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Cytosolic malate dehydrogenase 4 modulates cellular energetics and storage reserve accumulation in maize endosperm

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Keywords: Maize, cytosolic malate dehydrogenase 4, energetics, starch, zein, mitochondrion.

Abstract

Cytosolic malate dehydrogenase (MDH) is a key enzyme that regulates the interconversion between malate and oxaloacetate (OAA). However, its role in modulating storage compound accumulation in maize endosperm is largely unknown. Here, we characterized a novel naturally occurring maize *mdh4-1* mutant, which produces small, opaque kernels and exhibits reduced starch but enhanced lysine content. Map-based cloning, functional complementation and allelism analyses identified *ZmMdh4* as the causal gene. Enzymatic assays demonstrated that ZmMDH4 predominantly catalyses the conversion from OAA to malate. In comparison, the activity of the mutant enzyme, which lacks one glutamic acid (Glu), was completed abolished, demonstrating that the Glu residue was essential for ZmMDH4 function. Knocking down *ZmMdh4 in vivo* led to a substantial metabolic shift towards glycolysis and a dramatic disruption in the activity of the mitochondrial complex I, which was correlated with transcriptomic alterations. Taken together, these results demonstrate that *ZmMdh4* regulates the balance between mitochondrial respiration and glycolysis, ATP production and endosperm development, through a yet unknown feedback regulatory mechanism in mitochondria.

Introduction

Owing to its high value as animal feed, raw industrial material, food for human consumption and fuel through bioethanol production (Ranum *et al.*, 2014), maize (*Zea mays* L.) is widely distributed and cultivated throughout the world. Maize endosperm accounts for over 75% of the kernel dry weight and mainly contains starch and protein. Maize *starchy* endosperm mutants often exhibit changes in the structure and/or accumulation of starch granules and/or protein bodies (PBs), thus affecting grain yield and nutritional quality. Examples include the *opaque* (*o*) and *floury* (*fl*) mutants. *o1*, *o10*, *fl1* and *fl4* mutants have abnormal PBs and noticeable changes in the levels of zeins and alcohol-soluble prolamins, which are the main components of PBs (Holding *et al.*, 2007; Larkins and Hurkman, 1978; Lending and Larkins, 1989; Wang *et al.*, 2014a; Wang *et al.*, 2012; Yao *et al.*, 2016). Several other *opaque/floury* mutants, such as *o2*, *o6*, *o7*, *o11*, *fl2*, *fl3*, *Mucronate*, *De*-B30*, *Mto140* and *ocd1*, display reduced zeins and a compensatory increase in non-zeins, which result in elevated lysine content (Coleman *et al.*, 1997; Feng *et al.*, 2018; Holding *et al.*, 2010; Kim *et al.*, 2006; Kim *et al.*, 2004; Li *et al.*, 2017; Schmidt *et al.*, 1992; Wang *et al.*, 2011; Wang *et al.*, 2014b; Yang *et al.*, 2018). In case of *o5*, however, the *opaque* phenotype is caused by morphological alterations in starch granules rather than changes in protein and/or amino acid composition (Hunter *et al.*, 2002; Myers *et al.*, 2011). These alterations are due to mutations in the structural or regulatory protein genes of zeins. *opaque/floury* mutants, such as *o2*, *o11*, *fl2*, *fl3*, *Mucronate* and *De*-B30*, involve genes that regulate zein biosynthesis (Coleman *et al.*, 1997; Feng *et al.*, 2018; Kim *et al.*, 2006; Kim *et al.*, 2004; Li *et al.*, 2017; Schmidt *et al.*, 1992); whereas, other *opaque/floury* mutants, such as *o1*, *o10*, *fl1* and *fl4*, are controlled by genes that participate in the assembly of zeins into PBs (Holding *et al.*, 2007; Wang *et al.*, 2014a; Wang *et al.*, 2012; Yao *et al.*, 2016). In addition, the *o6*, *o7*, *Mto140* and *ocd1* mutants are impaired in amino acid metabolism or other primary metabolic pathways (Holding *et al.*, 2010; Wang *et al.*, 2011; Yang *et al.*, 2018). For example, *O6* (also known as *Pro1*) and *Mto140* encode Δ^1-pyrroline-5-carboxylate synthetase and arogenate dehydrogenase 1 that catalyse proline and tyrosine biosynthesis, respectively. Mutations of these two genes resulted in restricted proline and tyrosine supply for zein biosynthesis (Holding *et al.*, 2010; Wang *et al.*, 2014b). *O7* and *Ocd1*, however, are oxalyl-CoA synthetase and oxalyl-CoA decarboxylase genes that are involved in oxalate degradation. These mutations affect amino acid levels and zein accumulation (Wang *et al.*, 2011; Yang *et al.*, 2018) through an unknown mechanism. Together, these published data suggest a link between amino acid metabolism, zein biosynthesis and endosperm texture. Thus, the cloning and characterization of additional kernel mutants will deepen our understanding of how protein and/or starch biosynthesis are regulated in maize endosperm.

