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Review Article

Better Photosynthesis in Rice (*Oryza sativa* L.) by Introduction of the C_4 Pathway: an Evolutionary Approach Towards a Sustainable System

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Abstract

The 1st green revolution in the 1960s brought a rapid increase in the yield potential of rice up to more than 10 times that was sufficient to meet the then global food demand. In last four decades the yield of rice has not increased significantly, although the global population is growing rapidly. To meet food demand of this increasing global population, the production of rice must be increased by at least 50% within the next four decades compared to the present day's cultivars. It can be made possible, only if we designed the rice photosynthesis as C_4 type with the help of genetic engineering. It is proved that a C_4 type of photosynthesis mechanism is more efficient than a C_3 type found in all the present day's cultivars of rice and it might help in increasing the yield potential by alteration of the photosynthetic behaviour of the crops like sorghum and maize. This novel process will help us in producing more grain yield as well as higher water and nitrogen use efficiency particularly in the hot and dry environments. This review paper provides all the recent development of the factors that need to be altered in rice, so that the C_4 photosynthetic mechanism can be introduced successfully. Further the differences between the C_3 and C_4 type photosynthetic pathways in respect of anatomy, biochemistry and genetics are briefly discussed.

1. Introduction

Rice has been cultivated throughout the World for more than 9,000 years ago (Molina, 2011) and it is one of the major source of energy for more than half of the World's population specially in the Asian countries. The 1st green revolution in the era of 1960s led to an increase in the yield of rice from less than 1.5 t ha^{-1} (Jennings, 1964) to the at present yield potential of rice 8−10 t ha^{-1} (Prasad, 2013) although it varies widely among the rice growing countries. The global need of food grain as the World population has increased from 3 billion in 1961 to 7 billion within last five decades (FAOStat, 2014). The World population is likely to expect to reach about 9 billion in the next four decades. The major portion of this huge World population will increase from most of the rice eating Asian countries. To grow enough rice for this ever increasing population, there is a urgent need to develop new cultivars which can give sustainably more yield than traditional cultivars, under this present situation of decreasing per capita land holding, water and fertilizer inputs amid the predicted extreme change of climate. Even with the optimum uses of precious inputs like land, water, fertilizer, agro-chemicals etc. and standard management practices, the yield potential of the current varieties of *indica* have not able to exceed up to 10 t ha^{-1} (Kropff et al., 1993). One of the most important ways to overcome this current yield stagnation is by genetically introducing of the C_4 photosynthetic pathway in the present *indica* inbred of rice cultivars. A number of reviews has been carried on the importance of the C_4 pathway in rice (Sheehy et al., 2000). It was unanimously accepted in all of the present research works regarding the C_4 rice biology that C_4 photosynthesis mechanism is more advantageous and beneficial than the traditional C_3 rice growing cultivars in the areas under high temperature and bright sunshine, especially in the Asian countries. These exciting results led to an idea to upgrade C_3 to C_4 rice in the very recent years by several Rice biologists across the globe. There is no doubt that the changing of rice photosynthetic mechanism from C_3 to C_4 is a very complex process as multiple genetic changes are required to change the leaf biochemical, anatomical and physiological modifications

that occur during the evolution of C_4 plants from their traditional C_3 ancestors. The mechanism of C_4 photosynthesis has been evolved multiple times independently and efficiently during the evolution of plants (Sage, 2004) and has involved the duplication of genomes in whole or part which has created a redundancy in the genes, as evolution of *Kranz* anatomy (Rizal et al., 2012), a decrease in number of the Mesophyll cells between the veins, a shift in the metabolism of the Mesophyll and Bundle Sheath cells which include the relocation of carboxylase enzymes (Gowik and Westhoff, 2011) and this evolution of transporters to facilitate metabolic movement between the two cell types. But the absence of any C_4 traits in the close relatives of rice races prevents its incorporation by traditional breeding programmes. The advancements in the genetic engineering and development of plant biology at molecular level give us great possibilities to introduce C_4 photosynthesis in rice. The process of photosynthesis is widely studied in both of the C_3 and C_4 types of plants and the introduction of C_4 metabolism into Mesophyll cells of C_3 plants (i.e. in traditional rice varieties) has been attempted already (Miyao and Masumoto, 2011). This review paper reported all the strategies and development being adopted and progress made towards novel C_4 gene identification and introduction of known genes to incorporate C_4 type photosynthetic system into traditional relatively low yielding C_3 rice cultivars. The C_4 rice has shown higher yield potential, efficient use of water and also increased nitrogen use efficiency, particularly when grown in hot and dry environments of Asian countries (Sage and Zhu, 2011).

