



Designing Rice for the 22nd Century: Towards a Rice with an Enhanced Productivity and Efficient Photosynthetic Pathway

Rabin Thapa^{1,a,*}, Nabin Bhusal^{2,b}

¹Agriculture and Forestry University, Nepal

²Faculty of Agriculture, Department of Genetics and Plant Breeding, Agriculture and Forestry University, Rampur, Chitwan, Nepal

*Corresponding author

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ABSTRACT

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Rice (*Oryza sativa* L.) has been cultivated as an important cereal crop for more than 9,000 years and more than half of the world's population depend on rice as it is their primary source of energy. Almost 30% of the current world cereal production is represented by the rice alone. It is estimated that the world's population will reach 9.1 billion by 2050 i.e. 34 percent higher than today and for ensuring an ample amount of food and nutrition to such large population, global consumption of cereals will need to increase from 2.6 to 2.9 billion tonnes by 2027. On the other hand, the impacts of climate change in agriculture are expected to be negative, threatening the global food security. Besides, agriculture and global food security will be severely affected due to the COVID-19 pandemics as its after-effects are yet to be ascertained. The world needs an introduction of a new "Green revolution" in agriculture to increase crop production for food security and biofuel, because conventional breeding method have not brought much of gains not keeping its pace with the world population growth. Hence, the current study was done to review the various ongoing approaches and possible ways of designing a rice with enhanced productivity and photosynthetic capacity. One of the ways to increase yields, photosynthetic capacity accompanied by an increased Water Use Efficiency (WUE) and Nutrient Use Efficiency could be to introduce C4 traits into rice. Besides, genetic engineering using CRISPR-Cas9, molecular breeding, developing ideotype, heterosis breeding, developing apomictic rice, nitrogen fixing rice, use of nanotechnology as well as precision farming are the probable future approaches for designing a rice with high productivity. However, there are challenges and limitations in developing such rice and further research in this matter could help us get closer to developing the future rice.

^a rabinth007@gmail.com

^{id} <https://orcid.org/0000-0003-3625-2587>

^b nbhusal@afu.edu.np

^{id} <https://orcid.org/0000-0003-2330-0391>



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Introduction

Rice (*Oryza sativa* L.) is the staple food for almost half the people on world with global production of 782 mt (FAO, 2018). Rice has been cultivated as an important cereal crop for more than 9,000 years and more than half of the world's population depend on rice as it is their primary source of energy (Molina et al., 2011) while almost 30% of the current world cereal production is represented by the rice alone. It has doubled in the last 30 years, probably with the introduction of new varieties, but its present growth barely goes along with consumption. The number of people that rely on rice for their daily nourishment will reach 4.6 billion by the year 2025, while the consumption of rice around the globe seems to be rising in last ten years (Figure 1). A new surge in production is therefore anticipated (Gnanamanickam, 2009; FAOSTAT, 2018).

The renowned Green revolution (1960s) led to an enhanced yield of rice from less than 1.5-ton ha⁻¹ (Jennings, 1964) to the recent rice yield potential of 15-16-ton ha⁻¹ (Long-ping, 2014). Albeit success has been achieved by modern agriculture in increasing food production, it has also resulted in environmental degradation. Taking an example, the growing use of fertilizers has led to the quality degradation of aquatic sources in many regions (Foley et al., 2005). The burning issue of climate change on the other hand has been of major concern as Nelson et al. (2009), mention that although few positive results are expected in some regions of the world for some crops, as a whole the impacts of climate change on the agriculture are expected to become negative, threatening the global food security. It has been estimated that the world's population will reach 9.1 billion by 2050

(Figure 2) i.e., 34 percent higher than today and for ensuring an ample amount of food and nutrition to such large population, global consumption of cereals will need to increase from 2.6 billion tonnes to 2.9 billion tonnes by 2027. Although there is a need of an enhanced cereal crop production, it is pretty absurd that globally the rate of growth in yields of the major cereal crops has been steadily abating as it is projected that net imports of cereals in developing countries' will more than double from 135 million metric tonnes in 2008/09 to 300 million in 2050 (FAO, 2009; FAO, 2018).

The post COVID-19 situation could rather be of a greater concern for the world. "The recent COVID-19 pandemic resulted from the emergence of a disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)" (Henry, 2020). Agriculture and global food security will be severely affected due to the pandemics while the results will be complex (Torero, 2020), and many of the after-effects are yet to be ascertained and comprehended (Jambor et al., 2020). Despite growing reserved of cereals (FAO, 2020) it has been estimated that the number of people facing a food

crisis will grow from 135 million to 265 million by the end of 2020 (Anthem, 2020).

The world needs an introduction of a new "Green revolution" in agriculture to increase crop production for food security and biofuel, because conventional breeding method have not brought much of gains not keeping its pace with the world population growth (Sowjanya et al., 2019). The future is threatened by the after-effects of COVID-19 in agriculture, impacts of climate change, increasing population and the burning issue of food security. In this limelight, this study was done to understand the possible ways for addressing the major issues i.e., by assessing the possible ways of designing a rice with enhanced productivity and efficient photosynthetic pathway, to feed the future generation.

Materials and Methods

This review has been made consulting relevant research papers and various reports. Collected information was arranged and findings from them haven been summarized and presented in texts, tables, and figures under different headings.

