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Morphology and photosynthetic enzyme activity of maize phosphoenolpyruvate carboxylase transgenic rice

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ABSTRACT. The morphology and photosynthetic enzyme activity were studied in maize phosphoenolpyruvate carboxylase transgenic rice and non-transgenic rice. The results showed that compared with non-transgenic rice, phosphoenolpyruvate carboxylase transgenic rice was taller and had a stronger stalk, wider leaves, and more exuberant root system, with increased photosynthetic enzyme activity and improved yield components. Therefore, given the superiority of this plant type and heterosis, this is a novel breeding strategy for rice for the introduction of C_4 photosynthesis genes into high-yielding rice.

Key words: Phosphoenolpyruvate carboxylase gene; Rice; Photosynthetic enzyme activity

INTRODUCTION

Long-term reduction in photosynthetic efficiency caused by bright light is known as photo inhibition. In most C_3 plants, particularly rice, photo inhibition is a common phenomenon because of its strong photorespiration. C_4 plants possess the C_4 photosynthetic pathway, with a CO_2-concentrating mechanism enabling C_4 plants to achieve higher photosynthetic capacity and higher water and nitrogen use efficiencies. Thus, the transfer of C_4 traits to C_3 plants can be used to improve the photosynthetic performance of C_3 plants (Matsuoka et al., 2001). Previous studies have attempted to develop C_3 plants expressing C_4 traits using conventional hybridization (Brown and Bouton, 1993); however, these attempts have been unsuccessful. Recent progress in the development of molecular biology techniques, particularly the molecular engineering of photosynthetic genes, has resulted in the production of transgenic rice overexpressing phosphoenolpyruvate carboxylase (PEPC) using an agrobacterium transformation system (Agrie et al., 1998; Kums et al., 1999). In this study, the photosynthesis and morphology of the transgenic rice were analyzed and compared, providing a basis for the genetic improvement of rice.

MATERIAL AND METHODS

Plant materials

Maize was introduced into new japonica 18 to obtain transgenic rice. These plants were potted in the rice experimental fields of Henan Normal University under natural light conditions.

Extraction of leaf soluble protein and enzyme assays

First, 2 g rice leaf was ground in Tris-HCl buffer in an ice bath; after filtration through 8 layers of gauze, the filtrate was centrifuged for 20 min in 10,000 *g* at 4°C. The supernatant was the enzyme soluble extract. PEPC activity was assayed using the method described by Gonzalez et al. (1984). The carboxylase activity was assayed as described previously (Guo et al., 1988). For nicotinamide adenine dinucleotide phosphate (NADP)-malate dehydrogenase and ribulosebisphosphate carboxylase, the activity assay was separately performed using the methods described Li et al. (1987) and Kung et al. (1980).

RESULTS

Morphology

A series of transgenic rice of various shapes was obtained by introducing PEPC. Compared with japonica 18, PEPC transgenic rice was taller and had a more exuberant root system (Figure 1), wider leaves, and stronger stalks (Figure 2). The panicles per plant, ear length, grain weight, and yield per plant were increased by 30.2, 7.5, 8.9, and 32.1%, respectively, compared with the controls. This indicates that changes in shape were closely related to production.

Figure 1. Comparison of root gene transfer and control plant.

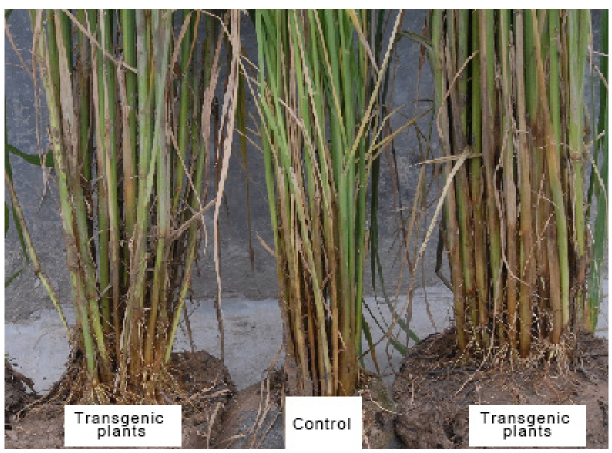

Figure 2. Comparison of stem of transgenic plants and controls.

Photosynthetic enzyme activity

Under natural light conditions (350 μmol·m^{-2}·s^{-1}, 21% O_2, 350 μL/L CO_2, 30°C, for 3 h), the PEPC activity of transgenic rice was significantly higher than in controls, while the activities of CA, NADP-malate dehydrogenase, and ribulosebisphosphate carboxylase were constant (Figure 3A).

