

Article Addendum

Transcriptional and post-transcriptional regulation of gene expression in submerged root cells of maize

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Abbreviations: ABA, abscisic acid; ADH, alcohol dehydrogenase; ALDH, aldehyde dehydrogenase; ANPs, anaerobic proteins; APs, aerobic proteins; ARF, auxin response factor; LDH, lactate dehydrogenase; miRNA, microRNA; SOD, super oxide dismutase; ROS, reactive oxygen species; TF, transcription factor

Key words: maize, *Zea mays* L., anaerobic stress, adaptation, gene expression, microRNA

Maize survival under the anaerobic stress due to submergence conditions is dependent on complex metabolic, physiological and morphological adaptation strategies. Here, we focus on gene expression regulation at the transcriptional and post-transcriptional level in submerged maize root cells. Early in progressive oxygen deprivation, root cells sense the low oxygen signal to trigger expressions of TF genes, anaerobic response genes and miRNA genes. The induced TFs, in turn, promote a broad spectrum of responses from morphogenetic to metabolic; these responses occur at later stages of the stress treatment. The selective translation of anaerobically induced transcripts and selective degradation of some APs are also suggested to be an important regulatory mechanism. In addition, miRNAs are possibly transcriptionally regulated in submerged root cells and involved in post-transcriptional control of target genes. Thus, regulation of gene expression in response to low oxygen involves in significant transcriptional and post-transcriptional control.

Introduction

Anaerobic or low oxygen conditions occur when maize plants are submerged or subjected to flooding of the soil. Maize survival under low oxygen conditions is mainly dependent on metabolic, physiological and morphological adaptation strategies. Under low oxygen conditions, a few ANPs are selectively synthesized, and most of the ANPs identified were found to be enzymes of glycolysis, such as aldolase, enolase, ADH and LDH.¹ The synthesis of these

enzymes indicated that oxidative phosphorylation of mitochondria is blocked, and cells undergo glycolysis and ethanolic fermentation, thus replacing the Krebs cycle in fulfilling the cellular demand for energy. Especially, during ethanolic fermentation, ADH is responsible for the recycling of the NAD⁺ needed for the glycolysis process to continue.² High levels of ADH accumulation and its activity is considered to be positively correlated with the magnitude of the stress injury, but negatively correlated with tolerant to anaerobic stress.^{3,4} Physiologically, alteration of metabolic pathways results in a change of cytosolic pH, accumulation of ROS and hormone homeostasis.⁵⁻⁸ Adaptation also occurs via a wide variety of morphological and anatomical responses to oxygen limitation of roots or entire plants, including formation of aerenchyma, fast underwater elongation of shoots or leaves, stomatal closure, adventitious root formation and lateral root development.^{3,9-11} Each of these reactions is mediated by plant hormones, such as ethylene, auxin and ABA.

Our discussion largely centres on recent work in our laboratory carried out on maize. In most of these studies, a progressive depletion of oxygen in roots was carried out by completely submerging seedlings that had developed three leaves, in growth culture buffer. Our results supported the view that submergence results in alterations of gene expression leading to metabolic, physiological and morphological changes, and that transcriptional and post-transcriptional regulation of gene expression is involved these adaptations of maize under submergence conditions.

Transcriptional Regulation of Gene Expression in Submerged Roots of Maize

Previous studies showed that mRNAs encoding ANPs, including important enzymes in glycolysis, accumulated under low oxygen conditions, e.g., pyruvate decarboxylase, ADH, glucose phosphate isomerase, and lactate dehydrogenase.^{1,12-16} Both high-throughput cDNA microarrays and suppression subtractive hybridization were employed in our laboratory to investigate the gene expression response in root cells of maize, and we found that numerous gene expressions were altered because of submergence treatment. These genes are involved in a broad spectrum of biochemical, cellular,

