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Rapid Communication

Phosphoenolpyruvate carboxylase activity in ear organs is related to protein concentration in grains of winter wheat

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Abstract

The relationship between phosphoenolpyruvate carboxylase (PEPC) activities in flag leaf blade and ear organs (glume and grain) and protein content of grain as well as grain weight after flowering were studied in different winter wheat (*Triticum aestivum L.*) genotypes. Results showed higher PEPC activity in the developing grain than in flag leaf blade and glume during grain development. For 16 of the genotypes studied, the mean PEPC activity in the developing grain or glume at 15 and 25 days after flowering was significantly and positively correlated with final protein content of grain. Enzyme activities in glume and flag leaf blade were positively correlated with final grain weight but the activity in developing grain was weakly and negatively correlated with grain weight. The overall data suggest that PEPC may be involved in protein biosynthesis during grain development, and it may have an important role in regulating carbon and nitrogen metabolism in the ear of wheat.
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Keywords: Ear organs; Phosphoenolpyruvate carboxylase; Protein content; Grain weight; Wheat

1. Introduction

Phosphoenolpyruvate carboxylase (PEPC) is a key enzyme for carbon dioxide (CO_2) assimilation in C_4 plants. It catalyzes the β-carboxylation of phosphoenolpyruvate (PEP) using HCO_3^- as a substrate to yield oxaloacetate and Pi (Champigny and Foyer, 1992). However, immunocharacterization studies indicated that PEPC was also present in C_3 plants, and it appeared to be distributed between the cytosol and chloroplasts of foliar parenchyma (Perrot-Rechenmann et al., 1982). As in C_4 plants, PEPC in C_3 plants is subject to complex regulation by metabolites and modification by reversible phosphorylation (Miyao and Fukayama, 2003). An abundance of PEPC was reported in the chaff and grain of wheat (Araus et al., 1993; Singal et al., 1986; Wirth et al., 1977; Ziegler-Jons, 1989). Although Araus et al. (1993) and Bort et al. (1995) considered that the C_4 pathway in ear organs of wheat to be absent or limited, many reports showed that ear organs have characteristics of C_4-like photosynthetic metabolism (Singal et al., 1986; Ziegler-Jons, 1989). The physiological functions of PEPC in C_3 plants have not been well understood, but it was suggested that the enzyme may play multiple roles (Oaks, 1994; Podestá and Plaxton, 1994) including a major anaplerotic function in replenishing the tricarboxylic acid cycle with intermediates (such as malate, oxaloacetate and so on) to meet demand for carbon skeletons for nitrogen assimilation and synthesis of organic and amino acids (Andrews, 1986; Chollet et al., 1996; Huppe and Turpin, 1994).

In recent years, relationships between PEPC activity and carbon and nitrogen metabolism, especially those between PEPC activity and synthesis of protein and oil in crops, have received much attention (Smith et al., 1989, 1992). Sugimoto et al. (1989) found a strong correlation between

Abbreviations: DAF, days after flowering; DTT, dithiothreitol; EDTA, ethylenediaminetetraaceticacid; MDH, malate dehydrogenase; NADH, ubiquinone oxidoreductase; PEP, phosphoenolpyruvate; PEPC, phosphoenolpyruvate carboxylase; Pi, inorganic phosphate; PVP, polyvinylpyrrolidone

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PEPC and protein content in seeds of 13 soybean cultivars, which indicated a possible rate-limiting role for the enzyme in accumulation of seed storage-protein. During soybean seed development PEPC and pyruvate kinase activities contributed to a complex interaction that regulated the metabolic flow of glycolytic carbon into precursors for both protein and oil biosynthesis (Smith et al., 1989). During the maturation of castor bean seeds, the increase in PEPC activity may be correlated with plentiful synthesis of oil (Smith et al., 1992). Using an electron-microscopic immunolabeling technique, Araus et al. (1993) detected a substantial amount of PEPC in protein bodies of immature durum wheat (*Triticum durum*) grains, where it might contribute to amino acid and protein biosynthesis during grain development. It is well known that grain protein is of primary importance in determining the nutritional and processed quality of wheat (Feil, 1997; Shewry and Halford, 2002; Weegels et al., 1996). Unfortunately, grain yield and grain protein content are often negatively correlated (Cox et al., 1985; Loffler and Busch, 1982; Terman et al., 1969). A common explanation for the negative correlation is competition for carbon assimilates during synthesis of starch and protein (Banziger et al., 1994). The biochemical control of the competition has not been well understood. We suppose that PEPC may be a key regulatory enzyme in the interaction between carbon and nitrogen metabolism in ear parts, and have an important role in determining the concentration of protein in wheat grains. To test this hypothesis, the PEPC activities of ear organs in different winter wheat genotypes during grain development were investigated and the correlations between PEPC activities and final protein content of grain were explored.