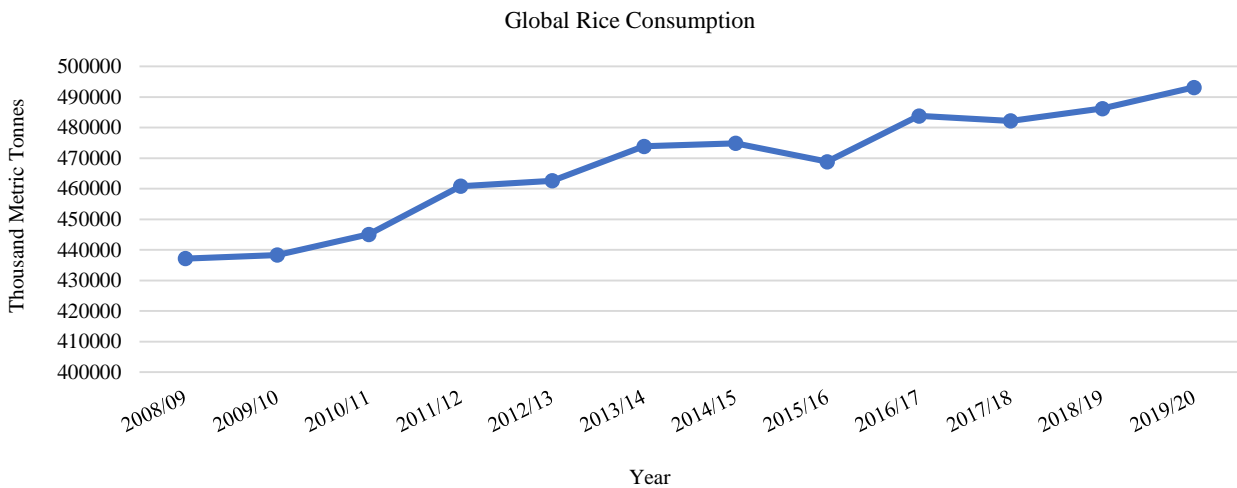


Figure 1. Global consumption pattern of rice in last ten years

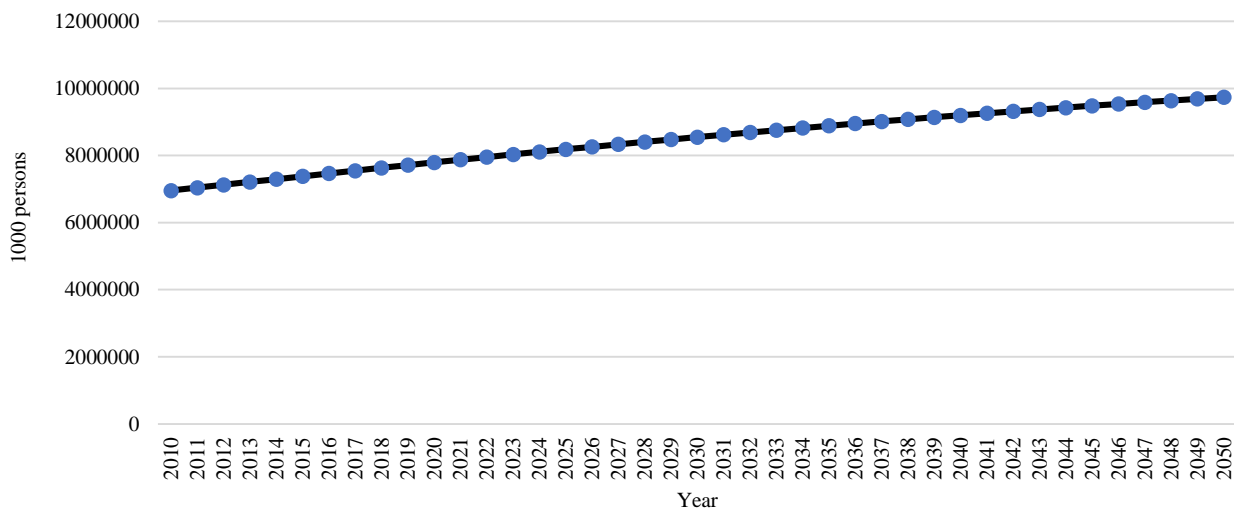


Figure 2. World total annual population projection

Thrust Areas for Improvement of Rice Productivity

Since the ingenious revolution brought by Norman Borlaug in the 1960s produced an enhanced yield of our major cereal crops, we have seen a steady drop in their annual yield growth. The genetic potential of the yield components viz harvests index and grain number, which were brought to limelight in the “green revolution” and subsequently by plant(cereal) breeders have largely been enhanced in our two macro cereal crops, rice and wheat. Despite the use of improved varieties and introduction of new technologies, the yield potential of rice cultivars at present has not improved significantly specifying the yield ceiling that these varieties have hit. As the growing population and climate change are putting an increased pressure on the global food supply, it is important that we continue improving crop performance, thereby grain productivity to suffice needs of increasing population (Akita, 1994; Furbank et al., 2015). The inputs of rice viz land, labor, water and fertilizer determine the production of rice significantly and these inputs will create a hurdle in increasing the rice production. Land for cultivation is fixed and hence the future gains in production of rice can be expected from an enhanced production per unit of land but not from increased area of cultivation. Similarly, with entry into the 21st century, demand of water has increased, not only from the agricultural sectors but also from industrial sectors and household purposes while the cost for establishment of irrigation system is escalating quickly. This implies the future rice production need to be attained from limited use of water. Urbanization and deviation from agriculture to more attractive jobs imply unavailability of labor in near future. The adoption of labor efficient technologies on the other hand may take way more time for adoption and most farmers do not fancy such economically less attractive techniques. The decreasing cost of fertilizers can be assessed in the world and hence increased yields through higher application of fertilizers, especially nitrogen in farmer’s field will require the introduction of new varieties. Thus, if rice can be converted into a C4 plant, this would then open new doors for creation of such new varieties that will give maximum production per unit land and utilize less amount of water but gives significantly high yield i.e., high-water use efficiency. Such conversion will not only address the issues of increasing population, climate change, food security but also help to alleviate poverty with zero hunger which are major goals of SDG 2030 (Dawe, 2000; UNDP, 2015).

Thus, one of the ways to increase yields accompanied by an increased Water Use Efficiency (WUE) and Nutrient Use Efficiency (NUE) could be to introduce C4 traits into C3 plants (rice). This idea came into its existence after it was proposed in the late 1990’s when transgenic experiments to broaden the knowledge regarding C4 gene function were initiated (Matsuoka et al., 2001). A prospective solution, therefore, is to introduce an efficient photosynthetic mechanism; the C4 pathway into rice. This is also the proposed goal of researchers in the international C4 Rice Consortium: to recognize and concoct the genes necessary to introduce C4 photosynthesis in rice (Hibberd et al., 2008). The thrust area, besides involving the improvement of photosynthetic capacity, should also be aimed for these (ICAR, 2013):

- Designation, development and ultimately testing the new plant types, next generation rice and hybrid rice that will opt for an enhanced yield potential.
- Identification of genes and their deployment for enhanced efficiency of inputs we use accompanied by a marked tolerance to various abiotic and biotic stresses.
- Development of a control strategy via proper understanding the pest genomes for evolution of a biotype, the survival of rice in off-season and molecular level host-pathogen/parasite interaction.
- Development of rice varieties which are nutrition rich (Vitamin A, Vitamin E, Zinc, Iron and protein) and which are climate resilient in varied rice ecologies.
- Developing a nutrient dense rice, resource use efficient rice, nitrogen-fixing rice, apomictic rice accompanied by exploitation of microbial resources, resource conservation technologies, use of nano-tech and precision farming.

