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An insight: Impact of reduced Rubisco on plant physiology and biochemistry

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ABSTRACT

Photosynthesis is a process of conversion of sunlight energy and atmospheric carbon to organic molecules with the help of a key and that is Ribulose-1,5-bisphosphate carboxylase/oxygenase. Ribulose-1,5-bisphosphate carboxylase/oxygenase (RubisCo) is one of the most abundant proteins in the biosphere and a key enzyme in the global carbon cycle and its assimilation. RubisCo has been extensively studied regarding its structure, kinetics, evolution, etc. But still, many questions remain an illusion such as why plants maintain a large pool of RubisCo protein and its many isoforms; how the different isoforms coordinate their functions altogether and how does RubisCo affect photosynthetic rate, biomass allocation and vegetative growth of plants, although much research has been conducted in the recent past to answer these questions. In this review, different physiological, biochemical, and molecular studies aimed to reduce RubisCo in plants will be discussed to answer above mentioned questions and to better understand it's functioning.

Key words: Photosynthesis, Plant growth, RubisCo

With a rising population of the world and rough addition of 83 million people every year (Desa 2019) it is estimated to be around 9.8 billion by 2050. To feed such a whopping population, there is an urgent need to improve crop yield to meet projected demand for agriculture produce, which is expected to double by 2050 (Alexandratos and Bruinsma 2012). According to an estimate we need to double the food production, and to meet this demand cereal production must rise from 2.1 million tonnes to 3 million tonnes (Alexandratos and Bruinsma 2012). Achieving such a production increase, under a changing climate with limited land and water resources is a great challenge for agricultural scientists and food security. Photosynthesis by the autotrophic organisms is the main source of biomass production. The performance of photosynthesis is an outcome of multiple enzymatic steps (Tyagi and Chandra 2006, Simkin *et al.* 2019). Understanding the performance of enzymes involved in photosynthesis is much-needed research to increase plant productivity and photosynthetic performance (Parry *et al.* 2013). Out of many key steps, assimilation reaction is controlled by Rubisco protein which is a much-talked enzyme of the photosynthetic system (Farquhar and von Caemmerer 1982, Sage 1990).

Ribulose-1,5-bisphosphate (Rubp) carboxylase/oxygenase (*RubisCo*, EC 4.1.1.39) catalyzes the photosynthetic assimilation of CO_2 into organic compounds. Although RubisCo is the most abundant protein in the biosphere making up 50% of the total soluble protein found in plant leaf tissues and only enzyme capable of total net assimilation of carbon has many limitations (Andrews and Lorimer 1987). RubisCo rate limitation in Calvin-Benson-Bassham (CBB) reductive pentose phosphate pathway is characterized by its lower catalytic rate (*kcat*) of ~5/sec, poor affinity towards CO_2 and a competing substrate reaction with O_2. These limiting factors for RubisCo are partially compensated by plants either by keeping an extra-large pool of RubisCO (15-35%) in C_3 plants (Evans 1989, Maheshwari 2020) or by an evolutionary mechanism such as utilization of Kranz anatomy (CO_2-concentrating mechanisms) in C_4 plants. A better understanding of RubisCo is very much necessary for better productivity and efficient resource utilization by plants (Parry *et al*. 2007).

Many studies were conducted in the past to understand the function, limitation, evolution, and structure of RubisCo at various levels (Pearce and Andrews 2003, Tabita *et al.* 2007, Andersson *et al*. 2008, Whitney *et al.* 2011, Stec B 2012, Gruber and Feiz 2018) and effect of RubisCo on photosynthesis, plant growth, nitrogen utilization (Stilt M *et al*. 1991, Lauerer 1993, Makino *et al*. 2000) etc. To understand the RubisCo impact and control on photosynthesis, plant growth, nitrogen utilization, studies were conducted by applying reduction approach (Maheshwari *et al.* 2020). The reduction approach provides an experimental strategy to understand the control flux through the pathway. Some of the studies were also conducted to understand the various isoforms of RubisCo and their differential expression to better understand the coordination among different isoforms

