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The Important Role of the Citric Acid Cycle in Plants

Lina Wang [1], Donghai Cui [2], Xueyuan Zhao [1], Miao He [1]
1 State Key Laboratory of Tree Genetics and Breeding, Northeast Forestry University, 51 Hexing Road, Harbin ,150040, China
2 Forestry College of Northeast Forestry University, Harbin, 150040, China
Corresponding author email: wanglina@nefu.edu.cn
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Abstract The TCA cycle is a central metabolic hub necessary for ATP production and for providing precursors used in many biosynthetic pathways. The TCA cycle also plays a very important role in plants.Thus, dysregulation of the TCA cycle flux is frequently observed in plants.The identification of transgenic poplar in several enzymes of the TCA cycle in plants demonstrated a direct connection between this metabolic pathway and the influence to TCA cycle. In this review, the main genetic and non-genetic alterations of TCA cycle in poplar will be described.
Keywords TCA cycle; The citric acid cycle; Plants

Introduction

The citric acid cycle was discovered by Hans Krebs in 1937 and was also called Krebs cycle or tricarboxylic acid (TCA) cycle (Steinhauser et al., 2012). Krebs received the Nobel Prize in physiology or medicine in 1953 for his discovery (Engelking et al., 2015). The TCA cycle occupies a central position in metabolism and meets most of cell energy requirement by the complete oxidation of acetyl-CoA, a key product in the catabolism of carbohydrates, fatty acids and amino acids, to CO_2 (Desideri et al., 2015).

Pyruvate dehydrogenase is a large enzyme complex in the mitochondrion consisting of 3 different types of enzyme subunits. It is the enzyme that connects the glycolytic pathway to the citric acid cycle (Igamberdiev et al., 2014). The pyruvate dehydrogenase complex has previously connected an acetyl group to the carrier coenzyme A, which holds it in an activated form. Citrate synthase pops off the acetyl group and adds it to oxaloacetate, forming citric acid (Schuller et al., 1990). For this to be achieved, the activity of the citrate synthase (CS) is fundamental to combine acetyl-CoA, obtained from both glycolysis-derived pyruvate and fatty acid, with oxaloacetate (OA) to form citrate (Ciccarone et al., 2017). This latter is in turn rearranged to isocitrate by aconitase (ACO2), also designated as mitochondrial aconitase to distinguish it from the cytosolic isoform ACO1 (Sweetlove et al., 2010). The citrate formed in the first step is a bit too stable, so the second step moves an oxygen atom to create a more reactive isocitrate molecule. Aconitase performs this isomerization reaction, with the assistance of an iron-sulfur cluster. Then, isocitrate is decarboxylated to α-ketoglutarate (α-KG) by the isocitrate dehydrogenase (IDH) enzymes (Fukunaga et al., 1980). IDH enzymes consist of two classes: IDH1 and IDH2, $NADP^+$-dependent, and IDH3, NAD^+-dependent. A further decarboxylation performed by the a-KG dehydrogenase (a-KGDH) complex drives the conversion of a-KG to succinyl-CoA. The real work begins in the third step of the cycle. Isocitrate dehydrogenase, removes one of the carbon atoms, forming carbon dioxide, and transfers electrons to NADH (Salazar-Roa et al., 2016). The following reactions of the cycle are deputed to the oxidation of succinyl-CoA to OA, thus regenerating the starting molecule which allows the cycle to repeat (Vegliante et al., 2016). In particular, succinyl-CoA synthetase releases guanosine and succinate. The latter is oxidized to fumarate by the succinate dehydrogenase complex with a concomitant reduction of FAD to FADH2, after fumarate hydration to malate by the fumarate hydratase, malate dehydrogenase, finally catalyses the oxidation of malate to OA producing NADH (Simcock et al., 2011). Schematic overview of the TCA cycle and the intermediate catalytic enzymes was shown in Figure 1.