Improving the Photosynthetic Capacity and Efficiency of Rice

Understanding the C3 and C4 pathways

The recent model explaining the process of C4 evolution which has been widely accepted, explains a stepwise series of changes leading to the evolution of C4 plants from C3 plants (Figure. 3). The changes seen here in each of the steps leads to a rather unique evolutionary advantage for the resulting species irrespective of whether it leads to the complete expression of the C4 like character. This consequence explains why the evolution of the complicated trait could occur multiple times in an independent way. The model explained here is in fact based on the relative analysis of the C3, C4 and the C3-C4 intermediates and an elaborative explanation can be assessed from Sage (2004) and Gowik and Westhoff (2011).

The enzyme Ribulose-1,5-biphosphate carboxylase oxygenase (Rubisco) is the enzyme associated with assimilation of CO₂ into a 3-carbon compound in case of the C3 plants like rice. The name, oxygenase at the end of the Rubisco suggests that besides the role of CO₂ assimilation, it also catalyzes the oxidation of Ribulose-1,5-bisphosphate (RuBP).

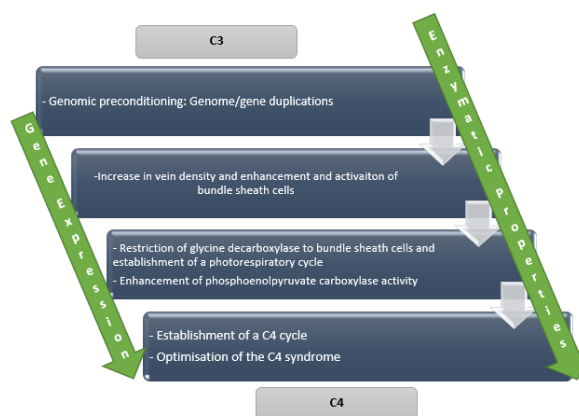


Figure 3. Evolution of C4 photosynthesis (a stepwise approach).

Note: A stepwise approach for evolution of C4 photosynthesis. Adapted from “The Path from C3 to C4 Photosynthesis.” By Gowik, U., and Westhoff, P. 2011, *American Society of Plant Biologists*, 110, p.1104.

The oxidation activity is in fact a wasteful activity referred to as photorespiration which can lead to a loss up to 25% of the carbon that was fixed already (Sage, 2004). This implies that the primary enzyme associated with fixation of CO₂ in rice is in fact a poor catalyst of CO₂ at existing circumstances in the atmosphere. The poor nature can be explained by the fact that this enzyme has confusing nature with respect to the selection of substrate i.e., a mere abundance of O₂ is accompanied by oxygenase of Rubisco and a poor turnover rate of only one or two fixations per second when CO₂ is major substrate. Unlike rice, many organisms including the cyanobacteria, algae and even some land plants have been found to develop an active mechanism involving CO₂ concentration in order to overcome the inefficient process of photorespiration (Badger et al., 2011). In the course of evolution, the C₄ photosynthesis was developed which takes place in different specialized tissue of leaves, the bundle sheath cells. What makes it distinct from the C₃ crops is, the CO₂ concentrating mechanism in the bundle sheath cells, thereby inhibiting the oxygenase action associated with the Rubisco. This means the distinctness lies in the efficiency of operation with respect to CO₂ fixation i.e., operation of the Rubisco at an optimal rate. This also explains why the C₄ crops (sorghum and maize) have better yields accompanied by an increased nitrogen use as well as water use compared to that of C₃ crops (like rice) (Borlaug, 2007; Hibberd et al., 2008). The evolutionary process explained above could be a probable explanation as to how the oxygenase activity of the enzyme could be a remnant of history considering its involvement 3 billion years ago. Later, it was rather impossible to revert the enzyme's action regarding oxygenase activity or replace the enzyme by another carboxylase. Nonetheless, plants acclimatized with this problem by evolving different mechanisms the best of which can be considered the C₄ photosynthesis (Gowik & Westhoff, 2011).

Current Photosynthetic Capacity of Rice in Comparison with C₄ Cereals

It is evident that, the action of oxygenase part of Rubisco (central role in Calvin cycle), which can't be avoided, makes photosynthesis in rice an inefficient one (Legwood, 2013). Unlike rice, inside the mesophyll cells of C₄ cereals, Rubisco formation is impeded and there is production of oxaloacetate followed by malate or aspartate

when the bicarbonate ion (HCO₃⁻) is fixed by the enzyme Phosphoenolpyruvate Carboxylase (PEPC) inside the mesophyll cells. The three possible mechanisms involved can be NADP-ME (Nicotinamide Adenine Dinucleotide Phosphate-dependent malic enzyme), PCK or NAD-ME (Nicotinamide Adenine Dinucleotide-dependent malic enzyme) that carry out the role of decarboxylation when the C₄-acids are diffused into bundle sheath cells which obviously have a low level of CO₂ thereby exalting the CO₂ level around the Rubisco to about five times more than ambient air (Furbank, 2011). In contrary, the C₃ plants like rice have CO₂ levels nearly half of ambient air concentrations in the chloroplast as the hindrances arise during diffusion due to stomata and mesophyll cells (Figure. 4). Thus, the photorespiration is efficiently prevented by this mechanism of concentrating CO₂ in bundle sheath cells thereby inactivating oxygenase activity and making C₄ cereals more productive than rice. (Caemmerer and Evans, 2010; Peterhansel and Maurino, 2011; Evans, 2013).

The physiology of rice is affected by higher temperature in case of rice cultivated areas (>30°C) in the tropics which is its typical range, the rate of oxygenation has a sudden upsurge reducing the photosynthetic efficiency by upto 40% (Ehleringer & Monson, 1993). This results in an inefficient photosynthesis of rice in the tropics and warm temperate regions. The potential of C₄ crops to reduce photorespiration, thrive hot, arid environments due to an enhanced CO₂ concentrating mechanism of leaves envisages a possible strategy to embark with crop improvement (Figure. 5). If the very system is introduced in the rice, besides an increased photosynthetic efficiency, an efficient utilization of scarce resources like water, fertilizer (Nitrogenous) and land can be ascertained ultimately leading to increased yield (Hibberd et al., 2008). A more obvious explanation of the efficiency of C₄ plants can be explained by the quantity of water required for production of 1g of biomass i.e., 650-800g of water is required by C₃ plants unlike C₄ plants which do well with just 250-350g of water (Ehleringer & Monson, 1993). The C₄ plants have thus enhanced their photosynthesis unlike C₃ plants even an enhanced radiation use efficiency as depicted in the Figure 5 below, although in the same ecology (Rizal et al., 1999).