To this end, we isolated a *starchy kernel* mutant with altered starch, zein and lysine content in the endosperm. The mutant phenotype was found to be the result of a 3-bp deletion in the cytosolic *NAD-dependent malate dehydrogenase* 4 (*Mdh4*) gene. MDHs are oxidoreductases that catalyse the reversible interconversion between malate and OAA in the cytosol and other organelles, including glyoxysomes, mitochondria, peroxisomes

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Identification of anther thermotolerance genes by the integration of linkage and association analysis in maize

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SUMMARY

Maize is one of the world's most important staple crops, yet its production is increasingly threatened by the rising frequency of high-temperature stress (HTS). To investigate the genetic basis of anther thermotolerance under field conditions, we performed linkage and association analysis to identify HTS response quantitative trait loci (QTL) using three recombinant inbred line (RIL) populations and an association panel containing 375 diverse maize inbred lines. These analyses resulted in the identification of 16 co-located large QTL intervals. Among the 37 candidate genes identified in these QTL intervals, five have rice or Arabidopsis homologs known to influence pollen and filament development. Notably, one of the candidate genes, *ZmDUP707*, has been subject to selection pressure during breeding. Its expression is suppressed by HTS, leading to pollen abortion and barren seeds. We also identified several additional candidate genes potentially underly QTL previously reported by other researchers. Taken together, our results provide a pool of valuable candidate genes that could be employed by future breeding programs aiming at enhancing maize HTS tolerance.

Keywords: maize, anther thermotolerance, high-temperature stress, linkage and association analysis, DUP707.

INTRODUCTION

Maize is one of the most widely planted crops in the world and is an important food, forage, and industrial material. Maize is thermophilic, with an ideal growth temperature between 20 and 30°C. Although maize can grow in temperatures ranging from 6 ± 1.1 to 42 ± 3.3°C, temperatures exceeding the optimal range, particularly above 35°C, can significantly reduce yield and affect pollen development. Extreme high temperature weather events are becoming more common (NOAA, 2024), and these events often occur during critical reproductive stages of maize, leading to pollen sterility, with an average decrease of 7.4 ± 4.5% per 1°C increase in global mean temperature (Lobell et al., 2011; Zhao et al., 2017). The proportion of worldwide maize growing areas experiencing high-temperature stress (HTS) for more than 5 days during the reproductive period is projected to increase from 15% in 2000 to 44% in 2050 (Gourdji et al., 2013). In Henan province of China, HTS resulted in 11%–53% yield reduction among 30 major maize varieties from 2013 to 2022. HTS during reproductive stages affected one-third of the maize growing areas in this province in 2022, with 13% of these areas yielding no harvest. A deeper understanding of the mechanisms underlying HTS responses during reproductive stages is essential and could lead to substantial improvements in maize yields (Sprague et al., 2022; Tigchelaar et al., 2018).

Successful stigma pollination, pollen tube growth, and fertilization of female gametes are critical for maize kernel development and yield. However, these processes are highly sensitive to high temperatures (Lizaso et al., 2018; Wang et al., 2019), which can cause sterile pollen with obvious morphological abnormalities, including early tapetum degradation, anther indehiscence, and deformity of pollen grains. These abnormities can cause up to 95% of yield loss in field conditions (Cicchino et al., 2010; Rattalino Edreira et al., 2011). The molecular and physiological

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Multi-omic characterization of the maize GPI synthesis mutant *gwt1* with defects in kernel development

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Abstract

Background Glycosylphosphatidylinositol (GPI) and GPI-anchored proteins (GAPs) are important for cell wall formation and reproductive development in *Arabidopsis*. However, monocot counterparts that function in kernel endosperm development have yet to be discovered. Here, we performed a multi-omic analysis to explore the function of GPI related genes on kernel development in maize.

