

# Upregulation of chloroplastic pyruvate dehydrogenase genes in rice leaf would potentially drive the in planta photorespiratory bypass for higher biomass

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## Research Article

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# Abstract

At ambient temperature (25–30 °C) and the prevailing atmospheric CO<sub>2</sub> levels (380 ppm), installing the C<sub>4</sub> photosynthetic machinery in a C<sub>3</sub> plant would potentially drive away the photorespiratory process through a carbon concentrating mechanism (CCM), thereby preventing oxygenation reaction of Rubisco. Development of C<sub>4</sub> rice is a global research priority, for enhanced water use efficiency (WUE) and yield. At optimal environment, the difference in the solar energy to biomass conversion between C<sub>3</sub> and C<sub>4</sub> plants is mainly due to photorespiration. So, photorespiratory bypasses are the potential alternatives than conversion to C<sub>4</sub>. Genetically transformed C<sub>3</sub> model plants with photorespiratory bypass had demonstrated higher biomass (under same environmental conditions) than its wild type. Using a transcriptome approach, we report here the differential expression pattern for photorespiratory genes and chloroplastic pyruvate dehydrogenase (*pIPdc*) gene between the leaves, peduncle, and the developing grain tissues in three rice genotypes. In addition to pyruvate, glycolate and glyoxylate also are the substrates for the *pIPdc* gene product and hence a suitable candidate for photorespiratory bypass.

## Introduction

At optimal environmental conditions, solar energy to biomass conversion efficiency is roughly 25% higher for C<sub>4</sub> plants than the C<sub>3</sub> ones, with key differences in photorespiration, while loss through respiration in light is unavoidable<sup>1–3</sup>. The C<sub>4</sub> trait, a carbon concentrating mechanism (CCM) that drives away photorespiration through preventing oxygenation reaction of Rubisco, is reported to have convergently evolved multiple times, nevertheless, same gene lineages were recruited in the C<sub>4</sub> trait evolution<sup>4,5</sup>. Converting a C<sub>3</sub> crop plant with a C<sub>4</sub> pathway to enrich the solar energy conversion efficiency for higher biomass is one of the research priorities to improve yield. Installing photorespiratory bypasses is also a viable alternative, demonstrated to improve efficiency with higher biomass through reduced photorespiration in model C<sub>3</sub> plants<sup>6–8</sup>. Also, it is important for plants to metabolize 2-phosphoglycolate (2-PG, formed through the oxygenation process of rubisco) and glyoxylate (key intermediate of photorespiration), to overcome the metabolite toxicity that inhibits photosynthesis and starch biosynthesis<sup>9,10</sup>. Installing a C<sub>4</sub> machinery or a photorespiratory bypass in C<sub>3</sub> plants would help enhance the assimilation rate under optimal environmental conditions<sup>7,8,11</sup>.

In addition to pyruvate, glycolate and glyoxylate – the intermediates of photorespiration – also acts as a substrate for the chloroplastic pyruvate dehydrogenase complex (pIPDC) in plants<sup>12</sup>. The PDC constitutes three components, E1 (pyruvate dehydrogenase in heterotetramer state-a2b2), E2 (dihydrolipoyl acetyltransferase, homodimer) and E3 (dihydrolipoyl dehydrogenase, monomer) with copy numbers of these components in the complex is variable<sup>13,14</sup>. Recent study highlights the E2 component's RNA binding activity with *psbA* mRNA coding for the D1 protein of the PSII reaction center<sup>15</sup>. To understand the expression pattern for genes of photorespiration and the C<sub>4</sub> pathway in leaf and

non-leaf (photosynthetic) tissues in rice, transcriptome analysis was performed in the three rice genotypes, with two biological replicates.

