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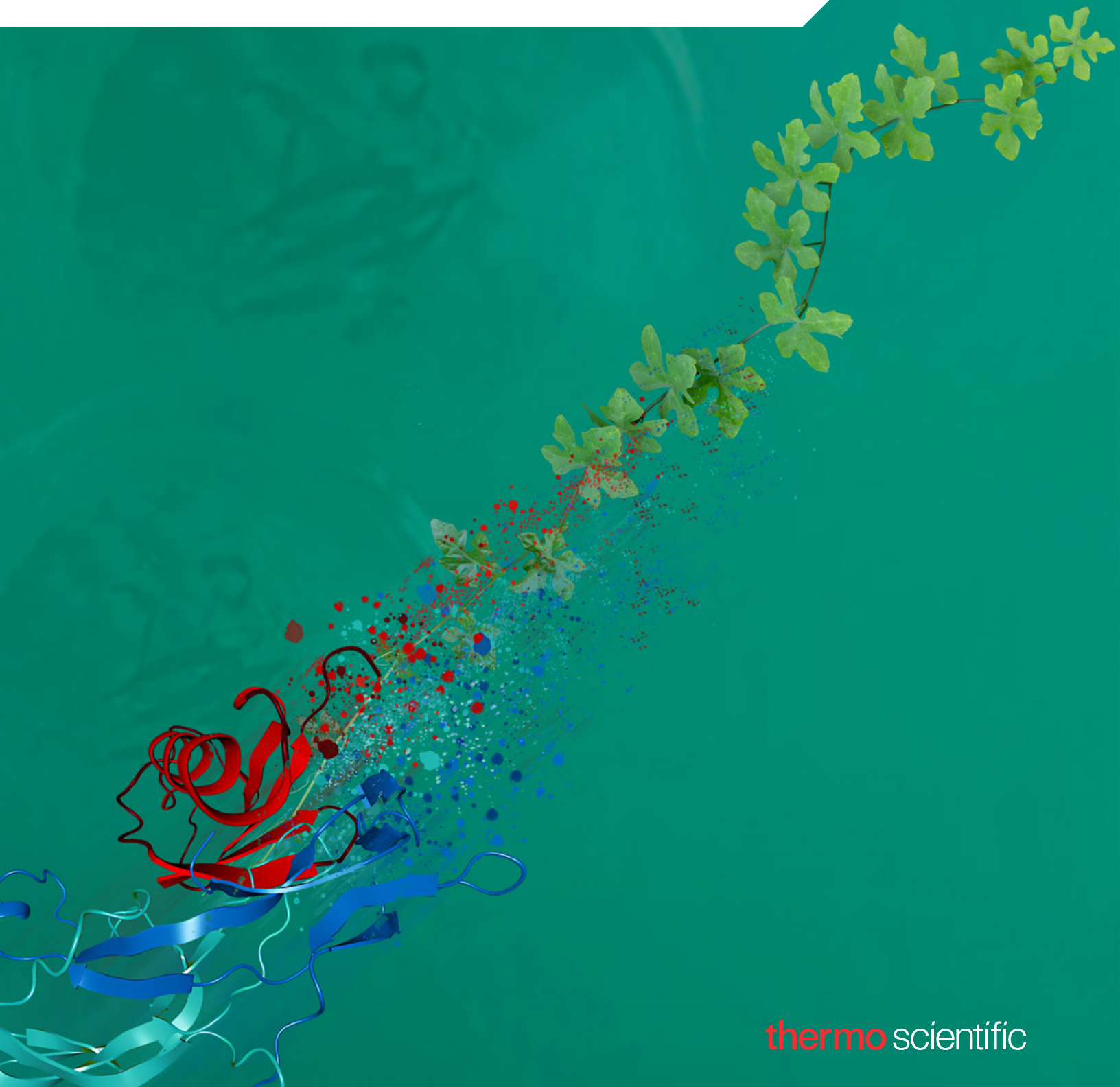
Plant Proteomics

Thermo Scientific™ Orbitrap™ Astral™ mass spectrometer



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Foreword

Plant proteomics is a rapidly advancing field that focuses on the large-scale study of proteins, particularly their structures and functions, within plant systems. Proteins are essential biomolecules that play crucial roles in virtually all biological processes, including growth, development, and response to environmental stimuli. By analyzing the proteome—the entire set of proteins expressed by a plant or a plant tissue—scientists can gain comprehensive insights into the molecular mechanisms underlying plant physiology and adaptation.