2. Supply and Demand of Rice in 21st Century

Around the middle of the past century, the classical plant breeding programmes developed numbers of cultivars of cereal with high yielding potential which improved the yield many times (FAOStat, 2014). This was popularised as the 'Green Revolution' throughout the World. The short, erect fertilizer responsive cultivars of rice that came to boost up production in the Asian countries, however, these traditional cultivars of rice has already reached a yield stagnation situation in recent years (Kropff et al., 1993) and the grains production associated with Green Revolution have now ceased. This is a serious fact that about 548 million Asians are undernourished (Sheehy et al., 2007). In the next 50 years, the Asian population will increase about 1.5 billion as well as the climate change will cause extreme variations in weather condition and play an adverse effect on the production of rice causing erratic growth pattern of rice and the water scarcity will come forward as a burning problem more commonly leading to a loss in the irrigated rice cultivation system (Bouman et al., 2007) and also the increasing of demand for fossil fuel and non-renewable energy will create abnormal competition between the grain for fuel and food production resulting in a huge increase in price of rice (Cassman and Liska, 2007). Currently, 75% of total production of rice (about 600 Mt) comes from irrigated rice cultivation system and about 22% only from rainfed area (Prasad, 2013) and rest are the deep-water rice grown in the coastal regions only. In the absence of additional agricultural land as the per capita land holding is gradually decreasing throughout the World as well as the demands of rice generated by the increasing population alone will require the yields of rice to rise at least 50% in all the ecosystems in the next half century throughout the rice growing countries. Number of theoretical models suggest that, the yield may be increased upto required to face the demand of projected population growth, can only be achieved by boost up the photosynthetic efficiency utilizing solar energy (Mitchell and Sheehy, 2006). The solar energy uptake use efficiencies of C_3 and C_4 plants generally differ about 50% in most of the cereal crops including rice (Kiniry et al., 2008).

The exceptional findings of C_4 photosynthesis mechanism has led to the radical suggestion that, C_4 photosynthesis pathway should be introduced into the traditional C_3 cultivars of rice. Introducing the C_4 pathway into the traditional cultivars grown in irrigated, rainfed as well as deep-water eco-systems of rice cultivation could be the potential solution to the problem of increasing the yield of rice in all rice ecosystems (Sheehy et al., 2007).

3. Comparison Between the Mechanism C_3 and C_4 Photosynthetic Pathways

Most of the cultivated plants species are either C_3 or C_4 type of photosynthesis based on the first stable compound formed in the assimilation of atmospheric CO_2. In the C_3 plants, atmospheric CO_2 is assimilated by Ribulose-1, 5-*bisphosphate Carboxylase Oxygenase* (RuBisCO) and forms 3-*phosphoglycerate* (3PGA), which is the first stable 3 carbon compound. This total process is completed within the Mesophyll cells of leaf which receives the atmospheric CO_2 via intercellular air spaces where ample amount of RuBisCO enzyme is present. RuBisCO enzyme fixes both CO_2 and O_2 depending on their availability in the mesophyll cell, thereby it is facilitating both carboxylation and oxygenation reactions in simultaneous process. The oxygenase activity of RuBisCO is also responsible for the increasing of the amount of harmful physiological process of plant viz. "photorespiration" (Bouman, 2007) and losses considerable amount of energy. In contrast, the RuBisCO enzyme present in C_4 plants is not expressed in the activity of the Mesophyll cells, hence its expression is restricted only to Bundle Sheath (BS) cells of the leaf. The atmospheric CO_2, which is absorbed by the plant, is first fixed in the mesophyll cells by Phosphoenol pyruvate carboxylase (PEPC) forming oxaloacetate (OAA), an organic compound with 4 carbon units