## Materials And Methods

Three rice (*Oryza sativa* ssp. *indica*) genotypes – Apo (EC734333), BAM4234 (EC497171), and Crossa (IC575838) – were grown under field conditions during *Kharif* season 2018 in triplicate at the research farm of the Division of Plant Physiology at IARI (New Delhi). Flag leaf, peduncle and developing grains (3–5 days-post-anthesis, dpa) were collected in two replicates, snap frozen using liquid nitrogen (-196 °C) and stored at -80 °C for transcriptome studies. The experiment was planned with two replicates since the study involves three genotypes. The expression levels between genotypes for most of the genes studied were insignificant (Supplemental File\_S1, hence genotypes could equally be considered as biological replicates, totaling to six (two replicates x three genotypes). So, technically, the expression pattern reported in the study for each tissue is supported with an equivalent of six biological replicates. All methods pertaining to this study were performed in accordance with the relevant guidelines / regulations / legislation as applicable.

Total RNA from the samples (80-100mg) was extracted using a RNeasy plant mini kit (Qiagen, USA) following the manufacturer's protocol. The quality and quantity of the RNA was assessed using a Bioanalyzer 2100 (Agilent technologies, USA) and spectrophotometer ND-8000 (Thermo Scientific, USA). The RIN values for the 18 samples (3 genotypes and 3 tissues, repeated twice) ranged from 7.0 to 9.5. RNA-seq libraries were sequenced on an Illumina platform (2x150bp paired-end reads). A total of 521 million pairs of reads were obtained. Adapter trimmed reads were quality checked using FastQCv0.11.8<sup>16</sup>. These pre-processed reads were mapped against the *indica* rice (ASM465v1) genome sequence<sup>17</sup>. Mapping and alignment against the reference were done using Tophatv2.1<sup>18</sup>. Summary statistics on the number and percent reads mapped were provided in the Supplemental File\_S2. Cufflinks v2.2.1 was used to assemble the individual transcripts for expression quantification<sup>19</sup>. The assembled transcripts were merged for the differential expression studies between each tissue (leaf vs peduncle, leaf vs grain, and peduncle vs grain) in every genotype and *vice-versa* (to confirm no significant differential expression for the genes / transcripts studied, between genotypes for the same tissue) using Cuffmerge<sup>20</sup>. The expression values (in RPKM) were tested for statistical significance at FDR 0.01 cutoff value using Cuffdiffv2.2.1<sup>19</sup>.

Including the key eight genes coding for the gene products being involved in photorespiration<sup>21</sup>, totally, 42 transcripts (with gene ids) were identified for the 11 genes involved in photorespiration viz., phosphoglycolate phosphatase-chloroplastic (*cpPGLP*), glycolate oxidase-peroxisomal (*pGOX*), glutamate:glyoxylate aminotransferase-peroxisomal (*pGGT*), serine hydroxymethyltransferase-mitochondrial (*mSHMT*), glycine decarboxylase-mitochondrial (*mGDC*), glycerate kinase-chloroplastic (*cpGLYK*), glutamine synthetase-chloroplastic (*cpGS2*), glutamate synthase (*cpGOGAT*), serine:glyoxylate aminotransferase-peroxisomal (*pSGT*), and hydroxypyruvate reductase-1 and -2 (*HPR-1 & -2*). The

corresponding transcript ids were identified and extracted from the plants ensembl database<sup>22</sup>. This is done since few genes were not functionally annotated. Expression profiles (in RPKM – reads per kilobase of transcript per million mapped reads) including statistical significance and log fold change details for the genes of interest were extracted from the transcriptome analysis (Supplemental File\_S1) and studied for its biological significance. Based on the results obtained, the expression profiles for transcript ids annotated with chloroplastic pyruvate dehydrogenase complex gene (*pdh*) were also studied from the transcriptome dataset and results are tabulated and given in Supplemental File\_S1. In addition, expression levels for Rubisco small subunit (*rbcS*) transcripts were also compared between the three tissues for all the three genotypes. Since the expression values of the *rbcS* transcripts are also significantly downregulated in developing grains (Supplemental File\_S1), when compared to leaves, ratio for expression level between leaf and developing grain' in each genotype has been worked out (excel sheet 'Ratio' in Supplemental File\_S1). For those transcripts with expression values significantly higher in leaves are greater than one. The *rbcS* transcript with highest expression in both leaf and developing grain is identified, and its ratio is used as the threshold ratio to identify the set of photorespiratory genes that are significantly downregulated, and simultaneously above the threshold ratio (cells highlighted in green, in excel sheet 'Ratio' in Supplemental File\_S1).