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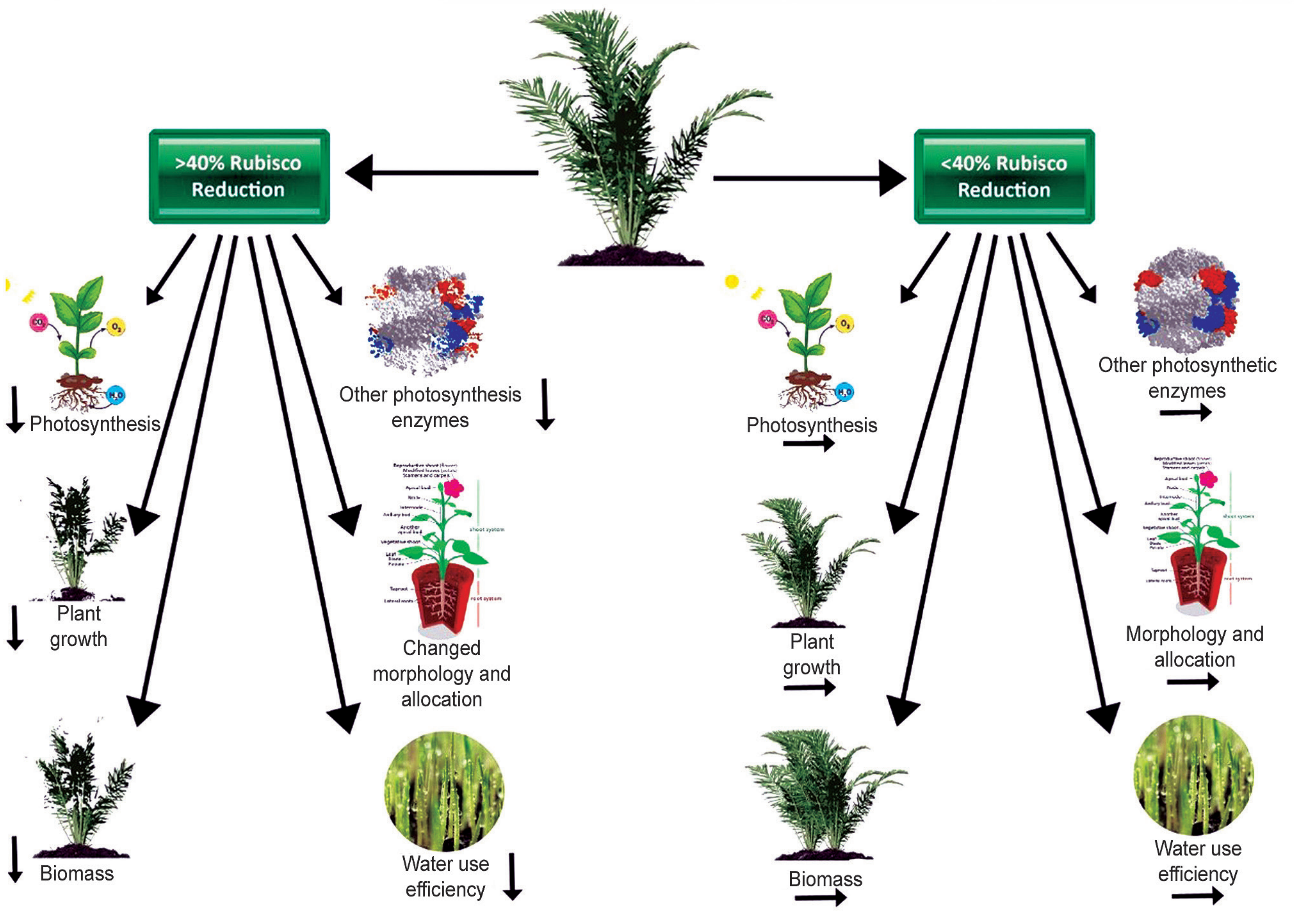

Fig 1 Depiction of overall impact of RubisCo on photosynthesis, plant growth and other growth parameters. Arrow showing the trend (Upward-Increase; Downward- Decrease and Horizontal-No change).

(Ren *et al*. 1991, Morse *et al.* 1995, Hansen *et al*. 1999, Tabita *et al.* 2007). In this review, we examined how reduction/suppression of RubisCo affects the plant at the physiological and biochemical levels (Fig 1).