(thus this type of plants called C_4 plant). Unlike the RuBisCO, PEPC is an oxygen-insensitive carboxylase which shows a much higher affinity in the fixation of the atmospheric CO_2 and can continue carboxylation process very efficiently, even when the amount of the leaf internal CO_2 concentration is very low compared to the C_3 plants (Ku et al., 1996). The CO_2 fixed in the form of 4-carbon organic acid compound is transported to Bundle Sheath cells where the decarboxylation process is done. The CO_2 released by this process after decarboxylation is again fixed in BS cells by RuBisCO. This decarboxylation of C_4 organic acid compounds creates a higher amount of CO_2 concentration around the RuBisCO. This mechanism to concentrate CO_2 in BS cells checks the harmful photo-respiratory oxygenation reactions unlike C_3 plants making C_4 plants generally more efficient to produce more photo-synthases products. It can be proved that the C_4 plants can also utilize maximum amount of sunlight because their rate of photosynthesis sharply increases with increasing light the intensity of sunlight and does not appear to saturate (Figure 1). Plants with C_4 photosynthesis are more efficient in using photosynthetic nitrogen than C_3 plants largely due to reduced amounts of RuBisCO protein required to achieve the same rate of photosynthesis (Sage and Zhu, 2011).

4. Genetic Factors Responsible for Controlling of the C_4 Leaf Anatomy

Numbers of researches have been carried out for exploring the C_4 photosynthesis mechanism. Various methods have been adopted by the researchers in the field of mechanism of C_4 biology (Rizal et al., 2012) to find out the genetic regulation of C_4 plants. The first approach was made in this respect to create a mutant C_4 plants (sorghum and *Setaria viridis*) (Figure 2) by

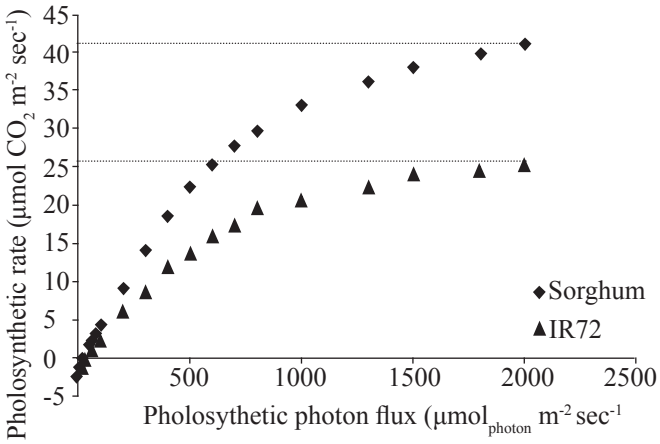

Figure 1: Compares in between light response curve of C_3 plant (rice, IR72) and C_4 pant (sorghum) using infrared gas analyser [Adopted from Rizal et al., 2012 under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/2.0)]

choosing randomly some of the C_4 characteristics which was helpful to identify which gene is responsible for controlling the C_4 mechanism (Figure 3a) (Sheehy et al., 2007). The second approach is to find the rice DNA activation for expressing the rice genes for introducing the C_4-like characteristics (Figure 3b) (Springer, 2000). Both of the mentioned two approaches require an extensive screening of large number of populations to find out appropriate phenotypic characteristics. For screening of large population, the scientist often used induced alterations in leaf Vein Density. The veins of C_4 cultivated plants are distributed much more closely than the C_3 plants. Experiment shows that alterations to vein spacing can create a number of anatomical changes including to the mesophyll cell number between the veins, BS size and vein size which is much essential for expressing C_4 characteristics in C_3 plants. These findings are first steps in creation of C_4 plants and, so identification of genes is very essential that disrupt or induce these desirable changes in C_4 (sorghum) or C_3 (rice) at the genetic level.

5. Introduction of Induced Mutation for Altering C_4 leaf Anatomical Mechanism

An induced mutation is very much helpful for gene discovery as well as the understanding of complex traits. This process is also helpful for boosting up speed of novel gene identification

