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Ponnaboina Sriraj
M.Sc., Department of Genetics and Plant Breeding, School of agriculture, Lovely Professional University, Punjab, India

Dr. Deshraj Gurjar
Assistant Professor, Genetics and Plant Breeding, School of agriculture, Lovely Professional University, Punjab, India

Corresponding Author:
Ponnaboina Sriraj
M.Sc., Department of Genetics and Plant Breeding, School of agriculture, Lovely Professional University, Punjab, India

Breeding effects in conversion of c3 rice plant to c4 rice plant

Ponnaboina Sriraj and Dr. Deshraj Gurjar

Abstract
With the increasing population, the food production started decline rapidly. To increase the food production mainly rice plant, the improvement in crop is very important. The improvement in photosynthetic capacity of rice is very important for inserting C4 photosynthetic pathway in C3 plants for increasing the crop yield. The crops having enhanced photosynthetic mechanism and can use the solar radiations and the solar energy is converted into biological energy which directly increases the crop yield. There are different types of pathways like C3 pathway of photosynthesis, C4 photosynthesis and CAM pathway of photosynthesis. Rice exhibits C3 photosynthesis but due to low yield the rice plant is converted into C4 type plant. Different types of intermediates, genetic tools, sources, genes etc. are used to convert C3 plant into C4 plants. As the genes and the genetic makeup of C3 plants resembles with the C4 plants so it is somehow possible to convert it. Many other breeding techniques like the use of gene pyramiding is also used for making clones like Golden braid system and Golden gate. Different genome editing techniques like CRISPER-Cas and TALENs etc. are used. This review paper provides the complete information about the conversion of C3 plant to C4 plants, factors needed for the modification, comparison of C3 rice with C4 rice plant with respect to bundle sheath cells and mesophyll cell, vein spacing, density, tools and genetic editing techniques used.

Keywords: Breeding, genetic, mesophyll, photosynthesis, pyramiding, rice

Introduction
Now a days the population has been increasing day by day and it was estimated that in 2050 the population of world will reach up to around 9 billion (www.unpopulation.org), and according to population the quantity and quality should be also increase of rice by 60% as it is a staple food of world. The grains of rice is edible which have many nutritional qualities around world 95% of population prefer rice, it is cooked in many ways according to their interests. During 1960s at the time of green revolution people were suffering from feminine so to overcome this problem breeders and farmers produced new variety of rice known as miracle rice it had many qualities such as disease resistance and high productivity (Bhagwat, 2005) [1]. There are various types of plants found in earth and have their own way of photosynthesis and basically they are divided into 3 ways C3, C4 and CAM, if we talk about C3 plants such as wheat, barley, rye, cotton, rice, tobacco, but among them rice is one type of C3 plant, in this type of photosynthesis first the carbon is breakdown in to 3 carbon compound by the enzyme called ribulose-1, 5-biphosphate carboxylase oxygenase (Rubisco). The catylaztion of the RuBP (Ribulose-1, 5-biphosphate) is also done by Rubisco and this process is known as photorespiration. As we know rice grow in the area where temperature is above 30°c due to high temperature the oxygen rate also increases which decreases the efficiency of photosynthesis by 40% in C3 plants (Evans & Caemmerar, 2000) [6]. Therefore the process of photosynthesis becomes inefficient in tropics of rice plant. On the other hand in C4 plants leaves have the mechanism by which carbon dioxide can concentrate due to which the process of photorespiration decreased and which lead to easily survive in hot and arid environment. The photosynthetic efficiency was increased in C4 rice by the mechanism of photosynthesis as the plants use scarce resources and use nitrogen effectively (Biswal *et al*., 2018) [4]. As C4 plants have the ability to perform well under any stress condition such as high temperature or scarcity of water along with nitrogen too, among there pathway C4 is the type of photosynthesis that fix atmospheric carbon dioxide and thus it evolved independently more than 66%. The maximum wastage of energy is due to photorespiration therefore, reducing or total eliminating the function of Oxygenase by the specialized photosynthesis can be very effective.