Impact of reduced RubisCo on photosynthesis

Rubisco accounts for up to half of the leaf nitrogen (). In the past research focused on various plant species with reduced RubisCo to understand its effect on photosynthesis. Strategies that were followed to reduce the RubisCo aimed at targeting the small subunits of *RubisCo* (rbcs) (Rodermel *et al*. 1988, Quick *et al.* 1991, Makino *et al.* 2000). Small subunits of *RubisCo*, which were encoded by a multigene family in the nucleus consist of 2–22 members, depending on the species (Spreitzer 2003). Although RbcS genes share a high similarity, differential expression was observed at the leaf and tissue level (Spreitzer 2003, Dean *et al.* 1989). Owing to a huge similarity, it has been assumed that RbcS genes do not have any functional difference (Morita *et al.* 2014). Many studies which were conducted on tobacco plants with reduced RubisCo to a tune of 20–60% relative to wild type reported a reduction in photosynthesis rate (Quick *et al.* 1991, Hudson *et al.* 1992, Andrews *et al.* 1995, Rodermel *et al.* 1988, Maheshwari *et al.* 2020). To understand the flux through a pathway of a particular protein, the best way is to bring the small change in the protein amount in question (say 20%) and if the flux also reduced by the same 20% then the enzyme alone is controlling the pathway. On the otherside, if the flux is reduced by say 10% then control of the pathway is shared by other enzymes (Kacser and Burns 1973). Flux-control coefficient (C_R) depends on short term conditions. Flux-control coefficient (C_R) of 0.1 with 50% RubisCo had marginal effects on photosynthesis under moderate light condition while in high light condition C_R value was 0.7 (Stitt *et al.* 1991). It has been also observed that with the increase of irradiance in transgenic tobacco plants with reduced RubisCo C_R increased slightly (Lauerer *et al.* 1993). In summary, C_R increased with increasing irradiance and humidity and decreasing external CO_2 (Stitt *et al.* 1991).Varying nitrogen supply too had an impact on C_R as plants grown in low nitrogen had an appreciably higher C_R compared to plants grown in high nitrogen supply (Quick *et al.* 1992, Fichtner 1993). A number of studies reported that impact of suppression of RubisCo on photosynthesis is compensated by various mechanisms such as an increase in RubisCo activity increased thylakoid energization, pH-dependent energy dissipation etc. (Stitt *et al*. 1991). Rice plants with a 10–25% decrease in RubisCo reported an improvement in photosynthesis at elevated CO_2 which justifies the extra amount of RubisCo (Kanno *et al.* 2017). Another experiment with a 35% reduction in RubisCo reported a 20% reduction in photosynthesis at lower CO_2 (36 Pa) and 5–15% increase in photosynthesis rate at elevated CO_2 (100Pa) (Makino *et al.* 2000). Studies on rice plants confirmed that at elevated CO_2 plants with 35% reduced RubisCo had high nitrogen use efficiency for photosynthesis (Makino *et al.* 2000). These studies confirmed the active role of RubisCo in controlling photosynthesis if RubisCo reduced beyond a certain level under unfavorable growth conditions such as low nitrogen supply, low irradiance and low CO_2. However, at elevated level of CO_2, an increase was reported in rice while no increase was observed in tobacco plants (Quick *et al*. 1991, von Caemmerer *et al*. 1994, Makino *et al*. 2000).

Impact of reduced RubisCo on metabolome

Metabolome refers to a complete set of metabolites present in a particular situation and time that provides a direct functional readout of cellular activity and physiological status (Khan *et al.* 2020). The metabolomics also helps to identify the function of the genes: how a particular gene affects the metabolic pathway and discovers different layers of regulation and interception between connected pathways (Sti). Photosynthesis which is an essential part of plant survival and only source of carbohydrate generation is also playing a major role in metabolites production in downside reactions (Sharkey 1989). RubisCo which is an essential enzyme involved in photosynthesis and also being present in an extra amount to compensate its limitations has been studied by various groups of scientists to understand how does RubisCo reduction affects the metabolome of plants. Antisense RubisCo plants were developed by group of scientists to understand the consequences of a lesion in photosynthetic carbon metabolism for nitrogen metabolism and secondary metabolism (Rodermel *et al.* 1988, Quick *et al.* 1991, Makino *et al*. 2000). A study on tobacco plants with reduced RubisCo (40%) had reported a decrease in starch, sucrose, glucose, and fructose content under low nitrogen supply while with high nitrogen these changes were not reported (Fichtner *et al.* 1993). A study on tobacco plants with reduced RubisCo reported a decrease in triose-phosphate, glucose-6-phosphate, fructose-6-phosphate, ADP and an increase in nitrate (Quick *et al*. 1991, Masle *et al*. 1993, Stitt and Schulze 1994). Quick *et al*. (1991) reported a decrease in nitrate accumulation in leaves of tobacco plants with reduced RubisCo along with an increase of ribulose 1,5-phosphate and decline in 3-phosphoglycerate. Metabolome analysis of rice plants with reduced RubisCo reported a decrease in organic acids, carbohydrate, ketones, aldehydes and a significant increase in amino acids. Although an increase in amino acids was reported the same was not reported in urea cycle related compounds such as putrescine, spermidine, and spermine. A decrease in RubisCo level in plants was accompanied by a decline in secondary metabolites such as nicotine, chlorogenic acid, and rutin (Matt *et al.* 2002). These studies had concluded that changes in RubisCo will affect the wide range of metabolites which are directly linked to photosynthesis, photorespiration and amino acid metabolism due to key importance of RubisCo in carbon-nitrogen economy balance in plants. Also, an inhibition of photosynthesis and decrease in sugar levels leads to a general inhibition of nitrogen metabolism and dramatic changes in the levels of secondary metabolites (Matt *et al.* 2002).