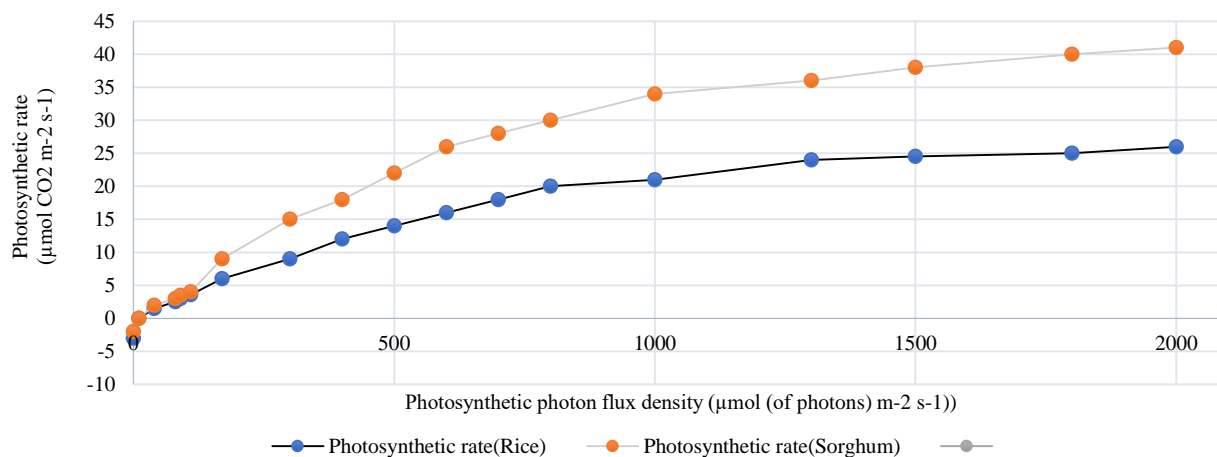


Figure 4. A comparison between light response curves of a C₄ plant (sorghum) and C₃ plant (Rice, IR72)

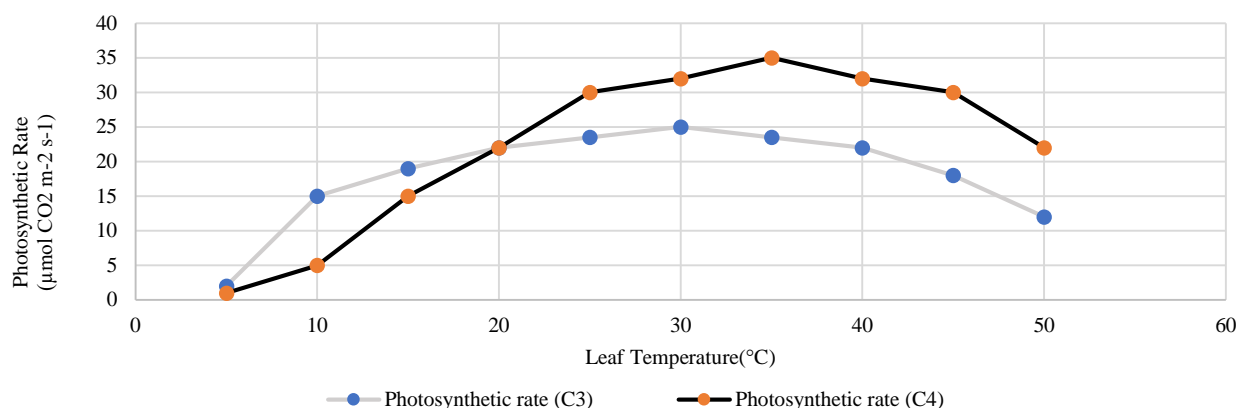


Figure 5. Typical temperature response of photosynthesis in C3 and C4 plants

Can Productivity Be Increased with an Enhanced Photosynthetic Pathway?

When trying to design high-yielding rice plants, several questions arise like; to what extent does photosynthesis shape yield? Sheehy and Mitchell (2013) proposed that breeders should aim for an advanced type of rice, different from the traditional or semi dwarf or new plant type (NPT) varieties currently available. In contrast, the C4 syndrome could increase yields by about 40% above the yields of semi dwarf cultivars in the tropics and by about 24% above the predicted yields for C3 rice in the subtropics. Experimental evidence indicates that improving photosynthetic capacity can lead to yield improvement (Long et al., 2015).

The CO₂ accumulating nature of C4 plants opts for an efficient photosynthesis accompanied by efficient use of nitrogen, water and also the radiation which are subsequently translated into higher yields in crops. Yield potential depends mainly upon four factors viz (1) amount of total solar radiation accumulated over the growth period (2) how efficiently is the photosynthetically active radiation (PAR) intercepted (3) how efficiently the PAR is transformed to dry matter or in other words, the radiation use efficiency and (4) the resources supplied to grain or the harvest index. The harvest index of crops and accumulation of PAR were the two major issues that were addressed by the Green Revolution but the extension of growth period is rather a trivial matter as management practices of crop is limited within the weather circumstances that limit the production within a stipulated time. The one thing that matters now is the resource use efficiency (RUE) as the primary source for genetic improvement bearing high significance. Theoretically the RUE of C3 crops can be enhanced by nearly about 50% if C4 photosynthesis is brought under action. This insists us to convert rice from C3 to C4 in order to mitigate the global food crisis (Mitchell and Sheehy, 2008; Zhu et al., 2010; Reynolds et al., 2011). With the engineering of C4 pathway, raising a question regarding how economic would it be, is rather obvious. It has been reckoned that the benefit-cost ratio of using a rice with C4 pathway is nearly the same as that of using dwarf cultivars that were introduced during the first green revolution which brought benefits to millions of people mostly in the poor regions of the globe. This leaves us with the opinion that no other evolutionary process persists that could be attributed to C3 rice which could ascertain yield as well as benefit (IRRI, 2012). Primary data show that two types of transgenic rice viz PEPC and PPDK transgenic rice have shown upto 20% and 35% increase in grain yield compared to other non-transgenic plants under field conditions which was the result

of increased number of tillers. Overall, the results explain why C4 rice has a huge potential for increment in the yield, photosynthetic ability and stress tolerance (Maurice et al., 2007).

Does the Sink in Rice Bear Space Enough for C4 Productivity?