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and physiological processes, such as glycolysis, energy metabolism, lipid metabolism, signal conduction, DNA transcription, protein biosynthesis/degradation, and photosynthesis.^{17–19} The spectrum of genes altered in the first 0.5 h of treatment was significantly different from that of the genes induced later 2–4 h.¹⁸ Comparison of the genes expression profile between a tolerant (Hz32) and a sensitive line (Mo17), showed that the response speed was different between the tolerant and sensitive line. The expressions of genes associated with fermentation were higher and earlier in the tolerant line compared to the sensitive line. A similar phenomenon was also revealed for genes involved in signal transduction, protein synthesis, protein trafficking and defense processes.¹⁸ Especially, a few TFs were identified to be more highly expressed in the tolerant line than that in the sensitive line. For example, during 0–1 h of treatment, an *rs2*-like TF, which encodes a type of Myb protein, was induced. Previous results demonstrated that the promoters of anaerobic response genes contain GT and CG motifs. The GT-motif resembles a Myb transcription factor binding site with a 5'-AAC-3' central motif and Arabidopsis Myb transcription factor AtMyb2 binds specifically to the GT-motif of *Adh1*.²⁰ Thus, we believed that *rs2* plays a key role during Hz32 roots' response to submerging stress. Another TF induced in Hz32 is *zm-bRLZ* which contains a bRLZ domain, a DNA binding domain and is highly homologous to the rice transcription factor, RISBZ1. Moreover, sequence variation in the promoter region of *zm-bRLZ* was analyzed, and 2 or 3 deletions in the promoter region of the sensitive line were found by comparison with that of the tolerant line. Similar variations also were discovered in promoters of other genes (unpublished). It is possible to partly elucidate the reason why the expression of *zm-bRLZ* is higher and earlier in tolerant line than in sensitive. We proposed that maize root cells under submergence firstly sense the low oxygen signal, and then promote the expressions of various TFs, which, in turn, likely regulate the expression of responsive genes to induce diverse mechanism of adaptation.

Post-Transcriptional Regulation of Gene Expression in Submerged Root Cells of Maize

A fact revealed by proteomics is that ANPs are accumulated with a concomitant increase in their transcripts, but some APs did not increase while their mRNAs accumulate in oxygen deprived cells.^{21–23} Subsequently, the accumulations of ANPs were shown to be tightly associated with efficient loading of anaerobically responsive transcripts with polysomes. Although a large number of aerobic transcripts are also induced in response to oxygen deprivation, fewer aerobic transcripts are loaded with ribosomes.^{24,25} Comparison of total mRNA populations with polysomal RNA populations reveal that low oxygen reduces the average proportion of individual mRNA species in large polysome complexes, including not only aerobic transcripts, but also a subset of known anaerobic-induced gene transcripts. Moreover, a large number of mRNAs displayed a significant decrease in polysome association without a concomitant decrease in steady-state accumulation. By contrast, a small group of abiotic and biotic stress-induced mRNAs showed a significant increase in polysome association, without a change in abundance.^{23,26} Evaluation of mRNA sequences demonstrated that a low GC nucleotide content of the 5'-untranslated region provides a selective advantage for translation under low oxygen.^{23,26} These evidences demonstrate that selected synthesis of anaerobic polypeptides involves transcriptional, as well as significant post-transcriptional, regulation of gene expression.

Another possible cause of this is that APs were selectively degraded by the proteasome. An interesting observation from cDNA microarray data is that a few genes, SKP1/ASK1-like protein and 20S proteasome subunit α -3, which are involved in protein degradation, were also upregulated.¹⁷ The SKP1/ASK1-like protein is a subunit of the SKP1, cullin/CDC53, F-box protein (SCF) complex, and plays an important role in selecting substrates for proteolysis by facilitating ubiquitin binding to specific proteins. In plants, the SCF complex can regulate phytohormone responses.^{27,28} The 20S proteasome subunit α -3, a subunit of a 700-kD multi-subunit protease, facilitates ubiquitin-dependent proteolysis, and participates in the breakdown of some proteins in the absence of ubiquitin.²⁹ At early submergence, the increase in the SKP1/ASK1-like protein implies an increase of the SCF complex. The induced expression of ubiquitin suggests that aerobic proteins bound to ubiquitin are selected as substrates and disassembled by 20S proteasome. The SKP1/ASK1-like protein and the 20S proteasome might play a crucial role in signal transduction by selective degradation of proteins involved in aerobic metabolism, implying that regulatory mechanisms at the translation level also play a crucial role in the low-oxygen response during early submergence.¹⁷

