al.*, 1997.

Another, more recent study, created a model projecting the influence of climate change on the production levels of different crops, based on IPCC projections (Iizumi *et al.*, 2017). In this model, the effect of temperature increase described for these four RCP scenarios on yield was calculated. Only 9% of rice production was projected to be negatively influenced by scenarios with higher temperature increases than the RCP2.6 scenario. 23% of the rice production would not stagnate under extreme warming conditions (RCP8.5). From the scenarios used in these reports (IPCC projections RCP4.0 and RCP6.5) the expectations would be that 93% of the rice production yields would not stagnate because of RCP4.0 conditions, and 71% would not be negatively affected by the RCP6.5 scenario. Yield increases were proposed to be most apparent at the RCP6.5 scenario in 2100 (fig. 4). This is mostly due to the fact that positive impact of the rise in atmospheric CO₂ seems to be greater than the losses due to increased temperatures for C3 species (Iizumi *et al.*, 2017).

In the previously mentioned study, projections for maize, a C4 plant, were made as well. In maize, 16% of the yield would not be reduced by the RCP4.0 conditions, and only 6 percent would not be negatively affected due to the projections made in RCP6.5. Maize crops that would be negatively affected by projections other than RCP2.6 are often cultivated at regions in which rice crops would not be negatively affected by RCP6.5. C4 species are expected to be negatively influenced by the changing climate conditions, which will cause shortened crop durations. (Iizumi *et al.*, 2017).

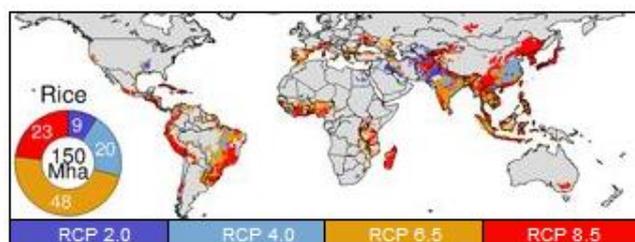


Figure 4: The temperature increase and corresponding RCP scenario for which the projected yield increase for 2100 was found to be the highest for these scenarios. Warming above this level would lead to yield stagnation. The pie diagram contains percentages of harvested area under the RCP warming levels. The figure originates from Iizumi *et al.*, 2017.

A shortened crop duration, or decreased growing time of a crop, is caused by unsuitable factors, which reduce the number of days in which crops are to grow. The main factors involved are temperature, light, and the availability of water. Increasing temperatures and drought are expected to decrease the number of suitable days for crops to grow (Mora *et al.*, 2015).

Rice with an C3 metabolism with an will still benefit under these circumstances, because the photorespiration cycle of plants with this pathway will still be less energy intensive. Furthermore, the C3 species with an will benefit from the increased atmospheric CO₂ levels, even though the AP mechanism itself already increases the CO₂ level in the chloroplast.

Even though models are a method for projecting future situations, there is always a chance that the outcome might differ from expectations. Therefore, findings based on experimental studies also need to be discussed.

A paper on the response of rice to climate change analysed the data generated by studies on this topic. The positive effect of elevated CO₂ levels is very evident, with an average of approximately 25% yield increase for crops grown at elevated CO₂ concentrations, based on 47 studies. The average ambient CO₂ concentration of these experiments was 362 ppm and the average increased CO₂ concentration of these studies was 627 ppm. The positive effect of high CO₂ concentrations is decreased in the presence of high temperatures. However, there is still an increase of approximately 10% on average compared to ambient conditions. Despite the mean increase, the extent of the confidence interval is very large (-6% - 29%), and even indicates that negative influence on yield has been found. The average ambient CO₂ concentration was 370 ppm, the average increased CO₂ concentration was 616 ppm for these experiments (Ainsworth, 2008). The average temperature increase was not reported.

The previous analysis of the available data did not clarify what the average increase of temperature for the experimental data which was used was, which makes it difficult to draw specific conclusions. Therefore, two specific experiments involving relevant temperature and CO₂ increases will now be discussed.