Clear inspection of the potentiality and capacity of rice is inevitable before installation of a new photosynthetic pathway. We are aware of the enhanced productivity of C4 cereals, but having been acquainted with such huge productivity, the existence of equivalent size of sink is also felt inevitable. The research by Sheehy et al. (2001), put a light on this matter where they showed that the capacity of rice for production of spikelets (>100,000 per sq.m) is more than twice the number of grains finally obtained at maturity (<50,000 per sq.m) (Table 1). The increment of yield by 30% in rice could be explained due to the enhanced photosynthesis for 33 days before heading, CO₂ enrichment being the obvious reason (Yoshida, 1973). These experiments direct their findings towards the same direction i.e., another 50% of the juvenile spikelets have the potentiality to be converted into grains in the rice with C4 photosynthetic pathway i.e., sink is enough for C4 productivity.

Table 1. Mean number of spikelets at different stages and thousand grain weight in crops of IR72

Items	Mean Number
Number of spikelets per sq.m (Juvenile stage)	113,848
Number of spikelets per sq.m (Maturity)	51,372
Filled number of spikelets per sq.m (Maturity)	38,793
Thousand-Grain Weight (g; Moisture Content = 14%)	24.0

Note: Mean number of spikelets at different stages and Thousand Grain Weight in crops of IR72. Adapted from "Spikelet numbers, sink size and potential yield in rice." By Sheehy, J., Dionora, M., and Mitchell, P., 2001, Field Crops Research, 71, p. 77-85

Designing a C4 Rice

With the discovery of a new photosynthetic pathway i.e., C4 in the 1960's, accompanied by consciousness of the fact that C4 is photosynthetically efficient than C3, there have been raised interests regarding the potential effect of photorespiration, carbon utilization, activity of RuBisCO and eco-physiological relation of C3 and C4 plants (Hatch et al., 1971). The discovery of a novel photosynthetic

pathway created an everlasting interest in increasing the plant yield in recent decades with more concern on feeding the increasing hungry mouths. It can be asserted that population increment has served as provocation to such research while efficiency of C4 plants has suggested an ensuring path to assist in the achievement of enhanced yields (Burriss & Black, 1976). Attempts have been made in the past to transfer the improvements of C4 photosynthetic pathway viz reduced photorespiration, enhanced use of nitrogen and water accompanied by increased efficiency of light use into C3 plants using the science of breeding. Although the method seems promising but due to the independent inheritance of genes referring to such characteristics of C4 plant like the Kranz anatomy, the compensation point of CO₂ and increased PEPC indicated it was not a good path to follow (Bjorkman et al., 1971; Bjorkam, 1976). The most promising research for engineering C4 photosynthesis is being led by the C4 Rice Consortium under International Rice Research Institute (IRRI, Philippines). Various projects viz. physiological phenotyping, identification of rice mutants with Kranz anatomy i.e., screening is underway to develop a C4 rice (Covshoff et al., 2012).

Concerns in Improving Photosynthetic Capacity of Rice

Many crops which have been domesticated, just like rice, fall within such a genus that belong to clades where only C3 species can be found. The sophisticated pathway of C4 plants increase the difficulty and make their integration into C3 leaves more challenging (Sage et al., 2011). It is arguable that the genomes of such species and the C4 photosynthesis are incompatible. The Kranz anatomy is integral part of C4 photosynthesis and this leaf anatomy is fundamental for such photosynthetic pathway but the genes associated with such leaf anatomy have not been identified. Also, the genes governing leaf characters like thickening of bundle sheath cells, the plasmodesmatal linkage between bundle sheath and mesophyll cells including the dimorphic chloroplast features have not been characterized yet (Kajala et al., 2011). There are still many gaps in our knowhows of C4 plants and these gaps need to be filled. The identification of transporters which are clearly associated with fluxes in the metabolism as well as the regulators and loci have not been identified yet. Cellular mechanisms and specialization of anatomy of

C4 plants still need to be identified that makes C4 plant unique. What has been achieved till now by genetic engineering has not been able to address the installation of the C4 pathway and on the other hand, the challenge of engineering C4 system will further aggravate as we are acquainted with a greater number of genes associated with C4. For the overall realization of the CO₂ fixation like that of C4 further challenges will arise and to address the challenge we need to realize the potential of installation in C3 plants like rice. Thus, there is a need to enhance our knowledge regarding how Rubisco can be generated and the performance of Rubisco under varied conditions viz temperature. The sustainability and maintenance of such system in C3 leaves via appropriate bioengineering is inevitable if the chloroplast system metabolism is to be facilitated (Sharwood et al., 2016). The accumulation of challenges regarding maintenance of a stable system of Rubisco is due to inability to maintain a progeny with transformed chloroplast that is heritable and also stable in none of the species of monocots (Khan & Maliga, 1999). However, Caemmerer et al. (2012) report that, in another 15 years of experiment, they will be able to enhance the phenotype and the C4 rice will be able to be cultivated in farmers' field.

To convert the rice, a C3 plant into C4 plant the following elements need to be brought to concern (Gangurde et al., 2016):

- Concentration of CO₂ in a compartment around the RUBISCO.
- CO₂ fixation system which is basically light driven.
- Supply of ATP for regeneration of PEP.
- A pool of metabolites and compounds for regeneration of PEP; the compounds being involved in transfer to mesophyll cells from bundle sheath cells at high capacity.
- Installation of decarboxylation enzymes for release of CO₂ from intermediate metabolite pool.
- Prevent leakage of CO₂ from bundle sheath and maintain ratio of mesophyll to bundle sheath cells (1:1).

The roadmap to the development of a C4 rice by IRRI is presented Table 2.

Table 2. Roadmap to the development of C4 Rice by IRRI and our projection

Phases	Commercial pathway of C4 Rice	Initial Time-frame (IRRI, 2012)	Tentative time-frame (Sage, 2004)	Our Estimates
Phase-I	Gene discovery and molecular toolbox, and characterize regulatory controls	2009-2011	2009-2011	2009-2011
Phase-II	Transform rice to express Kranz anatomy and the C4 metabolic enzyme	2012-2014	2012-2015	2012-2015
Phase-III	Optimize C4 function in transgenic rice	2015-2019	2016-2020	2016-2022
Phase-IV	Breed C4 from transgenic into local varieties	2020-2023	2021-2025	2023-2028
Product prototype development (Phase I-IV) (years)		15	17	20
Post-development (years)			5	7
Commercialization			2030	2035

Note: Roadmap to development of C4 Rice by IRRI and our projection. Adapted from "Compliance Costs for Regulatory Approval of C4 Rice." By Bairagi, S., and Mohanty, S., 2017, *Agriculture BioForum*, 20(2), p. 84-93.

