

Available online at www.sciencedirect.com

ScienceDirect



Enhanced tolerance to drought in transgenic rice plants overexpressing C_4 photosynthesis enzymes

Jun-Fei Gu, Ming Qiu, Jian-Chang Yang*

Key Laboratory of Crop Genetics and Physiology of Jiangsu Province, College of Agriculture, Yangzhou University, Yangzhou, Jiangsu, China

ARTICLE INFO

Article history:

Received 14 July 2013

Received in revised form

7 September 2013

Accepted 12 October 2013

Available online 24 October 2013

Keywords:

Pyruvate orthophosphate dikinase (PPDK)

C_4 -specific phosphoenolpyruvate carboxylase (PEPC) and PPDK (PCK)

Transgenic rice

Photosynthesis

Drought tolerance

ABSTRACT

Maize-specific pyruvate orthophosphate dikinase (PPDK) was overexpressed in rice independently or in combination with the maize C_4 -specific phosphoenolpyruvate carboxylase (PCK). The wild-type (WT) cultivar Kitaake and transgenic plants were evaluated in independent field and tank experiments. Three soil moisture treatments, well-watered (WW), moderate drought (MD) and severe drought (SD), were imposed from 9 d post-anthesis till maturity. Leaf physiological and biochemical traits, root activities, biomass, grain yield, and yield components in the untransformed WT and two transgenic rice lines (PPDK and PCK) were systematically studied. Compared with the WT, both transgenic rice lines showed increased leaf photosynthetic rate: by 20%–40% under WW, by 45%–60% under MD, and by 80%–120% under SD. The transgenic plants produced 16.1%, 20.2% and 20.0% higher grain yields than WT under the WW, MD and SD treatments, respectively. Under the same soil moisture treatments, activities of phosphoenolpyruvate carboxylase (PEPC) and carbonic anhydrase (CA) in transgenic plants were 3–5-fold higher than those in WT plants. Compared with ribulose-1,5-bisphosphate carboxylase, activities of PEPC and CA were less reduced under both MD and SD treatments. The transgenic plants also showed higher leaf water content, stomatal conductance, transpiration efficiency, and root oxidation activity and a stronger active oxygen scavenging system than the WT under all soil moisture treatments, especially MD and SD. The results suggest that drought tolerance is greatly enhanced in transgenic rice plants overexpressing C_4 photosynthesis enzymes. This study was performed under natural conditions and normal planting density to evaluate yield advantages on a field basis. It may open a new avenue to drought-tolerance breeding via overexpression of C_4 enzymes in rice.

© 2013 Crop Science Society of China and Institute of Crop Science, CAAS. Production and hosting by Elsevier B.V. All rights reserved.

1. Introduction

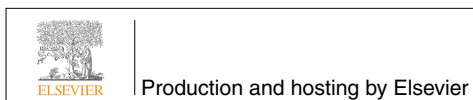
Many important crops including rice (*Oryza sativa* L.), wheat (*Triticum aestivum* L.), soybean (*Glycine max* L.), and potato

(*Solanum tuberosum* L.) are classified as C_3 plants, in which the first product of the Calvin cycle is 3-phosphoglycerate (3-PGA), whose production is catalyzed by ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco). However, competition of O_2

* Corresponding author.

E-mail address: jcyang@yzu.edu.cn (J.-C. Yang).

Peer review under responsibility of Crop Science Society of China and Institute of Crop Science, CAAS.



with CO₂ at the catalytic site of Rubisco results in a loss of up to 50% of carbon fixation via photorespiration [1]. Compared with C₃ plants, C₄ crops such as maize (*Zea mays* L.) and sorghum [*Sorghum bicolor* (L.) Moench] have evolved a C₄-metabolism system that concentrates CO₂ in the vicinity of Rubisco and thereby substantially increases the ratio of RuBP carboxylation to oxygenation. This strategy suppresses photorespiration by more than 80% [2] and is accompanied by higher photosynthetic rates, yields, and increased water- and nitrogen-use efficiency, especially under low CO₂ concentration, high temperature, high light intensity, drought, and other stresses [3–6]. Since the discovery of C₄ photosynthesis and its agronomic advantages, the genetic transformation of C₃ photosynthesis pathway into a C₄ system has become highly desirable.

The C₄ pathway in a C₄ crop such as maize (NADP malic enzyme (NADP-ME) C₄ cycle [7]) consists of three key steps: (i) initial fixation of CO₂ by phosphoenolpyruvate carboxylase (PEPC) to form a C₄ acid; (ii) decarboxylation of C₄ acid to release CO₂ near the site of the Calvin cycle in bundle sheath cells by NADP-ME; and (iii) regeneration of the primary CO₂ acceptor phosphoenolpyruvate (PEP) by pyruvate orthophosphate dikinase (PPDK) [8]. The transfer of C₄ key enzymes from C₄ plants to C₃ plants could contribute to introducing a C₄ system into C₃ plants, improving the rates of photosynthesis (P_n) and increasing crop yields [4,9]. By use of an *Agrobacterium*-based transformation system, genes that encode key C₄ enzymes such as PEPC, PPDK and NADP-ME have been successfully introduced and expressed in rice plants [9–14]. The transgenic rice plants have shown higher photosynthesis rates and often higher grain yield [4,10,15], although opposite results have also been reported [9,12,16,17]. In addition, enzymes involved in C₄ photosynthesis play important roles in plant defense responses to biotic and abiotic stresses [4,15,18–20]. However, the photosynthetic characteristics and grain yield of transgenic rice, especially under drought environments, have not been systematically examined. Few studies have been conducted under natural field conditions and normal planting densities to determine whether overexpressing C₄ photosynthesis in rice can result in a real improvement yield in terms of grain yield on a field basis [21].