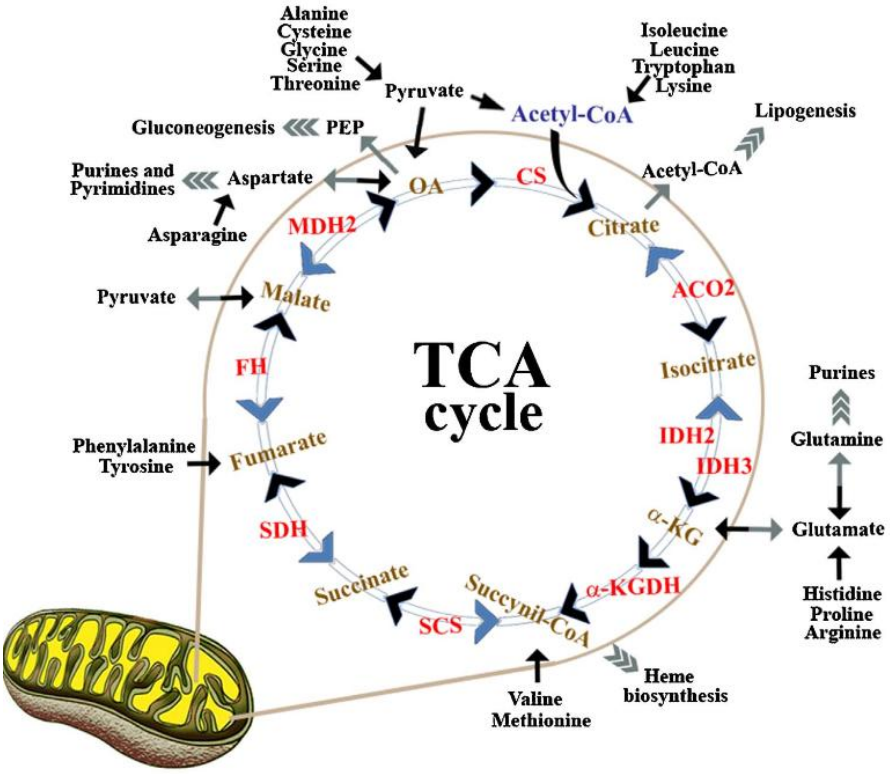

Figure 1 Schematic overview of the TCA cycle

Note: Black arrowheads indicate the "canonical" direction of metabolites through the cycle starting from pyruvate-derived acetyl-CoA; Blue arrowheads indicate the reverse steps of TCA cycle reactions; The enzymes and the metabolites of the cycles are depicted in red and brown (Ciccarone et al., 2017)

1 Results

The experimental results confirm that overexpression of *PEPCK1* increases the content of amino acids with TCA cycle disorders and breaks the balance of carbon and nitrogen metabolism in Poplar. Figure 2 shows analysis of differential gene enrichment differences in TCA cycle caused by *PtPCK1* at transcriptional level. Ten transcripts encoding for enzymes in the TCA cycle were significantly unregulated, including citrate cynthase (*Cs*), aconitate hydratase (*Aco*), malate dehydrogenase (*Mdh1*), succinate dehydrogenase (ubiquinone) flavoprotein subunit (*Sdh1*), 2-oxoglutarate dehydrogenase E1 component (*Ogdh*), dihydrolipoamide dehydrogenase (*Pdhd*), and succinyl-CoA synthetase alpha subunit (*Lsc1*), pyruvate-acetyl-CoA. A pathway was upregulated, which finally resulted in the upregulation of three enzymes that catalyzed the synthesis of pyruvate to acetyl-CoA: pyruvate dehydrogenase (DLAT), [EC:2.3.1.12]; pyruvate dehydrogenase E1 component alpha subunit (PDHA) [EC:1.2.4.1]; and dihydrolipoamide dehydrogenase (DLD) [EC:1.8.1.4]. The pyruvate dehydrogenase complex (PDHc; [EC:2.3.1.12]) is the rate-limiting enzyme for the metabolism of pyruvate to acetyl-CoA. The three enzymes play important roles in catalytic reactions for the synthesis of PDC through an "end-to-end" formation. This orderly combination successfully progresses to the reaction involved in the synthesis of acetyl-CoA catalyzed by the pyruvate dehydrogenase system.