Recently, microRNAs, a class of RNA that target mRNA for degradation, were also shown to be transcriptionally regulated in response to submergence in root cells of maize. We used a miRNA microarray to investigate miRNA populations in maize roots, and showed that the expressions of 39 different miRNAs, falling into four different patterns, are affected by submergence. A group of four miRNAs show early upregulation, another group of four miRNAs show early downregulation and later upregulation under submergence.³⁰ The alteration of the expression levels of miRNAs, in turn, directly regulates cleavage and translation of target mRNAs at the post-transcriptional level. The target mRNAs for the four upregulated miRNAs encode TFs. These TFs, mostly involved in root growth and morphogenesis, are in turn downregulated; most likely leading to an inhibition of elongation and a promotion of adventitious roots. We found that the IAA content increased during progressive submergence for 6 days, whereas ABA content decreased during 3 days of submergence, before rapidly increasing after 3 days of submergence (Fig. 1A). The early upregulated miRNA, miR167 targets auxin response factor 17 (*ARF17*), which is key factor in the Auxin/IAA signal cascade. *ARF17* negatively regulates the expression of *GH3* and downstream genes, such as *ramosa 2*. The RT-PCR profiles of *ARF17*, *GH3* and *ramosa 2* were coincident with the expectation mentioned above (Fig. 1B). The changes in expressions of these genes results in a promotion of adventitious roots (Fig. 1C and D). In addition, the miRNA osa-miR528-like, which was downregulated at the early stage and upregulated at the late stage of submergence, can lead to accumulation target mRNAs (*SOD* and *ALDH*) and their proteins. The results from RT-PCR of *SOD* (Fig. 2A), the protein band pattern (Fig. 2B) and *SOD* enzyme activity (Fig. 2C) support the above hypotheses. Thus, the overall pattern of changes is seen to enable the root to respond appropriately to submergence at all levels from morphogenetic to metabolic.

Conclusions

Complex mechanisms are involved in regulation of adaptation to low oxygen in root cells of maize. Under progressive oxygen