2. Experimental

Field experiments were carried out at the Experimental Farm of China Agricultural University, Beijing, China in 2003–2004 and 2004–2005 cropping seasons. The 0–20 cm soil layer at the experimental site contained 1.15% total organic matter, 0.087% total nitrogen, 37.7 mg kg^{-1} available phosphate and 90.1 mg kg^{-1} available potassium. In the 2003–2004 experiment, two winter wheat genotypes, Y15 and 6365, were sown on 8 October 2003. A randomized complete block design was used with three replications, and plot size was 12 m^2 (contained six rows of wheat, 20 cm apart). Irrigation was applied at jointing and flowering stages at the rate of 750 m^3 h m^{-2}. Fertilizer was applied before planting to provide 15.7 g m^{-2} N, 10.3 g m^{-2} P_2O_5, and 10.1 g m^{-2} K_2O. Routine cultural and management practices for pathogen and pest control were used. Plants flowering on the same day within each genotype were tagged. On 7, 14, 20 and 25 days after flowering (DAF), flag leaf blades and ear parts (glume and grain) of tagged plants were sampled, immediately frozen in liquid nitrogen and stored at −80 °C until the assay of PEPC. Grains were periodically sampled and dried to determine grain weight and protein content.

In 2004–2005 season, 16 genotypes (see Table 1) were sown on 7 October 2004, using the same experimental design and field management as in 2003–2004. The 16 genotypes which differ in protein content were used to examine correlations between PEPC activity in different organs during grain development and protein content of grain at maturity. Flag leaf blades and ear parts from tagged plants of each genotype were sampled at 15 and 25 DAF to assay PEPC activity. Dry weight and protein content of grains were determined at maturity.

The extraction of PEPC was carried out according to the method of Sayre and Kennedy (1979) with slight modifications. About 0.3 g of different organs (flag leaf blade, glume and grain) were ground with a mortar and pestle (2 °C) together with a small amount of sand and 3.0 mL of grinding media consisting of 0.1 mol L^{-1} Tris-HCl (pH7.8), 10 mmol L^{-1} $MgCl_2$, 1 mmol L^{-1} EDTA (ethylenediaminetetraaceticacid), 20 mmol L^{-1} mercaptoethanol, 10% (*w*/*v*) glycerin and 1% PVP (polyvinylpyrrolidone). This was followed by centrifugation at 15 000*g* for 10 min at 4 °C. For grains, the extraction was done two times and the combined supernatant was used for enzyme assay. PEPC activity was measured spectrophotometrically at 340 nm using Varine 100 UV spectrophotometer (Varian, USA) by coupling the reaction with the oxidation of NADH from malate dehydrogenase (MDH) according to Blanke and Ebert (1992). The enzyme extract was added to a 1 mL final reaction solution which contained 50 mmol L^{-1} Tris-HCl (pH7.8), 10 mmol L^{-1} $MgCl_2$, 0.25 mmol L^{-1} EDTA, 5.0 mmol L^{-1} $NaHCO_3$, 2.0 mmol L^{-1} DTT (dithiothreitol), 4U MDH, 0.1 mmol L^{-1} NADH, and 2.0 mmol L^{-1} PEP. The reaction was started by the addition of a tissue extract. Protein determinations were carried out following the method of Christensen et al. (1981).

Duncan's multiple range test was used to compare mean differences among organ parts and genotypes at the 5% probability level. A simple regression was performed to explore relationships between PEPC activity in each organ part and protein concentration and grain weight.

3. Results and discussion

Changes in PEPC activities of flag leaf blade and ear organs and in protein content and weight of developing grains after flowering of YN15 and 6365 in 2003–2004 are shown in Figs. 1 and 2. The parameters changed similarly in the two genotypes. PEPC activities in flag leaf blade, glume and grain increased progressively during the early stage of grain development, peaked at about 14 DAF and then declined markedly until 25 DAF for flag leaf blade and glume. The PEPC activity of grain was decreased from 14 to 20 DAF, but increased again at 25 DAF. González et al. (1998) also found increased PEPC activity during grain development, with a maximum level at 15 DAF. Protein content of developing grain exhibited a V-figure

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