An open-top chamber study on rice crops increased the CO₂ concentrations from 390 ppm to 550 ppm and increased temperature with 2.0 °C. Elevated CO₂ concentrations resulted in a 22.6% yield increase. This increase was reduced with 3% by increased temperature (Roy, Bhattacharyya, Neogi, Rao & Adhya, 2012). Contrary to these results, rice yields decreased in a free-air concentration enrichment (FACE) experiment by 17-35% at a CO₂ concentration of 500 ppm and a temperature increased with 1.5-2.0 °C, compared to ambient temperatures (Cai *et al.*, 2016). FACE experiments have more often found different results compared to other studies. A comparison was made for the influence of CO₂ increase between FACE experiments and enclosure studies, resulting in the conclusion that elevated CO₂ concentrations enhanced yield by approximately 50% less in FACE studies than in enclosure studies (Long, Ainsworth, Leakey, Nösberger & Ort, 2006).

The quantum yield of C4 plants is independent of both the intercellular CO₂ concentration and leaf temperature, for temperatures up to 40 °C (Ehleringer *et al.*, 1997). More recent studies found that the impact of increased CO₂ had very limited influence on C4 plants (Berg, de Noblet-Ducoudré, Sultan, Lengaigne & Guimberteau, 2013).

According to the majority of the experimental data, C3 species will increase their yields in future conditions and C4 species will not be affected to a great extent. The argument that crop duration will shorten is still relevant, because it was not taken into account in the mentioned studies.

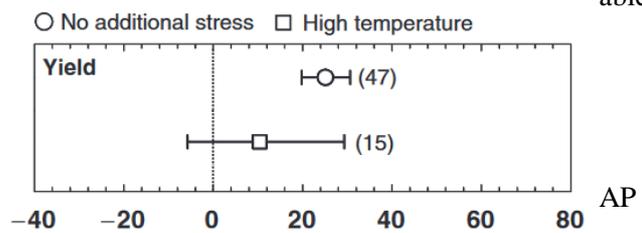


Figure 5: A comparison of rice yield research focussed on the change in yield at elevated CO₂ levels, with and without addition of high temperatures. The numbers between brackets represent the amount of different experimental studies that were used to calculate the yield percentages, the symbols are surrounded by a 95% confidence interval. The figure originates from Ainsworth *et al.*, 2008.

Conclusions

Based on model predictions, it can be concluded that the native C3 metabolism of rice will gain photosynthetic efficiency within the predicted temperature and atmospheric CO₂ increases projected by RCP4.0-RCP6.5 for 2100. The increased atmospheric CO₂ concentration is thought to have a large positive influence on yield, which will outweigh the negative influence of the increasing temperature. Photorespiration will be reduced, causing the C3 metabolism in rice to no longer be inefficient. The majority of experimental studies confirms these projections, by showing increased yields under elevated CO₂ concentrations in the presence of increased temperatures. Still, results from FACE experiments show reduced benefits of CO₂ increases, and even show decreases in yield at increased temperature. FACE experiments are mimicking natural situations, and therefore the lack of benefits of CO₂ enrichments in these experiments has to be taken into consideration.

C4 species in similar locations as rice cultivation areas were expected to have a decreased yield at the projected changes in temperature and atmospheric CO₂. Decreases were projected because increased temperatures are expected to decrease the crop durations, which will result in lower yields (Iizumi et al., 2017). Experimental studies found that the amount of atmospheric CO₂ will not result in significant benefits or losses for photosynthesis in C4 plants. Therefore, the negative influence of the decreased crop duration is not reversed.

There are not a lot of projections on how alternative glycolate pathways will be influenced by changing environmental conditions. These pathways increase the rate of photosynthesis by increasing the CO₂ concentration in the chloroplasts. The effect of this increase will be lower when atmospheric CO₂ levels will increase, and might no longer be significant. Despite this, APs can reduce the energy needed for the photorespiration reaction, through lower energy costs of the glycolate pathway, and the reduction of the production of toxic by-products. Therefore, APs will still increase yields compared to the native C3 metabolism in future conditions.

Based on these results, it can be concluded that the engineering of the C4 metabolism in rice does not seem like a project that will increase rice yields to a great extent in future conditions. C4 rice might be useful in areas where temperature increases are more severe compared to average conditions, but the majority of the places where rice is currently cultivated are not expected to gain efficiency from the C4 metabolism. Contrary to these results, rice with an alternated glycolate pathway has potential to facilitate large yield increases under future circumstances, and might therefore be of great importance in the near future.

Despite these conclusions, further research is needed to confirm the claims made in this report. This paper focusses mainly on temperature and atmospheric CO₂ increases, while there are other significant influencers on crop yields as well which will be influenced by changing environments. Other abiotic factors, like water and nitrogen, also have to be taken into account. Further research and analyzation of available data can lead to a more thorough understanding of the influence of the projected changes of all important abiotic factors combined.

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