Expression of *Bat-3* and phosphate stress induced genes in maize and sorghum

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Introduction

Phosphate unavailability is a growth-limiting factor for plants in many natural ecosystems. To thrive under Pi limited conditions plants have developed efficient mechanisms of phosphate acquisition and utilization of absorbed phosphorus in plant tissue. In order to decipher molecular determinants which could have a potential role in phosphorus acquisition and utilization efficiency, further studies on expression profiles of some of the phosphate responsive genes identified through microarray analysis were done. The advent of DNA microarray technology has made possible the analysis of global patterns of gene expression and revealed unexpected networks of coordinated regulation (Lockhart et al., 1996). The evaluation of expression of thousands of genes by microarray analyses in parallel has become an important tool in functional genomics. Recently, Girke et al. (2000) have identified genes which are transcriptionally regulated by different concentrations of phosphate. In collaboration with Pioneer HiBred Co. we have conducted a microarray analysis of a subset of genes expressed in maize. The microarray chips used were referred to as "metabolic chips" and "transcription factor chips". Analysis was done with RNA isolated from P+ and P- roots and cobs. A set of genes were selected for expression analysis and further characterization. Based on the results of RNA blot experiments, *Bat-3* gene was selected for further maize and sorghum analyses described in this study.

Methodology

Eight *Zea mays* and seven *Sorghum bicolor* lines developed and identified by Embrapa, maize and sorghum were used in these studies. The lines were categorized as phosphorus efficient or inefficient based on the comparative grain production when grown in dark red oxisols with low and high phosphorus levels (2mg/kg and 15mg/kg, respectively). Seeds of each genotype were germinated in 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 removed from the soil medium. The roots were washed and the plants were transferred to a hydroponics set-up containing half-strength modified Hoagland's solution (Liu et al., 1998). One week after transferring the seedlings to hydroponics the experimental treatments were initiated. Sets of plants were transferred to half strength nutrient solution with Pi (250 μM) or without Pi (0 μM). The solutions were changed every two days to maintain the concentration of nutrients and pH. Furthermore, after 15 days of growth under P+ and P- conditions, roots, stem, young leaves and old leaves were harvested separately for evaluating the spatial expression of genes in these tissues. Maize and sorghum plants grown in hydroponic culture were used to study the expression of *Bat-3* gene under Pi starvation conditions.

Results and Discussion

Distinct changes in the expression of phosphate starvation induced genes in maize and sorghum genotypes were observed. Interestingly *Bat-3* is strongly suppressed under phosphate deficiency. These genes exhibit temporal, spatial and phosphate deficiency associated patterns of expression. Although there were differences in the expression of phosphate starvation induced genes, no consistent pattern of expression associated with either phosphate efficient or inefficient genotypes of maize or sorghum emerged. It appears that phosphate efficiency in tropical maize and sorghum is a complex trait mediated by coordinated action of groups of genes either induced or

suppressed in response to nutrient deficiency. The majority of the gene expression studies during Pi deficiency are primarily focused on genes that are induced in response to Pi starvation. One interesting outcome of this study is the identification of a *Bat-3* homolog, expression of which is strongly suppressed during Pi deficiency. There is no assigned function for this gene in plants although there are indications that it may be regulated similar to heat shock proteins (HSPs) in animal systems (Baneji et al., 1990). To the best of our knowledge this is the first gene that is described to be down regulated during phosphorus starvation. Although the functional significance of this down regulation is not clear at present, this may serve as an indication of changes in Pi homeostasis during phosphorus deficiency or ressuply to plants. The suppression of this gene is not similar in all genotypes, especially for maize, where some genotypes had only a slight decrease in the transcript level whereas in others the expression was almost completly suppressed. In addition, sorghum genotypes showed consistent down regulation of this gene during Pi deficiency. Similar to other genes *Bat-3* expression was influenced by altered Pi levels in the media. The gene expression was completely restored when both efficient and inefficient genotypes were supplied with 100 μM of Pi. More studies are required to understand the regulation and function of this gene under phosphorus stress condition. Isolation and characterization of *Bat-3* gene from plants may help in this endeavor.

References

Baneji J, Sands J, Strominger JL, Spies T (1990) Proc Natl Acad Sci USA 87: 2374-2378

Girke T, Todd J, Ruuska S, White J, Benning C, Ohlrogge J (2000). Plant Physiol 124: 1570-1581

Liu C, Muchhal US, Mukatira U, Kononowicz AK and Raghothama KG (1998). Plant Physiol 116: 91-95

Lockhart DJ, Dong H, Byrne MC, Follettie MT, Gallo MV, Chee MS, Mittmann M, Wang C, Kobayashi M, Horton H, Brown, EL (1996). Nature Biotechnol. 14: 1675-1680.