Molecular Analyses of Glycerol-3-Phosphate Permease-G3P Genes Induced by Phosphate Stress in Maize

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ABSTRACT

Many essential biological processes are carried out inside sub-cellular compartments such as plastids and mitochondria. At some points almost all these processes require phosphorus (P). The impact of Pi starvation may be more severe in compartments such as chloroplasts that are actively exporting organic-P metabolites in exchange for inorganic P. Transporters that export products and supplies plastids with energy for biosynthesis including starch and fatty acids, and sugar transporters belonging to the major facilitator superfamily (MFS). Glycerol-3-phosphate permease (G3P), the topic of this study, is also a member of MSF. G3P is strongly induced under phosphate deficiency in maize.

INTRODUCTION

Many essential biological processes are carried out inside sub-cellular compartments such as plastids and mitochondria. These pathways often require an extensive transport mediated exchange of metabolites between compartments and surrounding cytosol (Eicks et al., 2002). At some points almost all these processes require P either as orthophosphate or in the organic form. Phosphate shortage may adversely affect transport across the membranes of these isolated compartments. The impact of Pi starvation may be more severe in compartments such as chloroplasts that are actively exporting organic-P metabolites in exchange for inorganic phosphorus. Plastids, like chloroplasts, utilize a variety of sugar transporters to maintain normal biological activities. Interestingly, many of these are antiporters that couple the efflux transport of a sugar-phosphate to the influx of Pi (Streatfield et al., 1999). Among these include a hexose transporter that exports the products of starch degradation from the plastids (Weber et al., 2000), an ADP/ATP transporter that supplies plastids with energy for biosynthesis of a wide variety of compounds including starch and fatty acids (Neuhaus et al., 1997), and sugar transporters belonging to the major facilitator superfamily (MFS) which function as antiporters exchanging Pi for C3 and C6 compounds (Griffith et al., 1992). Glycerol-3-phosphate permease, the topic of this study, is also a member of MSF.

MATERIALS AND METHODS

The genotypes of *Zea mays* (L.) were selected based on their response to and utilization of applied Pi. These genotypes were developed at Embrapa maize and sorghum, Brazil, through selection and conventional breeding. These genotypes were classified mainly into two categories namely efficient and inefficient types based on their yield potentials.

Seeds of maize were germinated in seedling trays containing Scott's ready earth plug mix (Scotts Co., Marysville, OH) and grown in the greenhouse for one week. One-week-old seedlings were gently washed with water to remove the soil medium off the roots. Seedlings were then transferred to one half-strength modified Hoagland's nutrient solution (Liu et al., 1998b). After one week, plants were again transferred to Hoagland nutrition solution containing different concentrations of Pi (0, 5, 10, 25, 50, 100 and 250 μM). During the course of the treatments, nutrients solutions were renewed on alternate days. After 15 days of treatment, roots were harvested from plants grown under different Pi concentrations, frozen in liquid nitrogen and RNA was extracted for Northern analysis. Since the evaluation of the expression of different phosphate transporters in maize revealed the induction at 0 μM of Pi and complete suppression at 250 μM of Pi, these two concentrations were treated as P- and P +, respectively for studying different aspects of Pi deficiency on the expression of selected genes of maize. For the time course study, roots from both P + and P- treatments were harvested sequentially after 1, 3, 5, 6, 7, 8, 12, and 15 days of growth. Furthermore, after 15 days of growth under P + and P- conditions, roots, stem, young leaves and old leaves were harvested separately for valuating the spatial expression of genes in these tissues.

RESULTS AND DISCUSSION

Increased expression of glycerol-3-phosphate permease

(G3P) was observed under Pi deficiency. G3P is induced in response to Pi starvation in maize plants. To adapt to Pi starvation, plant cells have developed a number of alternate metabolic pathways (Plaxton and Carswell, 1999). It is likely that, in addition to the alterations in metabolism, plant cells may have to utilize alternate modes of transport of phosphorylated compounds. G3P represents a typical sugar P/Pi antiporter possibly localized in chloroplast or mitochondria. The operation of this class of transporter may not directly impact the Pi concentration in the compartment in which it functions, as the net Pi change is zero. However, the protein does utilize Pi to move phosphorylated sugars across the membranes of cellular compartments. Normally Pi levels are tightly controlled in the cell and this control is likely to be even more stringent under Pi stress. Glycerol-3-phosphate itself is a major precursor in lipid synthesis as well as a major regulator of metabolism in the cell. Under Pi starvation the phospho-lipid composition of membranes is drastically altered (Essigmann et al., 1998; Hartel et al., 2000). Phospholipids are replaced by sulpholipids during Pi deficiency. Under these conditions the G3P permease levels may be altered to compensate for the reduced concentrations of phosphorylated sugars. The expression of G3P is strongly influenced by altered Pi levels in the media. A strong induction of the G3P gene was observed when the Pi concentration was reduced to 10 μM in maize for both efficient and inefficient genotypes. However, the concentration of Pi at which G3P is induced is much higher than the naturally occurring Pi concentrations in the soil (Barber, 1980). This suggests that phosphate starvation induced genes are activated well in advance of impending biochemical and physiological changes in plants. The induction of G3P is similar to the induction of phosphate transporters in maize. These results suggest that genes involved in phosphate starvation-induced rescue mechanisms are regulated by altered Pi concentration in plants. A detailed analysis of glycerol-3-phosphate permease in Arabidopsis and tomato showed that expression of the gene depended on the Pi nutrition status of the plant (Baldwin, 2003). The data presented here, taken together with earlier published reports, shows that many phosphate starvation induced responses are repressed and de-repressed by changing levels of Pi in plant tissues. In addition, expression of G3P in all parts of the plant is suggestive of its global role in enhancing Pi availability and possible recycling of organic P compounds. There are many questions that still need to be answered regarding cellular and subcellular localization of G3P. There is a need to evaluate whether G3P can transport other phosphorylated sugars in exchange for Pi.

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