The advent of advanced technologies such as mass spectrometry (MS), high-throughput protein sequencing, and bioinformatics tools has propelled the field of plant proteomics forward. These technologies enable the identification, quantification, and functional analysis of thousands of proteins simultaneously, providing a holistic view of the dynamic changes in protein expression and modification.

Plant proteomics has numerous applications, including the discovery of biomarkers for plant diseases, understanding stress responses, improving crop quality and yield, and elucidating the mechanisms of plant-microbe interactions. By integrating proteomics data with genomics, transcriptomics, and metabolomics, researchers can build comprehensive models of plant biology that facilitate the development of innovative agricultural practices and sustainable crop production strategies. Overall, plant proteomics is an essential and transformative field that contributes to our understanding of plant biology at the molecular level, paving the way for advancements in agriculture, environmental management, and biotechnology.

The Thermo Scientific™ Orbitrap™ Astral™ mass spectrometer combines faster throughput, deeper coverage, and higher sensitivity, while delivering accurate and precise quantitation over a wide dynamic range. Powered by the synergy of the high-resolution quadrupole mass filter, Thermo Scientific™ Orbitrap™ mass analyzer and novel Thermo Scientific™ Astral™ mass analyzer, this revolutionary instrument achieves unsurpassed performance with industry-leading usability. The combination of three mass analyzers enables rapid acquisition of exceptional quality high-resolution accurate mass (HRAM) data with high sensitivity and wide dynamic range. The performance characteristics of the instrument makes it ideally suited for plant proteomics with accurate and precise quantitation at an unprecedented depth of coverage at higher throughput.

In this eBook, we highlight some of the groundbreaking plant proteomics research being done using the Orbitrap Astral mass spectrometer. Enjoy exploring the cutting-edge advancements in this exciting field!

1. Deciphering Soybean Nodule Development: The Roles of NIN2 Signaling, GH3-Dependent Auxin Homeostasis, and Proteomic Insights

This article provides an in-depth exploration of the molecular mechanisms that regulate the formation and differentiation of symbiotic nodules in soybean plants. It focuses on the roles of NIN2 signaling and GH3-dependent auxin homeostasis, revealing how they interact to control nodule development, particularly in terms of zonation and cell differentiation.

Soybean plants form specialized structures called nodules on their roots, which when colonized by nitrogen-fixing bacteria, convert atmospheric nitrogen into a form usable by the plant. This process is crucial for plant growth, especially in nitrogen-poor soils. The authors explain that developing soybean nodules are divided into distinct zones: infection zones, where bacteria infect root cells, and nitrogen-fixation zones, where the bacteria convert nitrogen.

The study identifies auxin, a key plant hormone, as a critical regulator of nodule development, such that high auxin levels are essential for maintaining nodule infection zones. High auxin concentrations stabilize the NIN2a protein, a transcription factor that promotes the expression of GH3.1. GH3.1 is an enzyme that conjugates auxin to amino acids, thereby reducing its active levels.

The feedback loop described in the study is particularly significant. High auxin levels stabilize NIN2a, which in turn increases GH3.1 expression. The increased GH3.1 activity lowers the auxin levels, promoting the differentiation of cells in the nodule from infection to nitrogen-fixation zones. This feedback mechanism ensures a regulated transition between the different functional zones within the nodules, which is necessary for effective nitrogen fixation.

To investigate these processes, the researchers employed several experimental techniques. Hormones were quantified using a liquid-chromatography-mass mass spectrometry (LC-MS) multiple reaction monitoring (MRM) method.In addition to hormone quantification, the study also used RNA sequencing to analyze gene expression in the nodules. An important component of the research, the proteomics study, used Orbitrap Astral mass spectrometer-based LC-MS experiments to quantify and understand the molecular changes associated with nodule differentiation and development. In this case the protein expression profiles between wild-type (WT) soybean nodules and gh3Q-L2 mutant nodules, which have altered auxin levels were compared. The comparison revealed significant differences in protein expression between WT and gh3Q-L2 nodules. In the gh3Q-L2 nodules, 447 proteins primarily associated with nucleic acid metabolism and ribosome biogenesis processes were upregulated. This suggests an activation of cell-cycling events in the gh3Q-L2 nodules, indicating that high auxin levels may promote cellular processes related to growth and division. Conversely, 1,858 proteins were downregulated in the gh3Q-L2 nodules, pointing to a broad impact of altered auxin levels on various cellular functions.