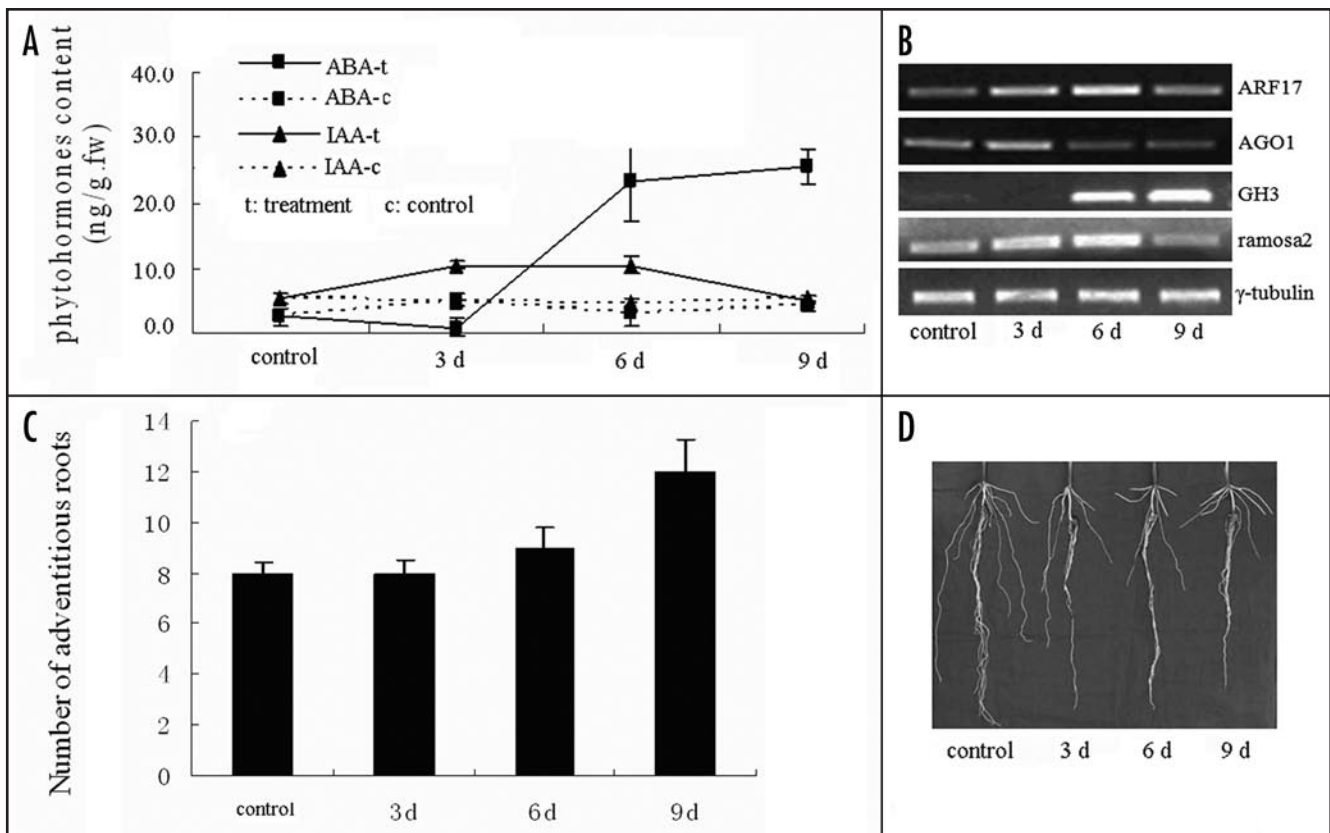


Figure 1. Morphological adaptation of maize roots under submergence conditions is regulated at the transcriptional and post-transcriptional level. (A) Hormone homeostasis is disturbed under submergence conditions. Hormone content was measured by enzyme-linked immunosorbent assay (ELISA) (reviewed in ref. 31). (B) Gene expression profiles involved in auxin cascade and regulation of adventitious root development at different time points of submergence treatments. (C and D) the number and conformation of adventitious roots.

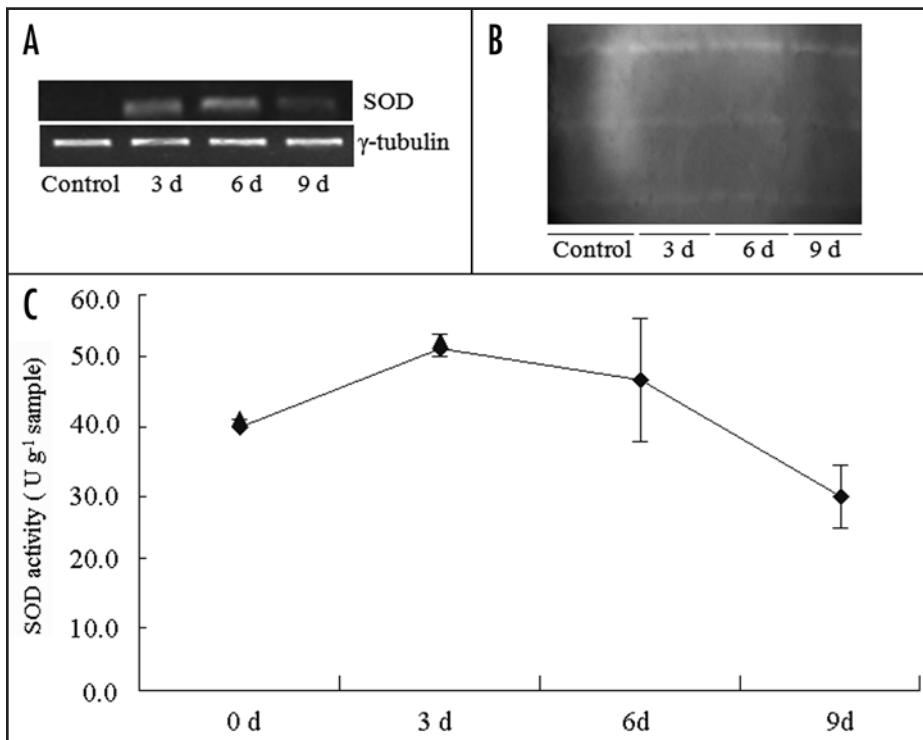


Figure 2. Accumulation of transcript and protein, and enzyme activity of SOD. Activity of SOD was measured by monitoring the inhibition of photochemical reduction of nitro blue tetrazolium (NBT) (reviewed in ref. 32). (A) SOD expression profile assayed by RT-PCR. (B) SOD isozyme bands on PAGE. (C) SOD activity at different time-points of submergence treatments in roots of maize.

deprivation, root cells sense the low oxygen signal to trigger expressions of some TF genes, genes encoding ANPs and miRNA genes. The induced ANPs are involved in modulation of glycolysis and energy metabolism. The induced TFs, in turn, promote a broad spectrum of responses from morphogenetic to metabolic, these responses occurring at the later stage of the stress treatment. The selective translation of anaerobically induced transcripts and selective degradation of some ANPs are also importantly regulation mechanisms. In addition, miRNAs are potentially regulated in submerged root cells at the transcriptional level, and are involved in post-transcriptional control of target genes such as *ARF17* and *SOD*. Thus, regulation of gene expression in

response to low oxygen involves significant transcriptional and post-transcriptional control.

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