Here we describe the photosynthetic characteristics and drought tolerance of transgenic rice overexpressing the maize C₄ PPDK enzyme independently or in combination with maize PEPC enzymes (PEPC + PPDK, PCK). By applying different levels of water stress during grain filling, we aimed to provide experimental evidence leading to an understanding of the mechanism underlying the enhanced photosynthesis and grain yield in these transgenic plants under drought environments.

2. Materials and methods

2.1. Plant materials and cultivation

Two independent experiments (field and cement tank experiments) were conducted at a research farm of Yangzhou University, Jiangsu Province, China (32°30' N, 119°30' E). The soil used in the experiments was a sandy loam (Typic Fluvaquent, Etoisol) with 24.5 g kg⁻¹ organic matter, 106 mg kg⁻¹ alkali-hydrolyzable N, 33.8 mg kg⁻¹ Olsen-P, and 66.4 mg kg⁻¹

exchangeable K. An untransformed wild type (WT, *Oryza sativa* L. ssp. *japonica* cv. Kitaake) and two transgenic rice genotypes, homozygous transgenic rice overexpressing the maize PPDK, PEPC + PPDK (PCK and provided by Prof. MSB Ku, School of Biological Sciences, Washington State University), were included in the study. Seedlings were raised in a seedbed and 20-day-old seedlings were then transplanted into both paddy field and cement tanks.

2.2. Soil moisture treatments

Both field and tank experiments were conducted. The field experiment was a three by three (three rice genotypes and three levels of soil moisture) factorial design with nine treatments, each with three replicates. Plot size was 4 × 3 m and plots were separated by an alley of 40 cm wide with plastic film inserted into the soil to a depth of 50 cm to form a barrier. Seedlings were transplanted at a hill spacing of 0.20 × 0.15 m with two seedlings per hill. N (60 kg ha⁻¹ as urea), P (30 kg ha⁻¹ as single superphosphate), and K (40 kg ha⁻¹ as KCl) were applied and incorporated just before transplanting. N as urea was also applied at mid-tillering (40 kg ha⁻¹) and at panicle initiation (25 kg ha⁻¹). All the genotypes headed on 13–15 July (50% of plants) and were harvested on 25 August. The water level in the field was kept at 1–2 cm until 9 days post-anthesis (DPA), when water stress treatments were initiated. From 9 DPA until maturity, three treatments including well-watered (WW), moderate drought (MD), and severe drought (SD) were applied. The WW regime was flooded with 1–2 cm water depth. Soil water potential was maintained at -25 ± 5 kilopascals (kPa) for the MD treatment and at -50 ± 5 kPa for the SD treatment. In each plot, four tensiometers (Institute of Soil Science, Chinese Academy of Sciences, Nanjing, China) consisting of a sensor of 5 cm length were installed to monitor soil water potential at 15–20 cm depth. Tensiometer readings were recorded every 4 h from 6:00 to 18:00. When the readings reached the desired values, tap water was added to the plot to maintain the values.

In the cement tank experiment, plants were grown in nine cement tanks in open-field conditions. Each tank (0.3 m height, 1.5 m wide, and 9 m length) was filled with sandy loam soil with the same nutrient contents as in the field experiment. Twenty-day-old seedlings raised in the field were transplanted into the tanks at a hill spacing of 0.15 × 0.20 m with two seedling per hill. N (8 g m⁻² as urea), P (4 g m⁻² as single superphosphate), and K (5 g m⁻² as KCl) were applied and incorporated before transplanting. N as urea was also applied at mid-tillering (5 g m⁻²) and at panicle initiation (3 g m⁻²). The three treatments of WW, MD and SD were imposed from 9 DPA till maturity. The treatment details were the same as in the field experiment. Plot size was 3.0 × 1.5 m and each treatment had three replicates. A rain shelter consisting of a steel frame covered with plastic sheeting was used to minimize the effect of rainfall precipitation on the treatments, and was removed after rain.

2.3. Sampling and measurements

Six flag leaves from each treatment were sampled at 14 and 28 DPA for measurement of leaf water content. Leaf water

Download English Version:

<https://daneshyari.com/en/article/2079596>

Download Persian Version:

<https://daneshyari.com/article/2079596>

[Daneshyari.com](https://daneshyari.com)