## Result And Discussion

The eight genes of photorespiratory enzymes *viz.*, phosphoglycolate phosphatase-chloroplastic (*cpPGLP*), glycolate oxidase-peroxisomal (*pGOX*), glutamate:glyoxylate aminotransferase-peroxisomal (*pGGT*), serine hydroxymethyltransferase-mitochondrial (*mSHMT*), glycine decarboxylase-mitochondrial (*mGDC*), glycerate kinase-chloroplastic (*cpGLYK*), serine:glyoxylate aminotransferase-peroxisomal (*pSGT*), and hydroxypyruvate reductase (*HPR*); two genes encoding for glutamine synthetase-chloroplastic (*cpGS2*), glutamate synthase (*cpGOGAT*), were studied and found to be significantly downregulated in the developing grains (ca. 3–5 days post-anthesis) than the leaves, in all the three rice genotypes (Supplemental File S1). Although it can be argued that the downregulated expression pattern in developing grains is an expected one when compared to leaves, to identify the biological significance, expression pattern for *rbcS* gene transcript was also studied (Supplemental File\_S1, 'ratio' worksheet). Those genes for which the expression pattern ratio between leaf and developing grains are greater than the ratio of *rbcS* gene, they were identified to play proportionately equal or higher role as in the leaves.

However, downregulation of photorespiratory genes might lead to cell toxicity due to the accumulation of 2-PG and glyoxylate, notably when the plant is under abiotic stress<sup>9,10</sup>. Conversion of these two metabolites into non-toxic compounds is primarily important to overcome the cellular toxicity, as well as to sustain the availability of ADP and NADP for accepting light energy<sup>23</sup>. Diversion of the 2-PG to bypass the photorespiratory process is reported to improve the plant biomass<sup>6–8</sup>. Alternatively, chloroplastic pyruvate dehydrogenase complex (pPDC) is reported to detoxify glyoxylate, producing CO<sub>2</sub> in chloroplast<sup>12</sup>, potential for a natural photorespiratory bypass to enrich the CO<sub>2</sub> for rubisco carboxylation process. In

addition to glyoxylate, glycolate also acts as a substrate for pIPDC, and CO<sub>2</sub> production from these metabolites are competitively inhibited in the presence of pyruvate<sup>12</sup>.

So, to understand the expression pattern at transcriptional level, we compared the expression levels of *pIPdc* (Supplemental File\_S1) and found that the transcript levels of *pIPdc* were significantly higher in the developing grains as compared to the leaves, in all the three genotypes. It gives an insight on the possible use of pIPDC to establish a photorespiratory bypass (Fig. 1). This would potentially aid in developing an efficient photorespiratory bypass, *in planta*, through enhanced *pIPdc* gene expression levels targeting for higher biomass or yield. Whether the upregulated *pIPdc* diverts the photorespiratory process or vice-versa, is unknown yet. Plants accomplishing C<sub>2</sub> photosynthesis have evolved for the preferential downregulation of *mGDC* (glycine decarboxylase, mitochondrial) or anatomical rearrangements (with more chloroplasts at the periphery) in mesophyll cells either reduce CO<sub>2</sub> release or provides high resistance to CO<sub>2</sub> efflux<sup>7,24</sup>. This is commonly called 'C<sub>2</sub> shuttle' and helps increase the plant productivity with high biomass or yield. With initiation of photorespiration through oxygenation reaction of Rubisco, conversion of the toxic metabolites 2-PG (through glycolate) and glyoxylate in chloroplast itself through pIPDC to release CO<sub>2</sub> will enrich the carbon flux for Rubisco's carboxylation process would simulate a natural photorespiratory bypass. Present study gives an insight for the probable existence of certain group of plants that have evolved to recapture the CO<sub>2</sub> released in the process of converting glycolate / glyoxylate to Acetyl-CoA through pIPDC in chloroplast itself, possibly having the shortest C<sub>2</sub> shuttle that also help enhance the plant productivity. Expression levels of Acetyl-CoA carboxylase (ACCase), the key enzyme that channelizes the carbon flux for fatty acid (FA) biosynthesis, is insignificant between leaf and grain tissues studied and suggesting for the expression of *pIPDC* is not associated with FA biosynthesis. Alternatively, these Acetyl-CoA pools formed through the action of pIPDC might possibly utilized for N-terminal acetylation through the action of plastidic N-terminal acetyltransferases (pINATs). This is in line with the reports suggesting ca. 30% of the plastid proteins are subjected to the action of NATs, especially the chlorophyll binding proteins and other enzymes of photosynthetic apparatus<sup>25-29</sup>.

