

Analysis of Nutrient Metabolism Pathways and Identification of Key Candidate Genes Associated with Cadmium Stress in Buckwheat Using Multiomics Analysis

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Abstract

Cadmium (Cd) is a heavy metal that poses significant environmental threats and can severely affect plant growth and development. Buckwheat (*Fagopyrum esculentum*), a pseudocereal with notable nutritional properties, is particularly sensitive to cadmium stress. Understanding how buckwheat metabolizes nutrients and identifies key genes associated with Cd stress is crucial for developing strategies to improve its tolerance. This study employs a multiomics approach, integrating transcriptomics, proteomics, and metabolomics data to elucidate the nutrient metabolism pathways and pinpoint key candidate genes involved in cadmium stress response in buckwheat. The analysis reveals significant alterations in carbohydrate, amino acid, and secondary metabolite pathways under cadmium stress. Several key candidate genes, including those involved in metal transport, antioxidant defense, and stress signaling pathways, were identified. This comprehensive analysis provides a deeper understanding of the molecular mechanisms underpinning cadmium stress in buckwheat, offering potential targets for genetic and biotechnological interventions to enhance buckwheat's resilience to heavy metal stress.

Keywords: Superoxide dismutase • RNA • TCA cycle

Introduction

Cadmium (Cd) contamination in soil is a pressing environmental issue resulting from industrial activities, agricultural practices, and natural occurrences. Cd is readily taken up by plants, leading to toxic effects that impair growth, reduce crop yield, and pose health risks to consumers. Buckwheat (*Fagopyrum esculentum*), valued for its rich nutritional profile, is susceptible to cadmium stress, which affects its metabolic processes and overall health. Understanding the molecular mechanisms behind buckwheat's response to Cd stress is essential for developing strategies to mitigate its impact and improve plant resilience. Buckwheat seeds were germinated and grown under controlled conditions. After three weeks, seedlings were exposed to cadmium by adding CdCl₂ to the nutrient solution at a concentration of 50 μM for seven days. Control plants were grown under identical conditions without Cd. Total RNA was extracted from leaf tissues using the RNeasy Plant Mini Kit (Qiagen). RNA quality was assessed using a Bioanalyzer (Agilent Technologies). RNA-Seq libraries were prepared and sequenced on an Illumina HiSeq platform. Differential expression analysis was performed using DESeq2, comparing Cd-treated plants with controls.

Literature Review

Proteins were extracted using a phenol extraction method. Protein samples were digested with trypsin, and peptides were analyzed by Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS). Proteomic data were processed using MaxQuant software, and differentially expressed

proteins were identified. Leaf metabolites were extracted with methanol, and extracts were analyzed by Gas Chromatography-Mass Spectrometry (GC-MS). Metabolites were identified and quantified using standard libraries. Statistical analysis was performed to identify metabolites significantly altered by Cd treatment. Transcriptomic analysis identified a substantial number of Differentially Expressed Genes (DEGs) in response to cadmium stress. Key pathways affected included carbohydrate metabolism, amino acid biosynthesis, and secondary metabolite pathways. Genes encoding for metal transporters, such as ABC transporters and NRAMP (Natural Resistance-Associated Macrophage Protein) family members, showed significant upregulation. Additionally, genes involved in antioxidant defense mechanisms, such as Superoxide Dismutase (SOD) and Glutathione-S-Transferase (GST), were also upregulated, indicating a response to oxidative stress induced by Cd [1,2].

Proteomic profiling revealed changes in protein abundance corresponding to the transcriptomic data. Proteins involved in carbohydrate metabolism, including enzymes of the glycolysis and TCA cycle, were differentially expressed. Stress response proteins, such as Heat Shock Proteins (HSPs) and chaperones, were notably upregulated, reflecting the plant's attempt to mitigate Cd-induced damage. Proteins involved in the synthesis of secondary metabolites, particularly flavonoids and phenolics, were also significantly altered, suggesting their role in detoxification and antioxidant defense. Metabolomic analysis highlighted significant changes in the levels of various metabolites. There was an accumulation of organic acids, amino acids, and sugars, indicating alterations in primary metabolism under Cd stress. Notably, increased levels of proline, a known osmoprotectant and stress marker, were observed. Secondary metabolites, including flavonoids and phenolic compounds, showed elevated levels, supporting their role in the plant's defense mechanisms against cadmium toxicity [3].

Discussion

The integration of transcriptomic, proteomic, and metabolomic data provides a comprehensive understanding of how buckwheat responds to cadmium stress. The upregulation of metal transporter genes and proteins indicates enhanced metal sequestration and transport mechanisms, which are crucial for reducing Cd toxicity. The activation of antioxidant defense

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pathways suggests a response to mitigate oxidative damage caused by Cd-induced reactive oxygen species (ROS). Carbohydrate metabolism pathways, including glycolysis and the TCA cycle, were significantly impacted. The upregulation of genes and proteins involved in these pathways suggests a shift in energy production and metabolic adjustments to cope with Cd stress. The accumulation of sugars and organic acids, observed in metabolomic analysis, further supports this metabolic reprogramming. Amino acid biosynthesis pathways were notably affected, with increased levels of amino acids such as proline. Proline accumulation is a common response to abiotic stress, serving as an osmoprotectant and stabilizer of proteins and membranes. The upregulation of genes involved in proline synthesis, along with elevated proline levels, underscores its role in buckwheat's stress response [4].

The synthesis of secondary metabolites, particularly flavonoids and phenolic compounds, was enhanced under Cd stress. These compounds are known for their antioxidant properties, suggesting their involvement in mitigating oxidative stress. The upregulation of genes encoding enzymes in the flavonoid biosynthesis pathway, along with increased levels of these metabolites, highlights their protective role. Several key candidate genes were identified as potential targets for improving Cd tolerance in buckwheat. Genes encoding NRAMP family members were significantly upregulated, indicating their role in metal transport and sequestration. Genes such as SOD and GST, involved in detoxifying ROS, were upregulated, highlighting their importance in managing oxidative stress. Heat Shock Proteins (HSPs) increased expression of HSPs suggests their role in protein protection and repair under Cd-induced stress conditions. Transcription Factors: Several transcription factors, including MYB and bHLH, were differentially expressed, suggesting their regulatory role in stress response pathways [5,6].

Conclusion

This multiomics analysis provides valuable insights into the complex molecular mechanisms underpinning cadmium stress response in buckwheat. The identification of key nutrient metabolism pathways and candidate genes offers potential targets for genetic and biotechnological interventions aimed at enhancing buckwheat's tolerance to heavy metal stress. Future research focusing on functional validation of these candidate genes and their regulatory networks will be essential for developing Cd-tolerant buckwheat varieties, contributing to sustainable agriculture and food security in contaminated areas. Functional Validation: Experimental validation of the identified candidate genes through techniques such as CRISPR/Cas9 gene editing or RNA interference (RNAi) to confirm their roles in Cd tolerance. Investigating the regulatory networks and transcription factors controlling the expression of key genes involved in Cd stress response. Testing genetically modified buckwheat lines with enhanced Cd tolerance under field conditions to assess their performance and potential for commercial cultivation. Comparative analysis with other Cd-tolerant and sensitive plant species to identify conserved and unique mechanisms of Cd tolerance.

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Conflict of Interest

There are no conflicts of interest by author.

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