Searching for C4-ness in Wild Rice

The best way to introduce C4-ness in the rice would be to find the genes for C4-ness in the wild varieties of rice. With a diverse genus and 24 species of genus *Oryza* only; only 2 species of which are cultivated ones while the rest belong to wild ones. The IRRI has collection of germplasm having more than 6,000 of wild rice accessions (IRRI, 2012). Although a detail study of the wild varieties needs to be done, the past study has shown some promising results in wild rice where characteristics of C3-C4 intermediates have been reported (Table 3). One of the most promising result can be observed where some accessions of species *rufipogon* exhibited a compensation point of 30 $\mu\text{mol mol}^{-1}$ and the

PEPC activity of about 3 $\mu\text{mol min}^{-1}\text{mg}^{-1}$ of chlorophyll. The most inefficient process in C3 i.e., photorespiration was found 25% lower in *rufipogon* species than the sativa species. Rice spikelets have exhibited some C4 characteristics while the lemmas carried out mainly C3 photosynthesis accompanied by CO₂ fixation using PEPC however, Kranz anatomy couldn't be found. The number of veins, total number of bundle sheath cells, number of chloroplasts in small veins were also found higher in some wild varieties of rice which need to be further screened (Vaughan, 1994; Yeo et al., 1994; Imaizumi et al., 1997; Sheehy et al., 2007).

Table 3. Percentage of bundle sheath cells (BSC) and BSC with chloroplasts in small vascular bundles

Species	Source	Bundle Sheath Cells with Chloroplasts (%)	Chloroplast area in Bundle Sheath Cells (%)
C4	Our collection	100	>80
C3	Cultivated rice <i>Oryza sativa</i> (IR64)	100	21
	Some Wild Varieties of Rice		
	<i>O. alta</i>	50	52
	<i>O. australiensis</i>	88	41
	<i>O. barthii</i>	64	41
	<i>O. longistaminata</i>	100	48
C3-C4	<i>Panicum milioides</i>	100	50

Note: Percentage of bundle sheath cells (BSC), BSC with chloroplasts in small vascular bundles determined via confocal microscopy. Adapted from "How the rice crop works and why it needs a new engine?" By Sheehy, J., Ferrer, A., Mitchell, P., Elmido-Mabilangan, A., Publico, P., and Dionora, M. (2007). in J. Sheehy, P. Mitchell, and B. Hardy, Charting New Pathways to C4 Rice (pp. 3-26)

Introducing Genes of C4 Photosynthesis into Rice

The identification of genes followed by their transfer and ultimately their expression in plants has been the source of breakthrough in the field of genetics. Talking about rice, the genomic sequence was completely identified in 2005 which then provided a new working environment under functional genomics to understand the trait expression better. With recent advancements, the phenotypes can be assessed at plant level while the interaction of alleles can be assessed at system level of plants. Identification of genes which associated with a particular phenotype using the whole genome sequencing approach, even in case of mutated genes has further opened door for limitless possibilities. (Furbank et al., 2009; Zhu et al., 2010; Wang et al., 2011).

The use of *Agrobacterium* mediated gene transfer is most common for gene transformation and (Agarie et al., 1998; Ku et al., 1999) have claimed the transformation of two rice varieties (Japonica) viz Nipponbare and Kitaake. The genes were transformed with 3 maize genes obviously providing C4 characteristics to the rice. One of the major approaches, besides gene transformation is the expression of appropriate genes like those associated with PEPC, PPDK and NADP-ME enzymes. Immunolocalization studies have exhibited that PEPC was only expressed in the cytosol whereas the other two enzymes were expressed in chloroplast which was pretty obvious. These enzymes are prevalent in C4 cereals, especially maize and are known to remain functional in transgenic rice plants. What makes it more insightful is that the activity of these enzymes showed positive correlation with protein amount from the enzymes (Ku et al., 1999). Studies suggest that the regulatory mechanism has been observed in transgenic rice

plants for the activity of the enzymes present in maize responsible for C4 characters. The organ-specific expression of such genes is absent in C3 rice but can be exhibited in transgenic rice (Maurice et al., 2007). Besides maize, recently the halophyte *Suaeda aralocaspica* have been recognized to perform a complete C4 photosynthesis within the individual cells (SCC4) which is in contrast with typical C4 crops. Presented the genome sequence of the SCC4 plant which can be highly useful for understanding the evolution of such photosynthesis and in fact capable of contributing towards establishment of a C4 mechanism. It is expected that genes present in C3 species can be re-engineered into cell-specific activities in the C4 pathway without any changes into their gene sequence. These results suggest the chances that only few parts of the C4 pathway might be recruited in rice for other parts to fall into place (ICAR, 2013; Wang et al., 2019). Xu (2016) have provided some promising results where they used CRISPR/Cas-9 mediated multiplex genome editing system for mutation of 3 major genes that negatively regulated the grain weight of rice. They concluded that the system used is highly efficient with respect to the mutagenesis frequency and can be used for rapid generation and pyramiding of beneficial alleles. The system can avoid the involvement of non-targeted genomic fragments, increasing the efficiency of breeding by allowing to select QTLs of interest thereby aiding for the pyramiding of necessary genes accompanied by multiplex gene editing. Such advancements in breeding with the help of CRISPR/Cas-9 complex can definitely to aid the transfer of genes and genome editing for engineering a C4 rice.

Introduction of Efficient Rubisco, CO₂ Concentration Mechanism and Lowered Photorespiration

Rubisco plays the role of central enzyme in photosynthesis and hence making it work harder is inevitable if we opt for greatly reducing the photorespiration. This can be done by increasing the concentration of CO₂ around Rubisco which subsequently is associated with light saturated rate of photosynthesis. We should aim for a continuous gain in fixed carbon by creating a primitive atmosphere conditions for Rubisco i.e., an enhanced concentration of CO₂ around it. The experiments carried out by scientists in past support this theory as enhanced concentrations of CO₂ have been found to increase photosynthesis in rice. The major aim of engineering a C₄ rice is to make the in-situ CO₂ available for itself by rice, the concentration in C₄ plants being 3-8 times higher.