Impact of reduced RubisCo on plant growth

Plant growth is a direct indicator of active photosynthesis and metabolism of plants. While many factors affect the plant's growth externally, internal factors (photosynthesis) do play a key role in the overall growth of plants. Photosynthesis gets affected by external (sunlight, water, nutrition, etc.) and internal (enzyme efficiency, substrate utilization, availability of substrate, etc.) factors. Internally photosynthesis rate is largely controlled by RubisCo which is a key regulator of the photosynthesis cycle. Earlier plants with reduced RubisCo and photosynthesis provided a study model to understand plant growth (Stitt and Schulze 1994). Many researchers have tried to answer the question, how RubisCo suppression by genetic manipulation affects plant growth in different species and cultivar? A study on tobacco plants with more than 50% reduced RubisCo brought many changes in plant growth such as a decrease in plant weight, root weight, and changes in leaf geometry (Quick *et al*. 1991). Relative growth rate which is estimated by dry weight increment per dry weight per day decreased in tobacco plants and showed a near-linear relationship with photosynthesis (Fichtner *et al*. 1993). Similar results, i.e. reduction in shoot and root mass were observed in rice plants with reduced RubisCo when grown at low CO_2 (Makino *et al*. 2000). Rice plants with reduced RubisCo, when grown at higher CO_2 reported higher biomass and relative growth rate (Kanno *et al.* 2017). Overall, these studies concluded that reduction in RubisCo affects plant growth only when reduction goes beyond 50%, which justifies the extra investment of plant nitrogen on RubisCo. Some of these studies also justify that at high CO_2 level plants with reduced RubisCo perform better than the wild type due to effective resources utilization, which is justified by an increase in net assimilation rate (NAR) and nitrogen use efficiency (NUE) (Makino *et al.* 2000, Kanno *et al*. 2017).

Effect of RubisCo suppression on gene regulation

In higher plants, RubisCo holoenzyme is a hexadecamer, composed of eight large (55-kD) subunits, coded by a single gene in chloroplast genome and eight small (15-KD) subunits coded by a multigene family in the nuclear genome (Dean *et al.* 1989). Few studies were conducted on how the suppression of RubisCo at the transcript level affects the expression of related genes. Suppression of *RBCS* in higher plants such as tobacco (Rodermel *et al.* 1988, Hudson *et al*. 1992), *Flaveris bidentis* (Furbank *et al*. 1996), rice (Makino *et al.* 1997) and Arabidopsis (Izumi *et al*. 2012) led to a coordinated reduction of RBCL and RBCS at the protein level. On the other side overexpression of *RBCS* in rice lead to an increase in *RBCL* (Suzuki *et al*. 2009). M. Ishizuka *et al.* (2004), reported a decrease in the activity or amount of Cp-FBPase, NADP-G3PDH, Ru5P kinase, and SPS in rice plants with 65% and 40% reduced RubisCo. In 2002, Mat *et al*. reported that decrease of Rubisco activity led to an inhibition of nitrate reductase activity in tobacco plants.

Conclusion

With these studies on board, it is concluded that RubisCo does have its functional limitations which gets compensated by its large pool in C_3 and C_4 plants. At the same time plants with reduced Rubisco by genetic manipulation and or through genetic or environmental factors provides an experimental system to evaluate the role of RubisCo in regulation of photosynthesis and plant growth. These studies confirm that at higher CO_2, nitrogen availability and irradiance plants with reduced RubisCo have in significant impact on plant growth due to efficient utilization of resources and their assimilation. Rubisco does have control over plant growth under limiting resources which justifies that plant growth and photosynthesis is controlled by many other external and internal factors besides RubisCo. Although much of the research work which is listed in the review conducted in near past, still there is a large scope of revisit this RubisCo science to understand it better in more refined way with new techniques on board.

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