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RESEARCH PAPER

High Light Tolerance is Enhanced by Overexpressed PEPC in Rice Under Drought Stress

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Abstract: Overexpression of phosphoenolpyruvate carboxylase (PEPC) gene in transgenic rice (*Oryza sativa* L.) may alleviate inhibition on photosynthesis under drought stress condition. In the present research, photosynthetic light curve, chlorophyll *a* fluorescence parameters, pigment content, and reactive oxygen metabolism were studied in 2 PEPC transgenic rice lines under drought stress at flowering stage. The results showed that under drought stress, especially severely drought stress, net photosynthetic rate decreased dramatically under high photosynthetic active radiation (PAR) in untransformed wild type rice, while maintained unchanging in the PEPC transgenic lines under high PAR higher than 1200 μmol m^{-2} s^{-1}. The photochemistry activities (F_v/F_m, $\Phi_{PS\,II}$, and q_P) decreased slightly under drought stress in both PEPC transgenic lines. These results indicated that PEPC enhanced the photoinhibition tolerance of rice under drought stress. The increased zeaxanthin content in leaves of the PEPC transgenic rice lines dispersed more light energy as heat under drought stress, thus decreased the O_2^- producing rate in photosystem II. At the same time, the activities of superoxide dismutase, peroxidase, and catalase were higher in the PEPC transgenic rice plants than the untransformed wild type under drought stress. These enzymes could effectively diminish the reactive oxygen species and reduce the membrane lipid peroxidation.

Keywords: PEPC transgenic rice; drought; photoinhibition; chlorophyll fluorescence parameter; reactive oxygen metabolism

Photosynthesis is very sensitive to stresses, which directly influences the photosynthetic capacity of crop to reduce crop production. Drought stress leads to stomatal closure, reduction of activities of enzymes involved in photosynthesis, thylakoid expansion, stroma lamellae disarrangement, and the cell ultrastructure destruction [1, 2]. The reduction of CO_2 and carbon assimilation of photosynthesis are influenced by stomatal closure, leading to decrease of carbon assimilation rate, excessive light energy absorbed by photosynthetic apparatus, and photoinhibition or photodamage [3, 4]. As a result, the net photosynthetic rate (P_n) was decreased ultimately. Under drought stress, the membrane system and antioxidant system are also influenced [5, 6], which result in the increase of membrane permeability, synthetic obstructing in root, and growth arresting. However, photosynthetic apparatus has tolerance to water deficit, by increasing photorespiration, thermal energy dissipation, and other ways to dissipate excessive excitation energy to effectively alleviate photoinhibition on photosynthesis, so as to form a variety of photoprotection mechanisms [7–9].

Phosphoenolpyruvate carboxylase (PEPC) is involved in photosynthetic carbon fixation and anaplerotic of tricarboxylicacidcycle acid cycle (TCA) [10]. Besides, many researchers have revealed that PEPC activity is enhanced under biotic and abiotic stresses; and this may result from the increases of gene transcription and protein synthesis, or just from the increase of protein phosphorylation [11]. For example, PEPC transcription and activity in rice (*Oryza sativa* L.) root were enhanced by NaCl, LiCl, and drought stresses [12]. There are different responses to salt and drought stresses among 4 *Atppc* genes in *Arabidopsis thaliana*. Transcriptions of *Atppc1* and *Atppc3* can be up-regulated under salt stress, but no response to drought stress; the transcription of bacterial type PEPC gene, *Atppc4*, is up-regulated under both stresses; and the transcription of housekeeping type PEPC gene, *Atppc2*, has no response to both stresses [13, 14]. Resistance to drought,

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salt, and photoinhibiton has been enhanced in maize (*Zea mays* L.) [15], *Arabidopsis thaliana* [16] and rice [17–19] due to PEPC overexpression. We have demonstrated that PEPC transgenic rice lines showed significant superiorities in photosynthesis under drought stress than the nontransgenic controls, and the drought-induced photosynthetic inhibition was reduced by PEPC [20–22]. However, the mechanism of PEPC in regulation of drought resistance and photosynthesis has been disclosed in rice.

In the present study, we analyzed the process of P_n in response to light and characteristics of chlorophyll fluorescence parameters and antixoidant metabolism in 2 *Zmppc* transgenic rice lines under different water conditions. The objectives were to clarify whether PEPC could alleviate the photoinhibition in rice caused by drought and to explore its physiological mechanism.