In terms of carboxylation, Rubisco contain by C4 plants is much more effective than Rubisco contain by C3 plants. There are many benefits of C4 plant as the water use efficiency is very high due to half opening of the stomata because by this technique the concentration of carbon dioxide gradient can be easily maintained due to which even at high intensity of light the efficiency of photosynthesis will not saturate also due to less requirement of Rubisco the nitrogen use efficiency will also get increased (Kajala *et al*., 2011) [12]. By taking the example of rice we can say that in higher temperature C4 plants are very productive (Evans & Caemmerar, 2000) [6]. So among these 3 types C4 type is mostly preferred so the modification of photosynthesis system in rice can be challenging and could be complex too. But today as per the situation again these problem can come. If the population is increased then automatically the demand of food will also be increased and food, water and land is the common source for all flora and fauna but day by day the land is getting degraded and establishment of industrialization is taking place replacing farmland. Therefore, many new techniques are introduced by the scientists in different fields such as physiology, breeding, entomology, genetics, bioinformatics, molecular biology, genetic engineering (Mitchell & Hardy, 2000) [20].

Comparison of C3 RICE and C4 RICE with respect to bundle sheath cells

The mesophyll cell which is located within the leaf contains 90% of the chloroplast on the other hand the chloroplast is located in both cell mesophyll and bundle sheath in C4 plants (Bianjiang & Demao, 2009) [2]. The process of photosynthesis takes place in mesophyll cell only in case of C3 plants while in C4 plants the process of photosynthesis is divided into two parts one is mesophyll cell and other is bundle sheath cell. The process start with the fixation of carbon dioxide in mesophyll cell in which the CO2 is divided into four different compounds which is known as oxaloacetate and this compound is further converted into malate which is a C4 acid and after that it is transported into the bundle sheath cell, in there the process of calvin cycle takes place which assimilate the carbon dioxide into carbohydrate (Nomura *et al*., 2005) [21]. Therefore, in C3 plants only one cell is responsible for photosynthesis i.e. mesophyll cell, while in C4 cell both mesophyll and bundle sheath cell is equally responsible for the photosynthesis as decarboxylation and calvin cycle involved in C4 pathway, because there is enlarged bundle sheath cells are present which include large number of chloroplast in it. In C3 plants the work of bundle sheath cells are different like the balance of hydraulic pressure is maintained by this cell, there is possibility of air entry in to the xylem by intercellular space so bundle sheath cell prevent this entry and during transpiration there is huge loss of buffer BSC also prevent it by providing a water reservoir along with this it maintain the entry of light which have high intensity and can hit the leaves vein (Raven *et al*., 2005) [22]. There are more function of bundle sheath cell in C3 plants as it acts as a transporter of nitrogen, carbohydrate, sulphur and also signalling pathway. In C4 plants both cell i.e. mesophyll and bundle sheath cell work co-ordinately which is more beneficial for the plant as the leaves of C4 plant have special kind of leaf anatomy so it can be a great step if we introduce C4 pathway to the rice by this method the photosynthetic efficiency will easily increase just we have to increase the number of chloroplast by increasing bundle sheath cell and it can be possible by introducing genetic elements such as GLK (golden 2 like genes) with the help of PEPC (phosphoenolpyruvate) which is a promoter of C4 gene (Bissinger *et al*., 2019) [3].

Comparison of C3 RICE and C4 RICE with respect to vein spacing and density

From the above discussion we get to know that in C3 plants the photosynthesis takes place only in the mesophyll cell thus in the leaves of the vein there are huge amount of mesophyll cell are present thus due to which there is more space between two veins and automatically the density of veins decreases and in case of rice there is total 6 veins/mm. on the other hand in C4 plants there is total 2 mesophyll cells in between the vein thus the vein density increases. If we look closely to the internal anatomy of C4 veins we can observe the pattern in which the sequence is repeating like BS-MC-MC-BS veins, and by this pattern we can easily see that the mesophyll cell is surrounded by the bundle sheath cell by the wreath like structure and this type of leaf anatomy is known as “kranz anatomy” which is given by G. Haberlandt a German botanist (Matsuoka *et al*., 1994) [18]. The dense cytoplasm is located at the bundle sheath cell of C4 plant which contain large number of chloroplasts and it is required that mesophyll cell and bundle sheath cell should be close contact with each other than only it can function efficiently (Matsuoka *et al*., 1998) [19]. Thus, in different crops the characteristics of C4 plants is introduced to C3 plants like in oat plants the chromosomes of maize is introduced by which the size of bundle sheath cell increases in oat and the density will increased. Similarly, in rice this characteristics can lead to a great difference in the field of agriculture and lead to high productivity (Ermakova *et al*., 2021) [5].