Under conditions of photo inhibition (1000 μmol·m^{-2}·s^{-1}, 2% O_2, 60 μL/L CO_2, 30°C, for 3 h), compared with the control group, the ribulosebisphosphate carboxylase activity of transgenic rice exceeded 5% (no significant difference), while the activities of PEPC, CA, and NADP-malate dehydrogenase exceeded 282.3, 23.1, and 25.6%, respectively (Figure 3B). The activities of all C_4 photosynthetic enzymes in transgenic lines increased and exceeded those in the control group following suppression of light. Thus, transformant plants had higher levels of PEPC activity, significantly improving photosynthesis.

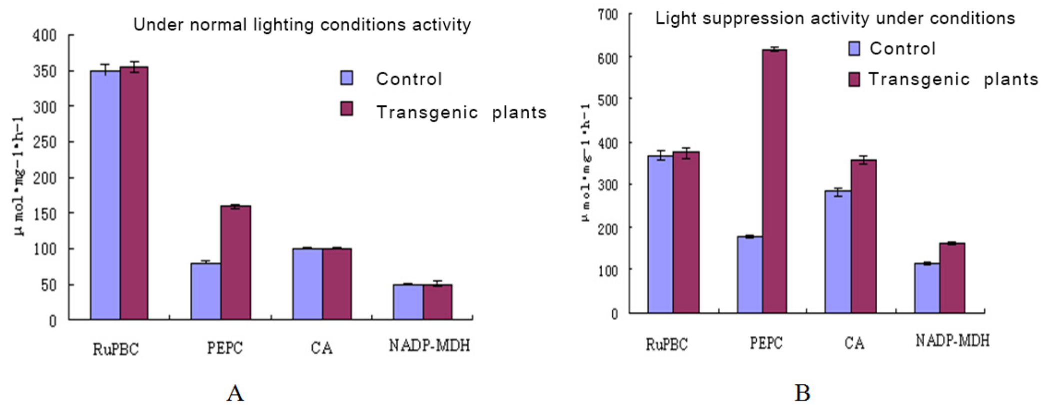

Figure 3. Leaves of transgenic lines and control of light synthase activity. **A.** Under natural light condition; **B.** under conditions of photo inhibition.

DISCUSSION

Whether the C_4 photosynthetic pathway exists in C_3 plants is unclear, as C_4 plant leaves possess the Kranz structure, while C_3 plants do not. It has been reported that the leaves of C_3 crops, such as soybeans (Li et al., 2001), wheat (Hata and Matsuoka, 1987), and rice (Wang et al., 2002), contain C_4 photosynthetic enzyme systems. Transforming C_4 cycle enzyme genes into C_3 crops is considered to be an important method for improving photosynthetic efficiency and crop yield. Chen and Ye (2001) found that the photosynthesis efficiency of spinach leaves was improved when the original products of the photosynthesis (oxaloacetate/malate) were added. This provides a basis for establishing the C_4 micro-circulatory system in C_3 plants to improve the efficiency of photosynthesis.

Conventional, physiological, and biotechnological breeding are traditionally used in the development of crop breeding (Austin et al., 1984), Contemporary plant physiologist and breeding workers give attention to the high light efficiency physiological breeding, particularly in the study of including C_4 photosynthetic capabilities into C_3 crops (Kum et al., 1996; Edwards, 1999). To investigate the microcirculation and its physiological function of C_4 in rice, the PEPC gene from maize was transformed into the new japonica rice 18. We obtained a series of transgenic lines with increased plant height, improved root development, broadened leaves, and thickened stems. Compared with the control group, in the transgenic lines, both the biological and economic yields were high, and photosynthetic enzyme activity was also greatly enhanced. The result agrees with that of Jiao et al. (2001). Zhou et al. (2001) found that overexpression of PEPC alleviated drought stress on rice. Ding et al. (2012) found that under drought stress, overexpression of PEPC enhanced the highlight tolerance of the rice, which is consistent with our results regarding the transgenic plants' morphological changes in developed root and thickened stem. Changes in the shape of transgenic lines may be the physiological basis of resistance and increase yield in PEPC transgenic rice. Therefore, given the superiority of this plant type and heterosis, this is a novel breeding strategy for rice for the introduction of C_4 photosynthesis genes into high-yielding rice.

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