1 Materials and methods

1.1 Plant materials and planting condition

Two transgenic rice lines, ZM07 and ZM24, were developed by transferring a PEPC gene from maize (*Zea mays* L.) into the recipient cultivar "Zhonghua 8". Phenotyping result showed no significant difference between the transgenic lines and the recipient cultivar (control).

Both transgenic lines and the control cultivar were grown in a net room in the campus of Chinese Academy of Agricultural Sciences, Beijing, China. On the noon of sunny days in July and August, the light intensity in the net room may reach 1800 μmol m^{-2} s^{-2}. Using a Vacuum Table-subpressure-type Soil hygrometer, the soil moisture was measured and the water potential was controlled to 0, –20, and –40 kPa, respectively. Each line was planted in 27 pots (25 cm in diameter and 30 cm in height) with 3 seedlings (hills) per pot. Irrigation was given normally after transplanting, and stopped since 15 d after transplanting. Other managements were the same as conventional practice.

1.2 Measurements

1.2.1 Gas exchange parameters The light curve of photosynthesis was drawn with data collected under 25 °C, (360±5) μmol mol^{-1} CO_2, (60±5)% relative humidity using a LI-6400 portable photosynthesis system (LI-COR, Lincoln, Nebraska, USA). The photon flux density (PPFD) was controlled by a LED light source equipped in portable photosynthesis system.

1.2.2 PEPC activity The activity of PEPC was determined using the method described in our previous experiment [21]. The reaction mixture was 1.0 mL in volume, containing 100 mmol L^{-1} Tris-HCl (pH 8.0), 5 mmol L^{-1} $MgCl_2$, 3 mmol L^{-1} PEP, 0.2 mmol L^{-1} NADH, 10 mmol L^{-1} $NaHCO_3$, 10 U MDH, and 20 μL crude extraction. The PEPC activity was determined by the oxidation of NADH at 340 nm. Protein concentration in enzyme extractions was determined according to the method of Bradford [23].

1.2.3 Chlorophyll fluorescence parameters The chlorophyll fluorescence dynamic parameters (F_o, F_m, F_s, F_m', and F_o') of flag leaf were measured using an FMS-2 fluorescence monitoring system (Hansatech, Norfolk, UK) according to the method described by Genty et al. [24]. The PS II primary photochemical efficiencies for dark adaptation (F_v/F_m) and photoadpatation (Φ_{PSII}) and the coefficients of photochemical quenching (q_P) and nonphotochemical quenching (NPQ) were calculated as follows:

$$F_v / F_m = (F_m - F_o) / F_m ;$$

$$\Phi_{PSII} = (F_m' - F_s) / F_m' ;$$

$$q_P = (F_m' - F_s) / (F_m' - F_o') ; \text{ and}$$

$$NPQ = (F_m - F_m') / F_m' .$$

1.2.4 Metabolism of reactive oxygen species (ROS) Superoxide dismutase (SOD) activity was determined according to the method described by Giannopolitis and Ries [25]. One unit of SOD activity was defined as the amount of enzyme required to cause a 50% inhibition of reduction of NBT. Peroxidase (POD) activity was determined using the method described by Kochba et al. [26], which was represented by the increase in absorbance at 470 nm (ε = 26.6 $mmol^{-1}$ cm^{-1}). Catalase (CAT) activity was determined as described by Cakmak and Marschner [27] using the mixture system containing 25 mmol L^{-1} phosphate buffer (pH 7.0), 10 mmol L^{-1} H_2O_2, and enzyme extract. The decomposition of H_2O_2 by catalase was followed at 240 nm (ε = 0.036 $mmol^{-1}$ cm^{-1}).

Malondialdehyde (MDA) content and superoxide anion (O_2^-) concentration were determined according to the methods described by Lin et al. [28] and Elstner et al. [29], respectively. The absorbance was measured under 530 nm, and standard curve for NO_2^- was established to calculate the production rate of O_2^- from the chemical reaction of O_2^- and hydroxylamine.

1.2.5 Contents of chlorophyll and zeaxanthin As described by Zhao [30], the contents of chlorophyll were calculated with the following equations:

$$Chl = 6.63D_{665} + 18.08D_{649} .$$

Relative zeaxanthin content was determined by A_{505}/A_{652} as described by Bilger et al. [31] and Zhang et al. [32].