2 Discussion

Also known as the Krebs cycle or the tricarboxylic acid cycle, the citric acid cycle is at the center of cellular metabolism. It plays a starring role in both the process of energy production and biosynthesis. The starting substrate for the TCA cycle is acetylCoA that can be derived from pyruvate, fatty acids through β-oxidation anf leucine and lysine. Citrate synthase inhibited by ATP, NADH, acyl CoA and succinyl CoA. Isocitrate dehydrogenase inhibited by ATP and NADH and activated by ADP. a-KG dehydrogenase inhibited by NADH & succinyl CoA. Oxaloacetate can be considered as a primary substrate of the TCA cycle. It is replenished from

pyruvate by the gluconeogenic enzyme pyruvate carboxylase. Since the TCA cycle intermediates are used for anabolism, their concentration varies according to the needs of the cell. Reactions that replenish the TCA cycle intermediates are called as anaplerotic reactions.

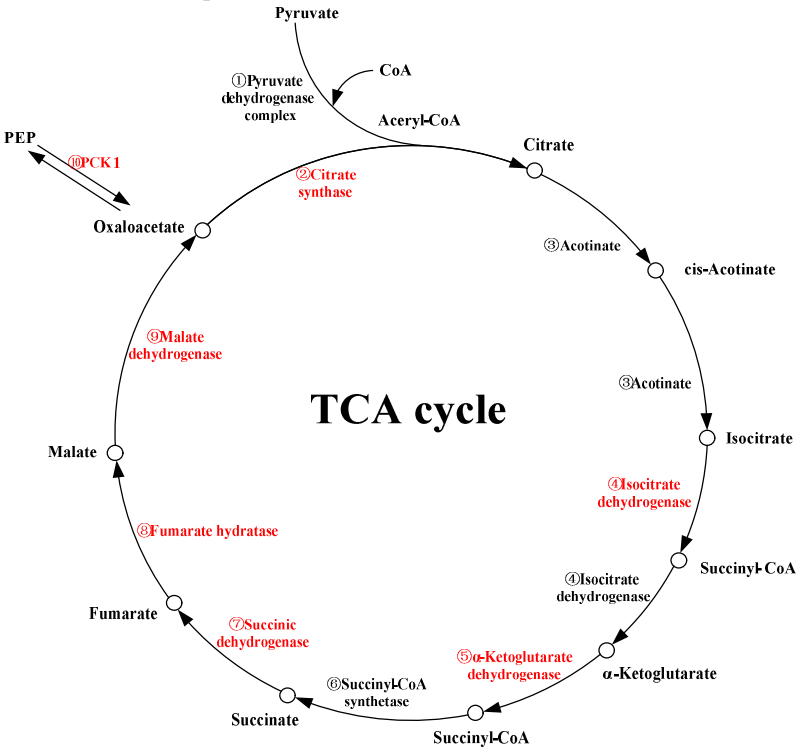

Figure 2 Gene enrichment of TCA cycle

Note: [EC:1.1.1.37]: malate dehydrogenase; [EC:2.3.3.1]: citrate synthase; [EC:2.3.3.8]: ATP citrate (pro-S)-lyase [EC:2.3.3.8]; [EC:1.8.1.4]: dihydrolipoamide dehydrogenase; [EC:2.3.1.12]: pyruvate dehydrogenase E2 component (dihydrolipoamide acetyltransferase); [EC:6.2.1.46.2.1.5]: succinyl-CoA synthetase alpha subunit; [EC:1.3.5.1]: succinate dehydrogenase (ubiquinone) flavoprotein subunit; Red shows upregulation, black means there is no change