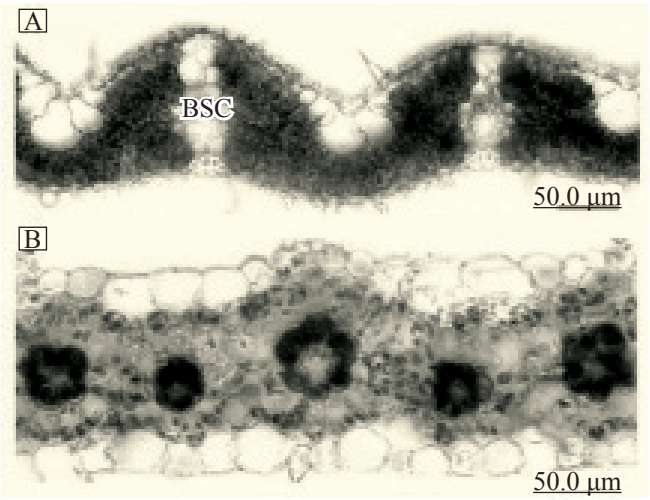

Figure 2: Anatomical differences between C_3 and C_4 leaves. (A) C_3 (*Oryza sativa* L., rice variety IR64) and (B) C_4 (*Setaria viridis*) leaf. Mesophyll cell (MC) of rice is filled with chloroplasts which is more than 90% of the total chloroplasts, whereas, the bundle sheath cells (BSC) have very few number of chloroplasts which account for less than 10% of the total chloroplasts in the rice leaves. In C leaf, chloroplasts are localized in BSC as well as in MC [Adopted from Karki, 2013 under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/2.0)]