According to the photosynthesis pathways opted by the plant, they are divided into various physiological groups. There are three different types of photosynthetic pathways which are C4 mechanism, C3 mechanism and CAM mechanism. These pathways are used by the plant for completing their life cycle. Mainly the plants exhibit C4 pathway of photosynthesis because this pathway is highly efficient and used to convert solar energy into biological energy. At about 25% of terrestrial plants in land, they exhibit the C4 pathway of photosynthesis and mostly the terrestrial plants exhibit C3 pathway of photosynthesis. The C3 pathway of photosynthesis is also known as Photosynthetic carbon reduction cycle or PCR cycle. The C3 pathway of photosynthesis first incorporates inorganically into (Rubisco) ribulose-1,5- bisphosphate carboxylase or oxygenase through catalysis which give rise to (RuBP) which is a five-carbon sugar phosphate, ribulose-1,5- bisphosphate. This product is a six-carbon compound and is unstable in nature (Sheehy, 2000) [23]. After hydration, this molecule breaks down and two molecules of three carbon compounds are formed known as (3 PGA) or 3-phosphoglyceric acid. This product is the first stable product of C3 cycle. Due to the formation of 3 carbon compounds this cycle is also known as C3 cycle. In C3 plants the process of photosynthesis always occurs in the mesophyll cells present on the leaf surface (Furbank *et al*., 2009) [7]. Firstly, the carboxylation process occurs where the Rubisco interacts with oxygen molecules at a very low affinity. At this time the atmospheric oxygen is available at a good amount approximately 20% and the Co2 is present in a very low

amount which is approximately 0.03%. Here, some part of Rubisco is involved with oxygen. The oxygenase activity of Rubisco produces 3-PGA compounds as compared to the carboxylase activity. With the process of photorespiration, phosphoglycolate also produces (Khush, 2013) [15]. Photorespiration occurs with the detoxification of mitochondria and peroxisome and this will lead to cause loss in energy. The involvement of Rubisco with atmospheric carbon dioxide causes some alterations in the oxygenase activity. Plants sometimes avoids the process of photorespiration, sometimes the plants use different enzymes for the fixation of carbon dioxide which does not reacts with the oxygen. While in some plants, bundle sheath cells are sequestered by Rubisco by developing the carbon dioxide concentrating mechanism. Firstly, the carbon dioxide is fixed in cytosol with the help of enzyme Phosphoenolpyruvate carboxylase and give rise to Oxaloacetate, which is also a four-carbon compound molecule (Liu *et al*., 2013) [17]. With the formation of four carbon compound, this cycle is named as C4 cycle. The enzyme Phosphoenolpyruvate carboxylase always interacts with the carbon dioxide and is sensitive towards the oxygen compound. After this process, the Oxaloacetate is converted into malate and diffused into bundle sheath cells with the help of enzymes Phosphoenolpyruvate carboxykinase. Due to the presence of carbon dioxide, the effectiveness of Rubisco also increases ten times and the effectiveness to survive in high temperature, arid conditions and harsh temperature in C4 plants also increases. With the reduction of Oxaloacetate, according to the decarboxylation modes, the plants are divided into three types that are Phosphoenolpyruvate carboxykinase, NADP malic enzyme and NAD malic enzyme. The C4 pathway of photosynthesis works with two types of cells, the bundle sheath cells and mesophyll cells. The bundle sheath cells help in increasing the concentration of carbon dioxide around Rubisco (Karki *et al*., 2013) [14]. This type of photosynthesis functions by providing expression to the genes in both the bundle sheath cells and mesophyll cells. The carbon assimilation pathway and the electron transport complexes of photosynthesis helps in the early development of leaves in Kranz anatomy (Hartzell *et al*., 2018) [10]. The other alternative to C3 cycle is the CAM plants that are also known as Crassulacean Acid Metabolism plants. This is the oldest method of photosynthesis and this type of pathway is mostly found in the plants which can adapt the extreme harsh climatic conditions. The plants of this pathway can also fix carbon dioxide just like the C3 plants by using the enzyme Rubisco. In this pathway of photosynthesis, at the night time, the carbon dioxide is produced from the malic acid which is stored internally. Because in light the malic acid releases the internal carbon dioxide, this is the reason that the plants fix atmospheric carbon dioxide at night time or at darkness. This uses a special PEPC system for storage of malate. So, in CAM plants the stomata are completely closed at day time (Gebauer, 1987) [8].

