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RESEARCH ARTICLE

Genome-Wide Expression Profile of Maize Root Response to Phosphorus Deficiency Revealed by Deep Sequencing

SU Shun-zhong[*], WU Ling[*], LIU Dan, LU Yan-li, LIN Hai-jian, ZHANG Shu-zhi, SHEN Ya-ou, LIU Hai-lan, ZHANG Zhi-ming, RONG Ting-zhao, ZHANG Xiao, TIAN Yue-hui, NIE Zhi and GAO Shi-bin

Key Laboratory of Biology and Genetic Improvement of Maize in Southwest Region, Ministry of Agriculture/Maize Research Institute, Sichuan Agricultural University, Chengdu 611130, P.R.China

Abstract

Phosphorus (P) is one of the three primary macronutrients that are required in large amounts for plant growth and development. To better understand molecular mechanism of maize and identify relevant genes in response to phosphorus deficiency, we used Solexa/Illumina's digital gene expression (DGE) technology to investigate six genome-wide expression profiles of seedling roots of the low-P tolerant maize inbred line 178. DGE studies were conducted at 6, 24 and 72 h under both phosphorus deficient and sufficient conditions. Approximately 3.93 million raw reads for each sample were sequenced and 6 816 genes exhibited significant levels of differential expressions in at least one of three time points in response to P starvation. The number of genes with increased expression increased over time from 6 to 24 h, whereas genes with decreased expression were more abundant at 72 h, suggesting a gradual response process for P deficiency at different stages. Gene annotations illustrated that most of differentially expressed genes (DEGs) are involved in different cellular and molecular processes such as environmental adaptation and carbohydrate metabolism. The expression of some known genes identified in other plants, such as those involved in root architecture, P metabolism and transport were found to be altered at least two folds, indicating that the mechanisms of molecular and morphological adaptation to P starvation are conserved in plants. This study provides insight into the general molecular mechanisms underlying plant adaptation to low-P stress and thus may facilitate molecular breeding for improving P utilization in maize.

Key words: maize, phosphorus efficiency, root, digital gene expression

INTRODUCTION

Phosphorus (P) is one of the essential macronutrients in plants and directly participates in a series of biological processes such as energy generation, nucleic acid synthesis, enzyme activation/inactivation, signaling, and carbon metabolism (Marschner 1995). Although soils may contain a sufficient amount of total P for plant growth, most of it is not available as it is tightly bound to soil particles and rapidly forms insoluble complexes with cations, particularly under acid conditions (Raghothama 1999). Maize is an important food and economic crop around the world and its production is seriously limited due to agricultural environments with low available P, such as in acid and calcareous soils.

In adapting to low P situations, plants have evolved a

series of morphological, physiological and biochemical adaptations to optimize P uptake and utilization (Raghothama 1999). The plant roots as an important organ for water and nutrient material absorption and is sensitive to small changes in P concentration in the soil. The root system is also highly plastic with the density and length of lateral root and root hairs increasing under P starvation to increase the exploratory capacity in the soils (Williamson *et al*. 2001). Large sets of genes have been found to have either increased or decreased expression by more than 1.5-fold in monocotyledonous plant roots under low-P stress (Calderon-Vazquez *et al*. 2008; Li *et al*. 2010). Phosphatases, ribonucleases, phospholipases, and P transporter encoding genes have shown higher expression levels in the roots of P deficient plants such as rich and maize (Calderon-Vazquez *et al*. 2008; Li *et al*. 2010). Biochemical pathways such as glycolysis and the tricarboxylic acid (TCA) cycle have also been implicated in dealing with P stress with increased expression levels of genes related to these pathways being observed during low-P treatment of rice and maize (Calderon-Vazquez *et al*. 2008; Li *et al*. 2010). Unexpectedly, a wide array of transcription factors (TF) and miRNA have been identified in various stresses such as low nitrogen and low P stress (Calderon-Vazquez *et al*. 2008; Zhao *et al*. 2012). Transcription factors PHR1 with a MYB domain was reported as a central regulator of downstream genes responding to P starvation in *Arabidopsis* and rice (Nilsson *et al*. 2007; Zhou *et al*. 2008). Gene orthologues of *OsPTF1* also have been considered as TF encoding gene responding to P starvation, and over-expression of *OsPTF1* can enhance the tolerance to P starvation in rice (Yi *et al*. 2005). Furthermore, studies have shown that the expression of a miRNA named *miR399* is induced by P starvation as its target *PHO2* and these have been implicated in the regulation of P transport in *Arabidopsis* (Aung *et al*. 2006; Hsieh *et al*. 2009). Evidence suggests that these genes are conserved in rice (Bari *et al*. 2006). Identifying candidate genes responding to P deficiency could therefore provide potential targets for molecular plant breeding.

Studies of low-P tolerance in maize have been mainly focused on the selection of tolerant genotypes, and morphological and metabolic alterations responsive to P starvation (Li *et al*. 2003; Liu *et al*. 2003; Zhang *et al*. 2004). However, information on genome-wide changes in gene expression adapted to P deficiency is yet to be clearly elucidated. Digital gene expression (DGE) profiling is a recently improved technology based on earlier massively parallel signature sequencing (MPSS) technology, which has been proved as a reliable tool for investigating expression profile of whole-genome in maize (Eveland *et al*. 2010; Shen *et al*. 2012a). Therefore, through this new generation, high throughput sequencing method, we have investigated root transcriptome profiles of the low-P tolerant maize line, 178, grown under normal and low-P stress conditions. This has resulted in the identification of differentially expressed genes responsive to low-P stress and facilitated the exploration of the extensive molecular modifications involved in different biochemical, cellular, and developmental processes involved during the adaptation to phosphorus deficiency.

RESULTS

Summary information of DGE

In this study, we used Illumina Genome Analyzer to generate digital expression signatures of maize inbred 178 roots grown under low-P stress conditions and control for 6, 24 and 72 h. In total, approximately 3 927 000 raw reads with 302 689 unique tags being sequenced for each sample (Appendix A). After filtering dirty tags from the raw data, we finally obtained 3 916 157 clean reads, of which 295 617 are unique tags with a length of 21 bp (Materials and Methods). The average percentage of low-quality tags of each sample was only 0.28%, which was within the normal range of the quality control. A total of 32 549 genes with 260 776 reference tags from the B73 genome (www. maizesequence.org) were taken as reference sequences. 98.08% of genes had CTAG sites and 91.83% of reference tags were found in the clean tag dataset. Around 62.71% of the clean tags and 36.18% of the unique clean tags for each sample were mapped to 23 551 genes (accounting for 72.36% of the reference genes). Unambiguous tags accounted for 57.08 and 32.66% of all clean tags and unique tags, and the number of unambiguous tag-mapped genes was 20 556 for each sample (accounting for 63.15% of the reference genes). In addition, there were 0.04, 0.01 and 14.57%