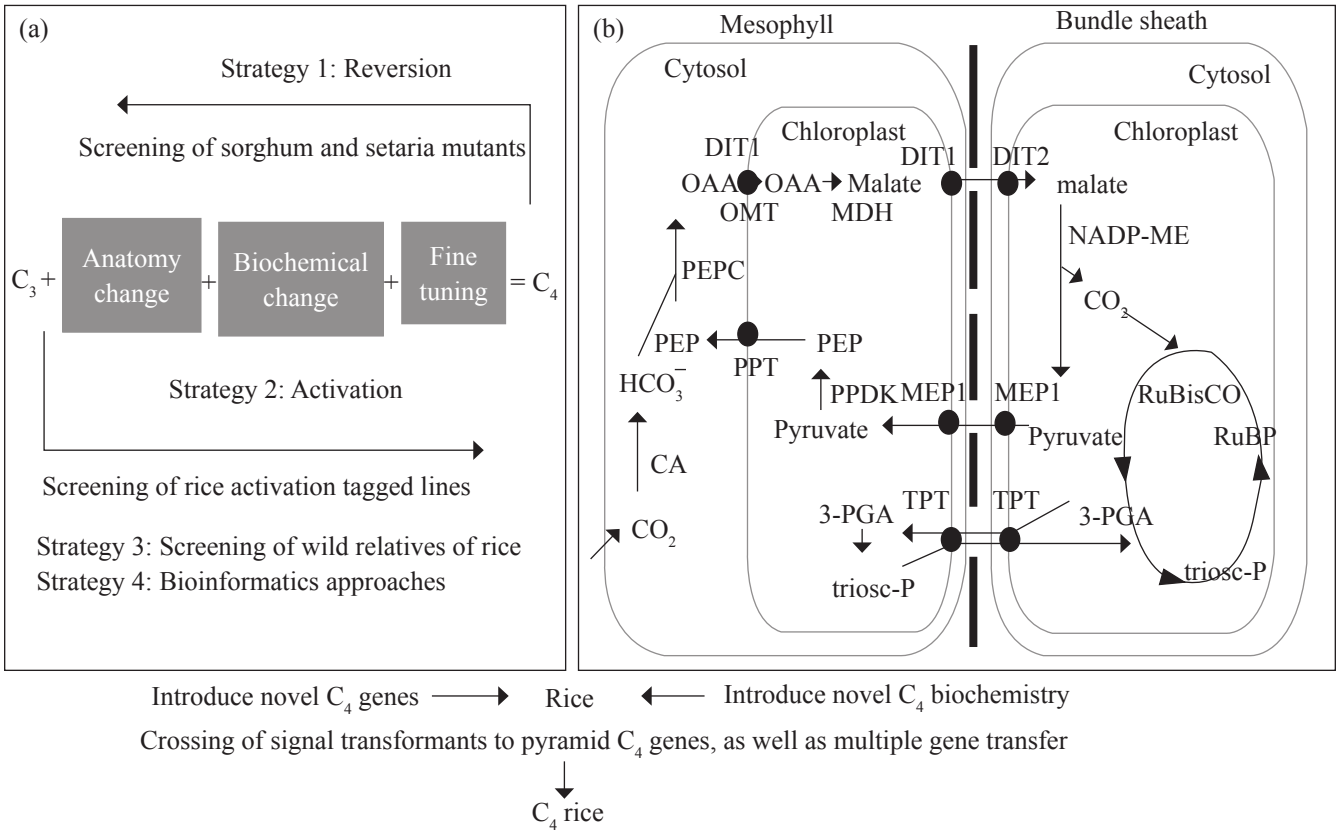

Figure 3 (a-b): Biochemical pathway of NADP-ME subtype of C_4 photosynthesis mechanism that is being genetically engineered into rice variety. PEPC does the first carboxylation in the MC producing oxaloacetate which is further converted to malate by MDH. This C_4 acid is transported from MC to the BSC chloroplasts where it is decarboxylated by NADP-ME to pyruvate and CO_2 is released to Rubisco to carry out the Calvin cycle reactions. In C_4 rice, Rubisco should be expressed in BSC and hence the increased CO_2 levels at its site will reduce its oxygenation activity subsequently reducing the photorespiration. Abbreviation: 3-PGA: 3-Phosphoglycarate; CA: Carbonic anhydrase; DiT1: Dicarboxylate translocator1; DiT2: Dicarboxylate translocator 2; MEP: Mesophyll envelope protein; NADP-MDH: NADP-Malate dehydrogenase; NADP-ME: NADP-malic enzyme; PEP: Phosphoenol pyruvate; OAA: Oxaloacetate; OMT: Oxoglutarate/malate translocator; PEPC: Phosphoenol pyruvate carboxylase; PPDK: Pyruvate orthophosphate (Pi) dikinase; PPT: Phosphoenol pyruvate phosphate translocator; Rubisco: Ribulose-1,5-bisphosphate carboxylase/oxygenase; RuBP: Ribulose-1, 5-bisphosphate; TPT: Triose-phosphate phosphate trans locator. [Adopted from Rizal et al., 2012 under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/2.0)]

procedure. It was found that induced mutation has caused a reduction in the vein density (Figure 4) (Rizal et al., 2012; Karki, 2013). Scientists have already developed the efficient technique of large-scale DNA sequencing for sorghum i.e. BT×623 (Rizal, 2012).

6. Development of C_4 Photosynthetic Mechanism in Mutant Rice Lines

One of the most important things in genetic engineering is to establish correlation between phenotype and genotype for a specific mutant plant species. The foreign DNA which is inserted in the mutant species, acts as a mutagen and a tag for the particular site of the insertion allowing for a function to a specific DNA sequence which is assigned. A considerable number of rice mutant lines has been developed using chemical mutagenesis throughout the World (Rizal et al., 2012). The first rice genes have been identified using the insertion of mutagen discovered by continuous using of forward and reverse (Agrawal et al., 2001) genetic screens. This valuable resource gives us extensive and diverse information in various genetic backgrounds of rice (Droc, 2006). This information about these mutant lines, advanced analytical techniques and developing the mechanism for photosynthetic system as well as leaf anatomy, cell biology and ultra-structure of rice is of extreme importance. It is reported that to date approximately 17,000 mutant lines have been screened and continuously

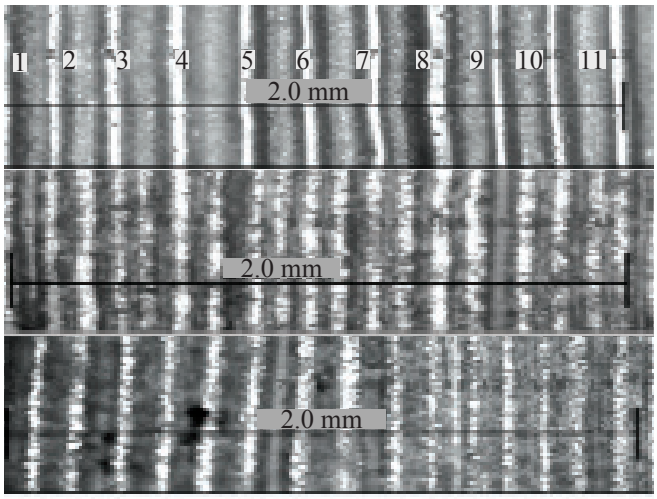

Figure 4: Variation in leaf vein density between C_3 and C_4 plants. Vein density of (A) C_3 (*Oryza sativa* L., rice variety IR64), (B) C_4 (*Setaria viridis*) and (C) C_4 (Sorghum bicolor) leaf sections. Rice has low vein density compared to the C4 plants like *S. viridis* and sorghum. [Adopted from Karki, 2013 under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/2.0)]