Conversion of C3 Plants to C4 Plants

For the conversion of C3 plants to C4 plants various intermediates are required. A step wise transition is there to convert the C3 plant to C4 plants. Various efforts are made to map the quantitative trait loci between the two species. Various crosses are made in between C3 to C4 but due to aneuploidy, it does not get success. In university of Toronto, Canada, Oakley *et al*., 2014 [3] made a cross between *Atriplex prostate* and *Atriplex rosea* which resembles the C3- C4 intermediates and also disrupts the C4 cycle (Gowik & Westhoff, 2011) [9]. From this experiment they generated F3 lines and for the generation of F3 lines new techniques are used. In modern techniques, biotechnological tools are used for hybridisation and for saving the embryo (Kamal & Ahmed, 2020) [13]. The genome sequencing technique is used to know the huge amount of data for understanding the difference between C3 and C4 plants. With the help of DNA and RNA sequencing the plants are converted into C4 type. Rice is a C3 crop and produces less yield as compared to C3 plants maize. To increase the yield potential of rice plant, the genetic engineering is used for increasing the rice yield with a good percentage. Various reasons are there for converting rice C3 to C4 plant. The C3 plants involves grasses and they have multiline lineages nature. With the help of convergent evolution, the C3 rice is converted into C4 rice plant. The enzymes and the genes present in C3 plants are also found in C4 plants, only 3% difference are there. To increase the rice crop yield, it is good to convert rice into C4 plant. Many other breeding techniques like the use of gene pyramiding is also used for making clones like Golden braid system and Golden gate. Different genome editing techniques like CRISPER-Cas and TALENs etc. are used (Li *et al*., 2011) [16].

Tools and Resources Used in Conversion of C3 to C4 Rice

To convert the C3 in to C4 different tools and resources are required and they are grouped in several categories such as; gene expression data, mutant resources, databases, genome sequence resources. Earlier this research has grabbed many attention of breeders and scientists and thus many related studies are also present which involves comparison of leaf transcriptome of both species i.e. C3 and C4, while other studies like gene expression which involve the development of vein. The study related to plant tissue relevant to C3 and C4 plants are also available, this kind of resource is also called BAR (bio-analytic resource) in which we can find out the plant biology along with this it also provides the information of maize gene expression particularly about mesophyll and bundle sheath cell and development of rice leaf gradient. The other study is genomic research which deals with the challenges faced during the conversion of C3 to C4. But researchers overcome this problem too by improving their method by tissue addressing and this database is known as plant homolog database. The last tool or resource is very important and known as genome sequence resources (Horton & Murchie, 2000) [11].

Conclusion

Rice is a very important food grain crop but due to increase in population, the rice crop does not completely meet the requirement of food. Due to reduction in yield as compared to the other grain crop, the genetic technique comes in mind to improve the photosynthetic pathway of the crop. Rice is a C3 crop but due to small leaf size or grass type crop, the yield gets reduced. So, to overcome from this technique with the help of genetic engineering and different breeding techniques the genetic photosynthetic pathway of rice is changed into C4 plants for increasing the grain yield. The plants with enhanced photosynthetic activity increase the nitrogen use efficiency, reduces water loss, utilizes solar radiation properly and results in high grain yield. This paper provides the information about

C3 plants, C4 plants, CAM plants and their differences. The breeding techniques used in the conversion of C3 plants to C4 plants, tools, sources and techniques like gene pyramiding, Crisper, Talen etc. used during conversion etc.

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