When it comes to increasing the concentration of CO₂, we should know the essentials of concentrating CO₂ mechanism. If we aim for making the bundle sheath cells of C₃ plants by increasing chloroplast number, introducing the necessary enzymes in the mesophyll and bundle sheath cells, it would still not be sufficing for initiation of C₄ photosynthesis as we would still lack a proper communication among the cells. This means, we have to look for alternatives viz introduction of carboxysomes and pyrenoids into C₃ plants (chloroplasts) or we could shift the location of photorespiration to bundle sheath cells which is evident in C₃-C₄ intermediate plants. We could also look for a proper alternative like, a better performing rubisco evidently from algae (a rhodophyte) or we could learn a new mechanism involved in *Suaeda aralocaspica* for carrying out C₄ photosynthesis independently. (Whitney et al., 2001; Winzer et al., 2001; Leegood, 2002; Wang et al., 2019). However, the chance of leakage of CO₂ is equally high which will squander our activities related to an efficient Rubisco. Long et al. (2006) have provided some suggestions regarding a faster regeneration of RuBP to increase rates of light-saturated photosynthesis. Raines (2006) emphasize on the possible improvements that could be brought by enhancing the heat stable nature of Rubisco activase at temperature more than 30 degrees or use of enzyme from cyanobacteria for concentration of inorganic carbon, whose precise function, once known, could be of utter significance.

Change in Morphology: Reduced Vein Spacing and Increasing the Vein Density

For an efficient C₄ photosynthesis, besides the role of enzymes, there might be the necessity of some essential features viz a high leaf vein density and reduced vein spacing. C₄ plants are reported to have such features making them a foundation trait for evolution of C₄ crops. The rapid exchange of photosynthates is permitted due to the amount and position of mesophyll cells and bundle sheath cells in the leaves. It implies that, if we opt for an efficient exchange of photosynthates then it is radical to introduce such features to rice. Feldman et al. (2014) carried out screening of mutant population of rice viz a deletion mutant library (cv. IR 64) and a T-DNA insertion mutant library (cv. Tainung 67) having 12,470 and 10,830 lines respectively. It was done to test for increase in leaf vein density and positive results were seen in case of 8 lines

where a significant increment was reported for leaf vein density. This experiment provided a lead on other findings that leaf width could also be looked for as a phenotypic marker for finding alterations in vein density accompanied by its use for speeding up vein density related screenings. Having been aware that vein density has been hypothesized as a foundational element in evolution of C₄ from C₃ photosynthesis, using the increased vein density in rice as an acceptor lines is highly relevant (McKown and Dengler, 2009; Kajala et al., 2011).

Other Potential Strategies Vis a Vis Yield Improvement

Genetic Engineering and Molecular Breeding

Rice genome sequencing was successfully completed in the year 2005 and after this the interest among researchers for understanding the functional genomics is ever-increasing. First of all, it is a pre-requisite to understand the traits followed by their manipulation. Genetic engineering can be highly useful for yield improvement as we can use the functional genomics, transcriptomics, proteomics and metabolomics. Protocol for transformation of rice is established providing with an opportunity to introduce genes which modify the yield related processes. Starch is the basic biosynthesized reserve which is governed by series of enzymatic steps. The limitation in storage of starch is found due to allosteric inhibition of enzymes (in the sinks). Thus, transformation should be aimed to affect the production of starch in the sinks with regulation of the enzyme by regulating expression of appropriate genes (Kishore, 1994). Similarly, genetic engineering can be useful for improving crop yields by slowing down the senescence process via introduction of "stay-green" traits in rice. In some genotype of rice, stay-green traits are accompanied by slower degradation of Rubisco which results in accumulation of photosynthates for a longer period i.e., an enhanced canopy photosynthesis and bumper yield (Khush, 2000).

Yield is a polygenically inherited character and is under the influence of environment. The assessment of genotypes from phenotypic expression should also look forwards for low heritability. With recent advancements, quantitative trait loci (QTL) have been identified for the different phenotypic expression among different populations. The use of QTL analysis is found to be more efficient for identification of genomic regions responsible for the expression of particular traits. For the development of rice with defined characters, we must identify, validate and finally combine QTLs/regions which govern traits either individually or in group (Tanksley et al., 1989). QTLs from diverse genetic sources from different landraces, wild genotypes and varied domain shall be identified and transferred into a single genotype using restriction fragment length polymorphism (RFLP), random amplified polymorphic DNAs (RAPDs) and microsatellites. Selection for yield can be done by using molecular markers that are closely linked (Tanksley et al., 1989; ICAR, 2013; Khush, 2000). These recent developments, can be used mainly for the development of saturated molecular map. The use of linkage analysis and molecular markers the location of such QTLs can be determined. Even more useful, is the use of CRISPR-Cas9 for revealing the gene function as well as for RNA guided gene editing. Besides

identification of QTLs CRISPR/Cas9 mediated targeted mutagenesis has been found to be successful in many crops and this feature can be used for crop improvement purposes as well (Junping et al., 2015).

Designer Plant (Ideotyping)

Crop ideotype involves all the morphological and physiological features that contribute to higher yield than currently existing crop cultivars. The concept of plant type was brought by Jennings in 1964 while the term 'ideotype' was introduced in 1968 by Donald. The ideal rice consists of 1. Semi dwarf stature 2. High tillering ability 3. Short, erect, thick and highly angled leaves (Sharma et al., 2013). It has been long recognized that dry matter production and harvest index are the two focal points to be worked out for future breeding prospects with the cereals like rice intending to hit the ceiling in terms of yield. These physiological parameters were brought to limelight after recognition of ideotype as base for crop improvement. In ideotype breeding, we opt for each of the traits, consequently aiming for a description of model crop with traits of interest (Rasmusson, 1987). Yield is the function of harvest index and dry matter—yield can be increased via improvement of accumulated dry matter and increased harvest index. The harvest index can in fact be improved by increasing the sink size. Hence the rice crop ideotype breeding should be focused on large panicles, increased grain denseness, increased number of filled grains, leaf canopy, duration of growth and root characteristics. Ideotype breeding was more focused by researchers after the introduction of IR8 rice (a cross between Indonesian variety "Peta" and "Dee Gee Woo Gen" from Taiwan) which embarked the green revolution. Hence for engineering a rice with ideal characters, we should focus on overall characters viz photosynthetic characters, yield attributing characters and also the modification of plant morphology along with breeding. The rice crop would require three major ideotype changes for overall improvement i) heavy panicle ii) super high yielding plant type and iii) super high yielding ideotype (Wu, 2008; Sharma et al., 2013). The overall ideotype building concept shall be centralized towards these goals:

- Maintaining moderate number of tillers (270-300 panicles per sq.m)
- Heavy panicles (weighing 5 g) and panicles drooping at maturity
- Plant height nearly a meter and panicle height of 0.6 meter at maturity
- Attributes of top 3 leaves:
- Flag leaf of length 50 cm and length of other 2 leaves 55cm
- All three leaves shall be above the height of panicle
- A 55, 108- and 208-degree orientation of flag leaf, 1st leaf and 2nd leaf respectively
- Narrow and V shaped leaves with a width of 2cm upon flattening
- Thick leaves with total specific weight of 55 g per sq.m (top 3 leaves)
- Leaf Area Index of top three leaves shall give a combined total of near 6.0
- Harvest Index shall be near to 0.55

Heterosis Breeding and Apomictic Rice

Hybrid rice have advantage over inbred rice in terms of yield varying from 10-15%. It is evident that indica varieties are mostly used for development of the hybrids in IRRI but the magnitude of heterosis in fact depends in the diversity of two parents (Tollenaar, 1994). The greater the magnitude the higher is the heterosis. However, use of indica rice varieties has slowed down due to less gene flow among the varieties. Thus, the development of hybrid using indica and japonica variety is felt necessary. It is considered that the improved varieties of japonica adapted in tropical region have been reported to have a higher heterosis. Development of hybrid rice is expected to enhance overall rice production in coming decades as different public and private sectors all over the world have made some progress (Yuan et al., 1989). The yield realization needs to be further boosted with a combination of heterosis and introgression of genes which provide tolerance to insect pests and diseases. Domain specific development of rice hybrid is absolute to develop a climate resilient rice hybrid. Hybrids for lowlands (rainfed) is felt necessary in South Asian countries. Development of suitable parental lines should be done to increase the degree of heterosis. On the other hand, molecular level understanding of hybrid development for plant modification can ensure a hybrid rice for future. Use of suitable technology provides with an opportunity to predict the best parents for hybrid development and hence opt for genetic improvement in yield and tolerance.

Despite the rapid advancement in development of hybrids, constraints involving adoption of hybrids, cost incurred, etc. seem to limit their usage. This challenges for development of apomictic rice hybrids. This allows the farmers to use the seeds from the crop harvested for the following year. Introduction of apomictic rice via introgression mutation and other genomic approaches is felt necessary along with a detailed study of diversity among species germplasm using some efficient techniques like Embryo-sac (ES) clearing, use of molecular markers and Flow cytometric Seed Screening (FCSS) (Kaushal et al., 2004). The use of such techniques will enable researchers to understand the role of traits and hence use them for breeding purposes. Apomictic rice development is a genuine need of farmers and its development with appropriate genes is necessary for wider adoption of hybrid rice (ICAR, 2013).

Nitrogen Fixing Nutrient Rich Rice

Use of fertilizers in rice has always been increasing after the Green Revolution, as the demand of fertilizers by the crop itself is high. Not only is the nutrient demand by hybrid rice a problem to small scale farmers but use of fertilizers has caused serious issues in the ecosystem as well. Contribution of fertilizers to the global carbon footprint of agriculture is ever increasing. Thus, engineering rice for the installment of a nitrogen fixing system is inevitable if we are looking for a sustainable production of rice.

The nitrogen fixation in rice will require nodule organogenesis and ultimately infection by nitrogen-fixing bacteria. We have to work out for maintaining a symbiosis between rice and the bacteria in order to develop a nodule and establish a mycorrhizal environment in the rice root system. A large number of diazotrophic microorganisms viz *Alcaligenes*, *Azoarcus*, *Serratia marcescens*, *Azorhizobium* have been found to be associated with the

roots of rice. Establishment of such system of rice need further genetic engineering and use of biotechnology for bacterial activity and nodule synthesis. With prediction of such huge rise in population, the demand for rice will definitely increase but we shall also look forward to making a rice that not only acts as a source of calorie but also provides protein and micronutrient. The rural and poor population suffering from malnutrition will need a rice that not only solves the problem of hunger but also prevents further malnutrition among the marginalized. The future rice demands biofortification which will help to combat against protein, calorie and micronutrient malnutrition (Bashir et al., 2013). All the conventional and recent technological approaches shall be directed towards utilization of genetic variation in order to create a rice with enhanced protein and micronutrient contents. The use of transgenic approaches also needs to be directed towards biofortification such that we can interfere with the current biosynthetic metabolism and direct end products towards synthesis of micronutrients, anti-oxidants, vitamins and protein (Bashir et al., 2013; ICAR, 2013).

Exploitation of Nanotechnology and Precision Farming

The use of modern technology has brought new achievements in different fields with flying colors while, in the agricultural sector, nanotechnology hasn't been exploited to the extent it shall be. Nanotechnology shall be focused primarily for early detection of nutrition deficiency, potential threats of insect pests and diseases, molecular level management of pests, a smart delivery system for increasing the input efficiency, etc. Agri-food nanotechnology is considered to be the next generation economic force in the field of agriculture. Nanotechnology can enhance the production of crops and its applications can be made for i) nanoformulations for application of chemicals and fertilizers ii) use of nano sensors for early warning and identification of diseases and pesticide residues iii) Use of nanotechnology for genetic manipulation iv). Diagnosis of plant diseases while precision farming techniques could further be of immense importance for crop improvement (Sekhon, 2014). Precision farming, on the other hand, is a systematic approach for management of soils and crops so as to prevent any sort of uncertainty with regards to decision making via in-depth understanding and management of spatial and temporal variation. Precision farming in rice can prove highly useful for significant improvement over crop yield, input use efficiency, profitability and reduced environmental impacts (Dobermann et al., 2004). With more recent advancement in site specific management viz GIS based irrigation management system, use of sensors, crop monitoring, crop models, etc., precision farming will act as a useful tool for taking decisions with respect to rice cultivation and management aspects (Dobermann et al., 2004; Rowshon and Amin, 2010).

Conclusion

The global food security is under great concern due to climate change, increasing global rice demands as well as the unpredictable impacts of post COVID-19 on cereal production. There have been promising approaches for designing a rice with enhanced photosynthetic pathway viz

introduction of C4 traits. It is evident that establishing C4 system in rice is a multidimensional approach that not only focuses on a particular gene but rather a combination of genetic engineering, breeding and use of recent technological advancements. While conversion of rice to C4 rice ensures an efficient photosynthesis, other approaches also need to go parallelly to ensure a high productivity for designing the future rice. The combined approach will definitely help to design an ideal rice for next century but we also need to overcome some constraints in the way.

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