maintained for further development (Danker, 2008) from the populations of more than 60 mutants lines have been isolated with distinct increased vain density (Hakata, 2010).

7. Exploitation of the Genetic Diversity of Rice

The most cultivated rice is from the genus *Oryza* of Poaceae or Gramineae family. Genus *Oryza* consist of 10 different genomes among them 23 are wild species and rest two are grown extensively for cultivation purpose (Shelly et al., 2007). It is reported that, there are no occurrences of C_4 like species within the all species of the genus *Oryza* till date. However, *Oryza* not only possesses a considerable amount of genetic variation for different traits but also within a single species (Hirochika, 2001). It has been reported that 56% genetic diversity can be found in the genome of rice (Ram et al., 2007). Currently *Oryza rufipogon* (46% of genetic diversity) and *O. nivara* (78% of genetic diversity) are being selected for developing C_4 mechanism as these species have shown a higher amount of intra-species genetic diversity (Juneja et al., 2006) as well as differences in the branching pattern, mesophyll cell conductance were found.

The Segment Substitution Lines (CSSLs) recently have been developed for cultivated rice by introgressions of small Wild rice chromosome which is very much useful for identifying Qualitative Trait Loci (QTL) as well as genes for different traits. These valuable genetic resources are still not properly utilized by the researchers for screening of physiology and photosynthesis character of different cultivars. Therefore, we have lot of alternative potentials to enhance the rice physiology by using these useful genetic resources for improvement of C_3 photosynthesis behaviour of rice or to make it more efficient for C_4 photosynthesis. Numbers of scientific researches are ongoing Worldwide for finding C_4 like properties in the wild species of rice. Number of studies suggests *O. rufipogon* as one of the suitable species for improving photosynthesis potential in rice (Karki, 2013). These results indicate that, the genes which are responsible for the alteration of leaf anatomy can change the observed CO_2 compensation point and can be used potentially to enhance C_4 pathway mechanism into the cultivated rice species.

8. Genomic Approaches for Introduction of C_4 Photosynthesis in Rice

In the recent developmental study, four important genes of the C_4 pathway have been introduced into rice Mesophyll cells to initiate the C_4 photosynthesis cycle between the chloroplast and cytoplasm of the C_3 rice plant (Dawe, 2007). This complex process needs to the different stages of (1) Discovery of additional genes that coordinate with the known C_4 genes for smoothly running the photosynthesis, (2) Transcription factors which is required for regulation of the activities of genes of network and (3) Introduction of the "gene silencing mechanisms" in the rice plant.

Number of functional genomics and bioinformatics approaches have developed to meet these objectives like discovery of genes associated with any phenotypic trait, gene expression profiling mechanism etc. (Ma, 2011). The mechanism followed in the regulation of the C_4 rice plants reported by numbers of experiment throughout the World is summarized briefly:

8.1. Transcription and alteration of the C_3 and C_4 leaves

The details analysis of the gene regulating the photosynthesis mechanism in both of the C_3 and C_4 plants, number of genes change their expression pattern in the evolution of the diverse C_4 species. It is reported by Brautigam et al. in 2011 that two such studies have been carried out between the C_3 and C_4 species of genera viz. *Cleome* and *Flaueria*. The research findings proved that in addition to core C_4 photosynthesis pathway, there are other some fictional genes are also affected (Rizal et al., 2012). Some of the classes like mRNA level included Calvin-Benson cycle, photorespiration, protein synthesis, primary metabolism showed lower steady state, while some other important photosynthetic classes of photo-system 1 (PSI) and cyclic electron flow, starch metabolism, nitrogen metabolism, cofactor synthesis, glucan metabolism and lipid transfer proteins showed higher level (Rizal et al., 2012). Identification of transporters is the most important

step, as it ensure the availability of metabolites to the enzymes which are present in different cell components (Sheehy, 2007).