Overall, our results show that, the significant downregulation of photorespiratory genes in the developing grains of rice as compared with leaves exhibit biological significance; with the ratio (leaf to developing grain tissues) for photorespiratory genes being greater than *rbcS* gene (Supplemental File\_S1). The significant upregulation of the chloroplastic *pdC* gene specifically in the developing grains, might convert glyoxylate / glycolate, to CO<sub>2</sub> in chloroplasts for carbon fixation (Fig. 1), thereby preventing carbon loss<sup>12,30</sup>. This finding provides an insight for possible development of an *in planta* photorespiratory bypass in the leaves of C<sub>3</sub> plants to envision for higher biomass or yield.

## Declarations

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**Conflicts of interest:** none.

**Availability of data and material:** Transcriptome raw reads for 18 samples (3 genotypes, 3 tissues and 2 replicates) associated with this article is available through Arrayexpress with accession number E-MTAB-8361. <https://www.ebi.ac.uk/arrayexpress/experiments/E-MTAB-8361/>

**Code availability:** Not applicable.

**Authors' contributions:**

**Conceptualization,** PR, DW, SM; **Formal investigation, analysis and supervision,** PR, DW, RS; **Funding acquisition,** SM, MJB; **Methodology,** PR, DW, RS, VC, PP, AB; **Resources,** VC, MJB, AR,KS; **Writing – original draft,** PR; **Writing – review & editing,** PR, VC, KS, RS, DW,MJB.

