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Revelation on early response and molecular mechanism of submergence tolerance in maize roots by microarray and suppression subtractive hybridization

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Abstract

Submergence stress results in oxygen limitation in maize roots. Studying differential gene expression pattern in comparison submergence-tolerance lines with non-tolerant lines is critical for discovering genes associated with submergence tolerance and elucidating the mechanism of submergence tolerance. In this study, two inbred with difference of tolerance, Mo17 and Hz32, were used to reveal early responsive genes in root cells under submerging condition. Suppression subtracted hybridization (SSH) and cDNA microarray experiments showed that a number of gene expressions were altered because of submergence treatment. These genes are involved in a broad spectrum of biochemical, cellular, and physiological processes, such as glycolysis, energy metabolism, lipid metabolism, signal conduction, DNA transcription, protein biosynthesis and digestion, cell component and photosynthesis. The spectrum of genes in the first 0.5 h of treatment was significantly different from that of the genes induced later 2–4 h. In addition, changes of gene expression in responses to submerging were different in the two inbred lines. Four transcription factors were expressed at different profile in Hz32 and Mo17. The metabolic and physiological conversions from aerobic to anaerobic condition were faster in Hz32 than that in Mo17. Based on the responsive genes and their functions, we propose that early response of maize roots to submergence stress may be a complex network involving multiple physiological and metabolic pathways, and regulation of transcriptional processes plays a critical role in metabolic adaptation of maize roots during early stages of submergence.

Keywords: Maize (*Zea mays* L); Submergence stress; Gene expression; Suppression subtracted hybridization (SSH); cDNA microarray; Regulation of gene expression

1. Introduction

Submerging stress during maize seedling leads to the reduction of oxygen content available to roots, then injuring roots through anaerobic stress. Previous studies showed that two sets of proteins were synthesized in maize roots under low-oxygen condition. One is transit peptides, synthesized in earlier stage (0–5 h) of stress and then markedly decreased. Another is anaerobic peptides (ANPs), continually synthesized after 1.5 h of low-oxygen treatment (Sachs et al., 1980). Many of ANPs are mainly identified as enzymes of glycolytic and fermentation pathways, such as ADH, LDH, PDC, ALD and etc. (Kelley and Freeling, 1984a,b; Kelley, 1989; Dolferus et al., 2003). More gene products, which are implicated processes other than glycolysis and fermentation, were identified to be involved in the response to low oxygen stress. It suggests that many other biochemical and metabolic processes are modulated during low oxygen stress, such as xyloglucan endotraansglycosylase (XET) (Sachs et al., 1996), alanine aminotransferase (AlaAT) (Good and Crosby,

Abbreviations: Adh, alcohol dehydrogenase; Ald, fructose bisphosphate aldolase; Eno, enolase; PFP, pyrophosphate-fructose 6-phosphate 1-phosphotransferase; RENT, regulator of nonsense transcripts; rs2, maize rough sheath 2; SSH, suppression subtracted hybridization; SMT, S-adenosyl-L-methionine:delta 24-sterol methyltransferase; Sicyp51, submergence inducible CYP51; TA, transcription activator; UTGs, tentative unique genes; Zm-bRLZ, Zea mays basic region leucin zipper; Zmzf, Zea mays zinc finger protein

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1989), glutamine synthase (GS) and nitrate reductase (NR) (Mattana et al., 1994a,b), Hemoglobin (Silva-Cardenas et al., 2003), CDPK (Morello et al., 1994) and SKP1/ASK1 like proteins (Zhang et al., 2005). Alteration of global expression profile in maize roots had not been known under submerging stress, but those genes responding to low oxygen treatment help us to understand metabolic and cellular adaptation of maize. Under a stressful environment, metabolic pathway transits rapidly from aerobic metabolism into fermentation in maize roots. Glucose is converted to pyruvic acid via the glycolysis pathways, pyruvic acid is then converted into ethanol (Drew, 1997). In addition, the formation of aerenchyma and adventitious roots in the vicinity of cotyledonary nodes indicates the presence of adaptive mechanisms (Kawase, 1981). Aminocyclopropane carboxylic acid synthase (ACC synthase), a critical enzyme in ethylene biosynthesis, is also induced (Olson et al., 1995). The interaction of auxin with ethylene is important in inducing adventitious root formation (McNamara and Mitchell, 1991).