The study also highlights the role of NIN2a protein in response to high auxin levels. It was observed that high auxin levels promote the accumulation and condensation of NIN2a-GFP in both soybean nodules and transiently transgenic soybean protoplasts. This indicates that NIN2a might play a crucial role in auxin-mediated regulation of nodule development.

Overall, the research findings provide understanding into the molecular mechanisms governing nodule development in soybeans. By understanding the roles of NIN2a, GH3.1, and auxin in nodule zonation and cell differentiation, researchers can develop strategies to enhance nitrogen fixation in legumes. This has important implications for agriculture, as improved nitrogen fixation can reduce the need for synthetic nitrogen fertilizers, promoting more sustainable farming practices. The insights gained from this research could lead to the development of soybean varieties with enhanced nodule function and nitrogen fixation efficiency, potentially increasing crop yields and reducing undesirable environmental impact.

Reference

Tu, T., Gao, Z., Li, L., Chen, J., Ye, K., Xu, T., Mai, S., Han, Q., Chen, C., Wu, S., Dong, Y., Chen, J., Huang, L., Guan, Y., Xie,

F., and Chen, X. Soybean symbiotic-nodule zonation and cell differentiation are defined by NIN2 signaling and GH3-dependent auxin homeostasis. *Dev Cell.* 59(16):2254-2269.e6. (**2024**).

2. Enhancing Foaming Properties of Seed Protein Isolate through Glucose Conjugation: Structural and Proteomic Insights

Trichosanthes kirilowii is an important crop and medicinal plant in several Asian countries. This article studies the effects of conjugating Trichosanthes kirilowii seed protein isolate (TPI) with glucose on the protein's structural and functional properties, focusing on its foaming characteristics. Foaming plays an important role in the appeal of foamed foods like ice cream. Though egg white and milk proteins are commonly used to enhance foaming, the possibility of using plant proteins instead is of interest due to consumer attention on health and diet, and ethical, religious and environmental concerns. The researchers employed various analytical techniques to characterize the structural changes and to understand the molecular mechanisms behind the improved foaming properties.

The study began with the conjugation of TPI with glucose via the Maillard reaction, a common glycosylation method that involves the reaction of protein amino groups and reducing sugars. The reaction resulted in the formation of TPI-glucose conjugates, which were then analyzed using several structural characterization techniques.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and high-performance size exclusion chromatography (HPSEC) were used to determine the molecular weight distributions of the protein subunits. The results indicated significant changes in the molecular weights of the conjugates compared to the native TPI, confirming the occurrence of glycosylation. The glycosylated proteins exhibited increased molecular flexibility, which is thought to be due to the unfolding of the protein structure, exposing hydrophobic groups and enhancing the protein's ability to adsorb at the air-water interface.

Further structural analyses using UV-Vis absorption spectroscopy and intrinsic fluorescence spectroscopy revealed interactions between the protein and glucose, leading to structural changes in the protein. Fourier-transform infrared spectroscopy (FT-IR) showed the consumption of some functional groups and the formation of new ones, indicating successful glycosylation. Circular dichroism (CD) spectroscopy confirmed changes in the secondary structure of the proteins.

The foaming properties of the TPI-glucose conjugates were evaluated by measuring the protein content adsorbed at the air-water interface, surface tension, and contact angle. The results showed that the glycosylated proteins had better foaming stability and capacity compared to the native TPI. This improvement was attributed to the enhanced diffusion rate and adsorption capacity of the glycosylated proteins, as well as increased steric repulsion between bubbles, which delayed coalescence and aggregation.

LC-MS based analysis played a crucial role in understanding the molecular mechanisms behind the improved foaming properties. Quantitative proteomics using data-independent acquisition (DIA) identified a total of 3139 peptides covering 898 proteins in the initial foam. The analysis revealed differential protein expression between the TPI-glucose conjugates and native TPI. Proteins that contribute to foam strength and stability, such as heat shock proteins and 14-3-3 proteins, were more abundant in the conjugates. These proteins are known to play essential roles in maintaining protein homeostasis and enhancing interfacial membrane strength.

In summary, the study demonstrated that glycosylation of TPI with glucose via the Maillard reaction significantly improved the foaming properties of the protein. This improvement was due to structural changes that increased protein flexibility, enhanced adsorption at the air-water interface, and altered protein-protein interactions. The proteomic analysis provided insights into the specific proteins and mechanisms involved in this enhancement, highlighting the potential of glycosylation as a method for improving the functional properties of food proteins.