8.2. Alteration of the mesophyll and bundle sheath cells of leaf

In the comparison of the mesophyll and bundle sheath cells of C_3 and C_4 plants is that genes expression is missing in different locations. To identify such type of genes a more precise and efficient method of experimental design is highly needed. Isolation and identification of RNA from Mesophyll and Bundle Sheath cells is essential for developing the C_4 pathway in rice as several components has already been partitioned from the two types of cells including secondary metabolism complexes as well as light harvesting, respiration, and transport complexes. A near-perfect correspondence has been observed between the cell-specific enrichment an as well as a high correlation between the differential expression ($0.68<r<0.98$) in the analysis of transcriptome data with the proteome datasets of the C_4 maize plastids (Rizal et al., 2012). So, it is assumed that genes involved in C_4 photosynthesis mechanism in the plants are highly controlled at the transcriptional level.

8.3. Discovery of cis-elements

The current development of the cis-elements involved by C_4 genes has shown some important findings. It was reported from various experiment that genes from C_3 plant species contain cis-elements, which is sufficient for development of the Bundle Sheath specificity in C_4 1eaves (Peterhansel and Maurino, 2011).

The availability of location of the cis-elements is quite low amount in several C_4 genes. The high amount of the cis-element is required for successful introduction of C_4 mechanism in rice. In recent years promoter-deletion assay provides some important information regarding this matter but in quite low amount (Sage, 2004) which require extensive research effort.

8.4. Gene silencing mechanism and non-coding RNAs

C_4 differentiation occurs with a developmental gradient in the undeveloped pro-plastids which are generally found in cells at the leaf base of C_4 Mesophyll and Bundle Sheath cells chloroplasts at the leaf tip. C_4-specific genes were from pre-existing C_3 genes to encode the components which are necessary for the C_4 photosynthesis mechanism. The successful establishment of the C_4 cycle in C_3 plants requires multiple enzymes activity in different cell types and there functional response to diverse environmental conditions. For the selective expression of specific C_4 pathway genes in the particular location of cells different regulation mechanisms required (Scholze and Boch, 2011). The cis-acting DNA elements of C_4 plants are important as gene regulator as well as chromatin configuration, also plays a vital role. It is reported that the expression of the PEPC has been linked to epigenetic control, and chromatin remodelling and also histone modification in maize (Danker, 2008; Rizal et al., 2012). These histone modifications mechanisms contribute significantly to gene regulation by the acetylation process of specific histone-lysine residues and also this pattern is not dependent on activity of gene.

8.5. Molecular engineering approaches for the development of C_4 rice

The conventional approach for transferring of C_4 photosynthesis mechanism to the C_3 plants is quite unsuccessful. Unfortunately, most of the C_3–C_4 hybrids produced under the classical breeding approaches showed infertility due to incomplete chromosome pairing and also due to some other genetic barriers (Thakur et al., 2011). Therefore, a genetic engineering approach appears as a most appropriate method to transfer C_4 photosynthetic characteristics into the C_3 plants. Although it is reported that "isoforms" of genes of some C_4 enzymes are also present in the C_3 plants, in very low concentration and also in wrong cell location (Upadhyaya et al., 2006). By the recent progress in plant molecular biology and also in the genetic engineering we are now able to introduce the desired genes encoding C_4 enzymes into the C_3 plants by using transgenic techniques (Miyao and Masumoto, 2011). This progress provided valuable information about the functions and evolution of these C_4 genes and their introduction in the C_3 plants. This novel technique helps the researchers to express higher level of enzymes involved in the C_4 pathway and in desired locations even in the leaves of C_3 plants which are to be manipulated. The C_4 pathway can be divided into 3 subtypes based on primary C_4 acid decarboxylation enzymes: NAD-malic enzyme, NADP-malic enzyme and PEP carboxykinase (Rizal et al., 2012). In a typical NADP-ME C_4 type plant (maize), 21% of genes are expressed differentially between Bundle Sheath and Mesophyll cells (Wan et al., 2009). Promoters with Bundle Sheath and Mesophyll cells from the C_4 plants can be successfully used to drive tissue specific transgene expression in rice. This experimental result proves that some of the C_4 specific genes localized in Bundle Sheath cells can maintain their cell functionality even in a C_3 plant suggesting that C_3 plants still able to maintain a regulatory mechanism for the gene expression of Bundle Sheath cell. Experiments showed that Agrobacterium mediated transformation of immature embryos of *indica* rice cultivars were highly efficient. The experimental results made it possible for researchers to introduce of C_4 genes into the *indica* rice varieties rather than in past where most of the transgenic plants were developed with C_4 genes using japonica varieties.