Results In maize, 48 counterparts of human GPI synthesis and lipid remodeling genes were identified, in which null mutation of the glucosaminyl-phosphatidylinositol O-acyltransferase1 gene, *ZmGWT1*, caused a kernel mutant (named *gwt1*) with defects in the basal endosperm transport layer (BETL). We performed plasma membrane (PM) proteomics to characterize the potential GAPs involved in kernel development. In total, 4,981 proteins were successfully identified in 10-DAP *gwt1* kernels of mutant and wild-type (WT), including 1,638 membrane-anchored proteins with different posttranslational modifications. Forty-seven of the 256 predicted GAPs were differentially accumulated between *gwt1* and WT. Two predicted BETL-specific GAPs (*Zm00001d018837* and *Zm00001d049834*), which kept similar abundance at general proteome but with significantly decreased abundance at membrane proteome in *gwt1* were highlighted.

Conclusions Our results show the importance of GPI and GAPs for endosperm development and provide candidate genes for further investigation of the regulatory network in which *ZmGWT1* participates.

Keywords Maize endosperm development, Transcriptomics, Proteomics, Membrane proteomics, GPI anchored protein

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Zhang et al.
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Involvement of the intracellular β-glucosidase BGL1B from *Aspergillus niger* in the regulation of lignocellulose-degrading enzymes' synthesis

Zhen Zhang[1], Hua Li[1], Feiyu Dong[1], Hui Lin[1], Yanan Li[1], Kun Cheng[1*] and Hongge Chen[1*]

Abstract

Background *Aspergillus niger* is an important lignocellulose-degrading enzyme-producing strain. Multiple regulatory factors regulate the synthesis of lignocellulose-degrading enzymes in *A. niger*. We previously found that *A. niger* possessed an intracellular β-glucosidase BGL1B, and the intracellular localization of BGL1B and its active transglycosylation action prompted us to explore whether BGL1B was involved in the regulation of the synthesis of lignocellulose-degrading enzymes in *A. niger*.

Results In this study, by investigating the production of lignocellulose-degrading enzymes of *bgl1B* knockout strain (Δ*bgl1B*) and overexpression strain (OE::*bgl1B*), it was found that BGL1B exhibited a repressive role on the expression of lignocellulose-degrading enzyme genes through carbon catabolite repression (CCR) way. On the other hand, BGL1B's transglycosylation products sophorose and laminaribiose were proved to be able to induce the expression of lignocellulose-degrading enzyme genes, which explained why OE::*bgl1B* showed the same enhanced enzyme activity and gene expression as Δ*bgl1B* strain compared to the starting strain (WT).

Conclusions The present study demonstrates that BGL1B plays dual regulatory roles in the regulation of the synthesis of lignocellulose-degrading enzymes in *A. niger*: the repressive role caused by BGL1B's hydrolysis product glucose and the induction role caused by BGL1B's transglycosylation products sophorose and laminaribiose. This study broadens the understanding of the regulatory network of the synthesis of lignocellulose-degrading enzymes in *A. niger*. Also, it provides a strategy to create an engineered strain with high production of lignocellulose-degrading enzymes.

Highlights

- Intracellular β-glucosidase BGL1B in *A. niger* was proven to be involved in the regulation of the synthesis of lignocellulose-degrading enzymes.
- Sophorose and laminaribiose were confirmed to have an induction effect on the synthesis of lignocellulose-degrading enzymes in *A. niger*.

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Article

Overexpression of *Myo*-Inositol Oxygenase (*TaMIOXA*) Enhances the Drought and High-Temperature Resistance of *Triticum aestivum* L.