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## Figures

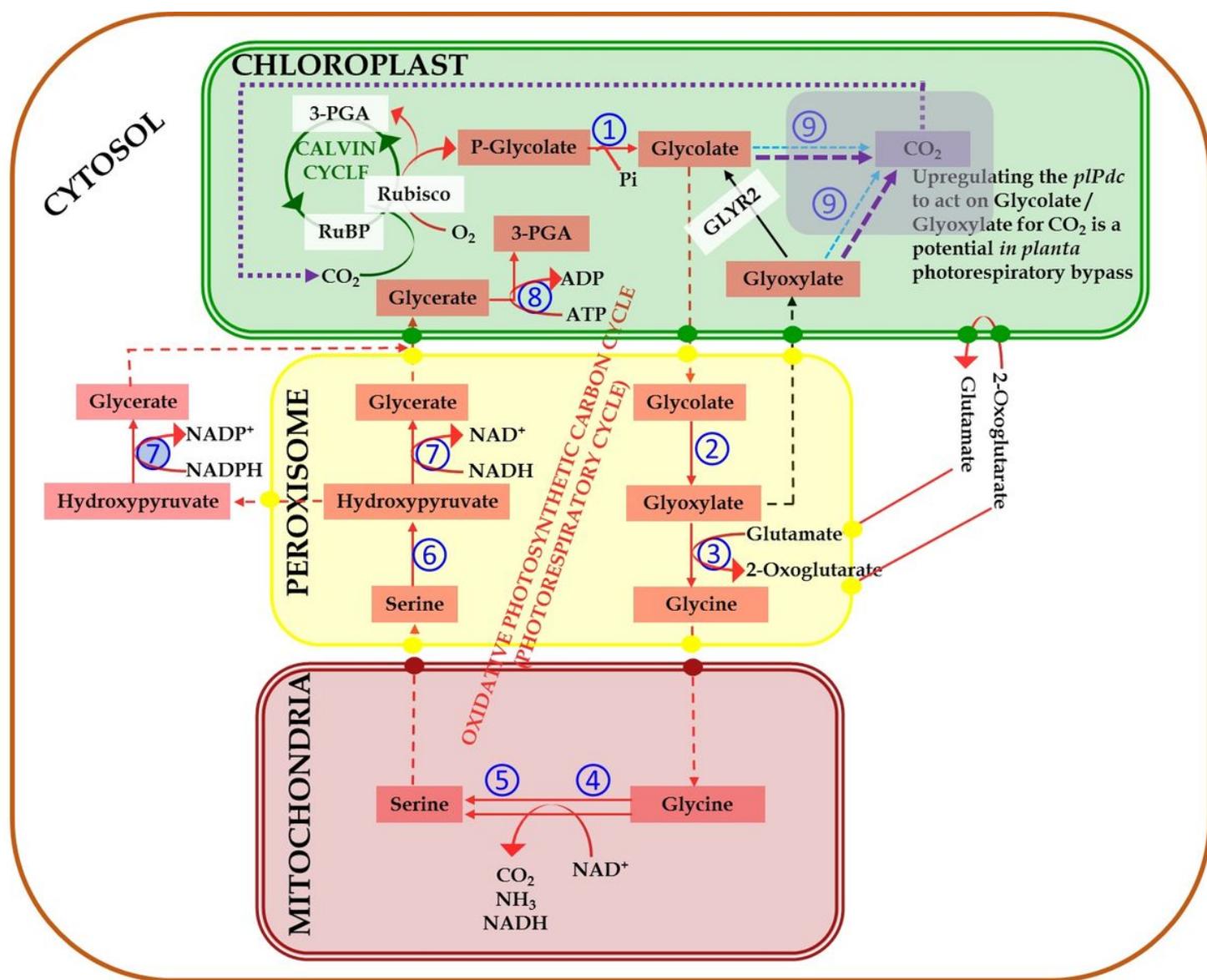


Figure 1

Insight from transcriptome data to modulate photorespiratory cycle for developing C2 rice with high biomass. Calvin cycle (green), photorespiratory pathway (red), conversion of intermediates of photorespiratory pathway (light blue), proposed strategy for C2 rice (purple), bold purple arrows (adjacent to light blue arrows) to suggest for upregulating the existing pathway for better biomass accumulation through enhanced assimilation. 1: chloroplastic, phosphoglycolate phosphatase (cpPGP); 2: peroxisomal, glycolate oxidase (pGOX); 3: peroxisomal, glutamate:glyoxylate aminotransferase (pGGT); 4: mitochondrial, serine hydroxymethyltransferase (mSHMT); 5: mitochondrial, glycine decarboxylase (mGDC); 6: peroxisomal, serine:glyoxylate aminotransferase (pSGT); 7: peroxisomal, hydroxypyruvate reductase (pHPR), highlighted 7 in cytosol is cHPR; 8: chloroplastic, D-glycerate 3-kinase (cpGLYK); 9: chloroplastic, pyruvate dehydrogenase complex (cPDC); 3-PGA: 3-phosphoglycerate; GLYR2: glyoxylate reductase-2; Rubisco: ribulose-1,5-bisphosphate carboxylase/oxygenase; RuBP: ribulose-1,5-bisphosphate.

## Supplementary Files

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