Gene expressions in response to submergence are regulated on transcriptional, post-transcriptional and translational levels (Fennoy et al., 1998; Zhang et al., 2005). Submergence results in a dramatic reduction in overall protein synthesis, selected synthesis of ANPs and selected degradation of aerobic protein. Accumulation of mRNAs, which are involved in glycolysis and ethanolic fermentation, and regulation of specific genes at the level of RNA synthesis, were conferred by the finding of the anaerobic responsive element (ARE) in promoter region of *Adh1*, *Adh2*, *Ald* and *Sh1* (Dennis et al., 1988; Olive et al., 1991; Walker et al., 1987). The ARE was found to contain all the sequences necessary for hypoxic induction, and when the ARE linked to a second gene, it could confer hypoxic responsiveness (Olive et al., 1991; Hoeren et al., 1998; Dennis et al., 2000). However, the efficient translation of induced mRNA is determined by sequences in the 5′ and 3′ portions of the message (Bailey-Serres and Dawe, 1996) and other trans-acting elements. Accumulations of SKP1/ASK1 like protein and ubiqutin mRNA under submergence provided indirect evidence of the degradation of an aerobic protein in maize roots (Zhang et al., 2005).

Plant species with different tolerance have identical metabolic pathways in response to submergence stress, but the different regulation ability to metabolism (Subbaiah and Sachs, 2003; Drew, 1997). There is a significantly different tolerance to submergence in the maize inbred. For instance, Hz32 is a tolerant line identified from about 120 inbred collections, however, Mo17 is a line sensitive to submergence (Jiang and Zhang, 1999). In theory, the tolerance difference among inbred should also be a representation of different regulation ability to metabolism under low oxygen. In the first early stage (0–4 h in maize, 0–0.5 h in Arabidopsis), it includes the rapid induction or activation of signal transduction components, the initial signal reception response, in turn, activating the next stage, such as metabolic and cellular adaptation (Dennis et al., 2000; Klok et al., 2002). Thus, gene expressions in the first stage play a critical role on regulating gene expressions, which are involved in physiological adaptation. Gene mining expressed in the first stage is a significant effort to understand molecular mechanism on different tolerance among inbred to submergence. In this study, a set of early responsive genes (0–4 h) was confirmed by cDNA microarray and SSH. The results suggest that rapidly metabolic conversion and response under submerging treatment could be a critical mechanism of tolerance in Hz32, and that regulation of gene expression in response to submergence involves significant transcriptional level control, especially during earlier stress treatment periods.

2. Materials and methods

2.1. Plant materials, tissues collection, total RNA extraction and poly(A+) RNA purification

Mo17 is the second cycle line from CI187-2 × C103 from the USA, but Hz32 was selected from a mutant plant of Hz1 in China, the extensive genetic difference is revealed by morphological and molecular marker assay, and a commercial hybrid was developed by the combination of the two inbreds (Huang and Li, 2002). The two inbreds were germinated in an incubator individually, and then planted in a pot until these seedlings developed three leaves. During this period, seedlings were irrigated with 1 × nutrient solution [$Ca(NO_3)2$ 820.7 mg/L, KNO_3 505.6 mg/L, $MgSO_4{\cdot}7H_2O$ 616.2 mg/L, KH_2PO_4 272.2 mg/L, Fe-EDTA 13.02 mg/L, H_3BO_3 2.860 mg/L, $MnSO_4$ 1.015 mg/L, $CuSO_4{\cdot}5H_2O$ 0.079 mg/L, $ZnSO_4{\cdot}7H_2O$ 0.220 mg/L, H_2MoO_4 0.090 mg/L]. At the stage of three leaves, seedlings per inbred were divided into two sets and planted at room temperature. A set of seedling was submerged under 1 × nutrient solution; another was used as control and irrigated with 1 × nutrient solution based on need of plant growth. After 0, 1, 2, 4, and 8 h of submerging, the roots were collected. Root samples (2–3 g) were collected to extract total RNA using TRIzol reagent (Life Technologies, United States). Poly (A^+) RNA was purified from total RNA using a polyATract® mRNA Isolation System IV kit (Promega, United States).

2.2. Suppression subtractive hybridization (SSH)

SSH was performed according to the protocol of the PCR-Select™ cDNA Subtraction Kit (BD Biosciences Clontech, CA, United States). For SSH analysis, the mixed RNA from treatments 1–8 h and control were used. RNA of Mo17 treatment subtract that of control to form reverse subtracted library of Mo17, in contrast, RNA of Mo17 control subtract that of treatment to form forward subtracted library of Mo17. Similarly, forward and reverse subtracted cDNA library of Hz32 were constructed. Randomly 380 clones from per library were screened to identify the differentially expressed clones according to Sambrook and Russell (2001).