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Abstract

Wheat (*Triticum aestivum* L.) is the most widely cultivated staple food crop globally. As a primary food source for 35–40% of the world's population, the stability of its yield is directly linked to global food security. However, extreme weather events triggered by climate change have led to reductions in wheat yield, resulting in an urgent need to enhance the stress tolerance of wheat against drought and high temperatures. In this study, we successfully isolated and cloned a *myo*-inositol oxygenase gene from wheat. Further research revealed that high temperatures and drought stress significantly increased the expression level of the *TaMIOXA* gene in wheat leaves. A batch of overexpressing lines was obtained via Agrobacterium-mediated transformation. Compared to the control group, wheat plants with molecularly modified *TaMIOXA* overexpression exhibited stronger resistance to high temperatures and drought. This significantly increased their survival rates by 10% to 40%. The cumulative amount of hydrogen peroxide decreased from 7.86×10^{-4} to 1.54×10^{-2} mmol/g, and that of malondialdehyde decreased from 8.42×10^{-7} to 2.21×10^{-6} mmol/g. This confirms that overexpression of *myo*-inositol oxygenase significantly enhances wheat's tolerance to drought and high temperatures. This study offers valuable genetic resources for wheat stress tolerance.

Keywords: wheat; *Myo*-inositol oxygenase; drought; high temperatures; stress resistance

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1. Introduction

Wheat (*Triticum aestivum* L.) is the most widely cultivated staple crop worldwide [1], spanning a broad latitudinal range from 60° N to 40° S and occupying 30.7% of the global grain-cropping area [2]. It serves as a primary food source for 35–40% of the world's population [3], supplying approximately 20% of humanity's caloric and protein requirements [4]. Consequently, the stability of wheat yields is directly linked to global food security [5]. However, extreme weather events driven by climate change are threatening wheat production systems at an unprecedented rate [6]. Current studies indicate that for every 1 °C rise in global temperature, the environmental adaptability of existing wheat varieties declines by 8.7% [7]. High temperatures directly damage the photosynthetic apparatus of wheat leaves, disrupting their chloroplast structure [8] and reducing Rubisco activity by 55–70%,

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In situ generation of reduced graphene oxide on 3D Cu—Ni foam as high-performance electrodes for capacitive deionization

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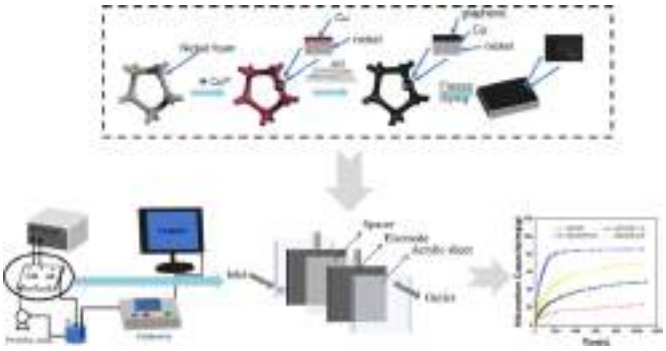
HIGHLIGHTS

- Graphene oxide was in situ reduced and loaded on copper decorated nickel foam.
- rGO/Cu-Ni foam was directly employed as anode and cathode in a CDI cell.
- The introduction of copper improves the pseudocapacitance of the electrodes.
- The composite electrode shows excellent desalination performance.

GRAPHIC ABSTRACT



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ABSTRACT

Capacitive deionization (CDI) is one of the high-efficient technologies for desalination of brackish water. In this paper, a composite electrode with 3D Cu—Ni foam (CNF) as current collector and reduced graphene oxide (rGO) as active material was synthesized, in which graphene oxide was reduced in situ and immobilized on the 3D CNF. Obtained rGO/CNF electrodes were directly employed as anode and cathode in a CDI cell. The effects of different substitution reaction time on the electrodes were investigated by SEM, XRD, Raman and XPS. Results showed that the existence of Cu on CNF could adjust the morphology and the defect content of rGO. The specific capacity of rGO/CNF-1.5 electrode (Note: 1.5 stands for the substitution reaction time) reached 352.5 $F \cdot g^{-1}$ at 5 $mV \cdot s^{-1}$ in 1.0 M NaCl electrolyte. During desalination, the rGO/CNF-1.5 electrode has a high salt adsorption capacity (SAC) of 84.6 mg/g in 250 mg/L NaCl solution at 1.2 V, which is 2.23 times higher than that of rGO/NF. The high performance of desalination can be attributed to the formation of electric double layer capacitance and

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