PEPCK1 is well known for its role in gluconeogenesis. However, in our results PEPCK is also a key regulator of TCA cycle (Figure 2). The TCA cycle integrates glucose, amino acid, and lipid metabolism depending on cellular needs. In addition, biosynthetic pathways are crucial to plant growth which requires the TCA cycle for the processing of glucose and glutamine derived from carbons (Huang et al., 2015). Moreover, cytosolic PEPCK plays an important role in regulating energy homeostasis and flux through the TCA cycle (Araújo et al.,2013). PEPCK1 is at the intersection of carbon and nitrogen metabolism involving more biological processes and genes. A change in PEPCK will inevitably lead to changes in the corresponding genes and metabolic pathways, coupled with carbon and nitrogen metabolism. These results suggest that plants have their own ways to defeat the disorders of key metabolic pathways to ensure the normal growth.

3 Matirials and Methods

3.1 Agrobacterium suspension preparation and Agrobacterium infection

A single colony from Agrobacterium strain was inoculated separately into 10 mL YEB broth amended with 50 mg mg/L kanamycin and 10 mg/L rifampicin. The cultures were incubated for 18 h in an orbital shaker with 180 rpm at 28 C. The *Agrobacterium* cultures were multiplied by sub-culturing 0.1 volume of bacterial culture into 200 mL YEB broth containing the above-mentioned antibiotic and incubated at 28 C in an orbital shaker set at 180 rpm until the bacterial broth reached an OD_{600} of 0.8. The bacterial cells were harvested by centrifuging the culture at 5000 rpm for 10 min. The resulted pellets were suspended in 500 mL of 1/3MS medium containing 3% sucrose supplemented with concentration of acetosyringone (200 uM) and the optica density was adjusted to an OD_{600} of

0.6.

3.2 Plantlet rooting and transplanting

Healthy shoots differentiated from green calli with 3-4 true leaves were excised from the culture and transferred to rooting media. Rooting media consisted of MS salts, 30 g/L sucrose, 6.5 g/L agar, and pH adjusted to 5.8 and subjected to autoclaving as previously described.After 2-3 weeks,plantlets with a fully developed root system were transplanted to 10 cm × 15 cm pots with a 3:1 (v:v) ratio of peat:perlite in a 24°C glasshouse with about 80% relative humidity (The Northeast Forestry University, Haerbin, China). Survival rates were recorded two weeks after transplantation.

3.3 Library construction and Illumina sequencing

Library construction was performed in our experiments.and sequenced by BioScience. The mRNA libraries were prepared based on the preparation protocol of Illumina RNA Seq Library Preparation Kit for Transcriptome. Thereafter, the poly-A mRNA was subjected to two steps purification using Illumina poly-T oligo-attached magnetic beads. In the second step of poly-A RNA purification, the mRNA was fragmented and reverse transcribed into first strand of cDNA using random hexamer primers. The RNA template was removed and second strand of cDNA was synthesized utilizing DNA polymerase I and dNTPs. The ends of first and second strand of cDNA were repaired, dA base was added and Illumina adapters were ligated to both ends. Finally, 15 cycles of PCR was carried out using primers designed for the ends of adapters. The concentrations and size distribution of achieved libraries were validated using the Agilent BioAnalyzer 2100. The purified cDNA libraries were utilized for cluster generation onto HiSeq Flow cell and then sequenced using High-throughput RNA sequencing (Illumina Next-Generation Sequencing) on Illumina HiSeq 2000.

3.4 RNA-seq data analysis

The adaptor sequences and low-quality sequence reads were removed from the data sets. Raw sequences were transformed into clean reads after data processing.

Authors' contributions

Wang L.N. conceived, designed and performed the experiments. Cui D.H. analyzed the data and supervised the project. Zhao X.Y. and He M. offered statistical analyses. All authors read and approved the final manuscript.

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