The major objective behind the development of C_4 rice is the higher production efficiency of grain under higher temperature and water stress conditions. As *indica* rice cultivars are generally grown under such conditions, thus introduction of

the C_4 photosynthesis mechanism in those indica cultivars is more beneficial. Scientists have chosen IR64, a high yielding indica variety for introduction of C_4 genes such as PEPC, PPDK, NADP-ME from maize by the *Agrobacterium* mediated genetic transformation (Rizal et al., 2012). The successful C_4 cycle cannot be stabilized only with introduction of core C_4 enzymes (Sheehy et al., 2007). Therefore, the recent approach is to ensure a proper facilitation of exchange of all metabolite fluxes into the right position of the Bundle Sheath and Mesophyll cell by the appropriate transporters of the C_4 pathway. In rice, numbers of C_4 genes have been successfully transformed by the help of genetic engineering from maize and other closely related C_4 plant species that have successfully integrated into the genome of C_3 rice and can be maintained over the several generations.

9. Future Prospect of C_4 Rice

It is unanimously accepted that C_4 rice is one of the most challenging subjects for transgenic rice research as well in the field of genetic engineering. C_4 rice research is very tidy job due to wide inter relationship between the anatomy and genetics in between C_3 and C_4 plants. However, this technique is quite important to boost up the present status of the rice yield. To develop transgenic drought-tolerant 'climate-ready' C_4 rice, there is urgent need of genetic manipulation of the rice by introducing some foreign genes, but this is very complex. We all know that the commercialization and popularization of genetically modified rice is still quite difficult in market. As the C_4 rice gives higher grain yield unit^{-1} of water transpired which is an important contribution to manage the global water scarcity in future especially in the Asian rice growing countries, which would be a tremendous contribution to poverty alleviation as well as to reduce environmental loads. C_4 rice is an improved nitrogen fertilizer user, thus it can reduce the production costs and help the environment by efficient utilization of nitrogenous fertilizer and less expenditure of fossils fuels. Furthermore, current trends show that there will be an increasing trend in the next 20 years in the price of the raw rice in the Asian countries (FAOStat, 2014). So, there is lot of uncertainty surrounding any projections of the global demand and the price. The possible adverse effects of climate change like globally increasing temperature, rise of sea water level, changing of annual season cycle have also not been considered in the current prediction, considering such type of uncertainty. In such circumstances, it is urgent to provide solid funding for extensive research in development of C_4 rice as soon as possible. Considering the current global situation like increasing population, hiking of the demand of fossil fuel, increasing pollution; the increased productivity from the C_4 rice can play an important role in alleviating World poverty as well as global hunger. Thus C_4 rice would able to provide an important buffer mechanism to not only increase the global cereal production but also stabilize the unpredictable fluctuation in the changing of the price of raw rice in the Asian countries.

Hence, genetic engineering of C_4 rice is very critical as well as labour and time consuming process. While our recent information of the regulatory reactions controlling the photosynthesis mechanism is well understood, but this knowledge has still not been incorporated. So, there is an urgent need to understand all the fundamental processes of plant physiological process at the molecular level, and the incorporation of this knowledge to understand how a plant works. This technique needs to include in the alteration and manipulation of the canopy architecture, the growth and development process of the leaf as well as the biochemistry of the photosynthetic regulating apparatus in addition to just inserting C_4 enzymes in the C_3 rice plant only.

10. Conclusion

Rice production needs to keep pace with the increasing rate of global population. With the help of advanced technologies and sustainable funding, C_4 rice can be available in the market in coming decades. However, the total process is very complex, thus only extensive research effort can help to overcome this constraint. There should be a holistic approach to understand the complex mechanism of C_4 photosynthetic pathway and successful introduction of this pathway into the rice genome.

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