Reference

Peng, D., Huang, W., Bao, H., Ding, W., Pan, X., Li, G., Dong, L.,

Li, W., Chen, J., Li, P., and Du, B. **Glucose-induced glycation enhances the foaming properties of Trichosanthes kirilowii seed protein isolate: Insights into structure, interfacial behavior, and proteomics**. *Food Hydrocolloids*. 157, 110444. (**2024**).

3. Multi-Omics Analysis Reveals Key Role of AtuMYB306 in Enhancing Drought Tolerance through Flavonoid Metabolism Regulation in Chinese Chive

This research integrates transcriptomic, metabolomic, and proteomic analyses to understand the molecular mechanisms of chive seedling responses to drought stress.

RNA sequencing and differential gene expression analysis provided transcriptomic data. LC-MS/MS-based metabolomics analysis of the chive seedling treatment group showed that flavonoid compounds significantly increased with drought stress. Flavonoids play a crucial role in plant adaptation and response to various stresses.

The researchers next used the Orbitap Astral mass spectrometer to quantify proteins in chive seedlings under different drought stress conditions. A total of 7127 proteins were quantified across various treatment groups. The proteomes of the three biological replicates for each treatment group showed high correlation, indicating consistent results. Principal component analysis (PCA) demonstrated distinct proteomic profiles for drought-treated seedlings compared to control groups, suggesting significant changes in protein expression due to drought stress.

Proteomics analyses identified differentially accumulated proteins (DAPs) across seven comparisons, including different degrees of drought stress and recovery stages. For instance, compared to the control, 866 proteins increased and 875 decreased in the mild drought (MiD) vs. control (CK) comparison. Similarly, substantial changes were observed in moderate (MoD) and severe drought (SD) conditions. Gene Ontology (GO) enrichment analysis on the DAPs revealed that metabolic processes, particularly those related to polysaccharide and glucan metabolism, were crucial for the drought response in chive seedlings.

The proteomic data were consistent with transcriptomic findings, which also highlighted the importance of metabolic processes during drought stress. The integration of transcriptomic, proteomic, and metabolomic data provided a holistic view of the molecular mechanisms involved, emphasizing the role of specific metabolic pathways in the drought response.

Additionally, the study explores the role of the transcription factor AtuMYB306 by generating transgenic Arabidopsis and chive hairy root lines that overexpress AtuMYB306. These transgenic lines showed increased flavonoid content and improved osmotic stress tolerance. The study demonstrates that AtuMYB306 directly binds to and activates the promoters of key flavonoid biosynthetic genes under drought stress, thereby enhancing drought tolerance.

In summary, the article provides detailed insights into the proteomic changes in chive seedlings under drought stress, highlighting the significant alterations in protein abundance and the critical role of metabolic processes. The findings underscore the complex molecular interactions and regulatory mechanisms that enable chive seedlings to cope with drought conditions.

Reference

Li, T., Wang, Z., Chen, Y., Yao, P., Zhang, Z., Cai, S., Zhu, Y., Yu, Y., Liao, C., Liu, D., Yang, X., Wang, L., and Ma, X. **Multi-omics analysis reveals the transcription factor AtuMYB306 improves drought tolerance by regulating flavonoid metabolism in Chinese chive (Allium tuberosum Rottler)**. *Plant Stress*. Volume 14, 100591. (**2024**).

4. The Role of SlMKK4 and SlMPK20 in Tomato Pollen Development: Insights from Transcriptomic, Proteomic, and Metabolic Analyses

The growth of tomato flowers and fruits is compromised by high temperatures, drought, and salt stress. These stresses can cause abnormal pollen development, which in turn can affect in pollination, fertilization, and fruit set. This article explores the intricate relationship between SlMKK4 and SlMPK20 in tomato

plants, focusing on their roles in pollen development through the modulation of auxin and sugar metabolism, as well as signal transduction pathways.

The study identifies SlMKK4, a MAP kinase, as an upstream regulator of SlMPK20. This interaction was confirmed through various biochemical assays, including yeast two-hybrid (Y2H) assays and in vitro pull-down assays. The SlMKK4 protein was found to specifically interact with SlMPK20, a plant-specific group D MAP kinase, which has a critical role in post-meiotic pollen development.

To further understand the functional implications of this interaction, the researchers conducted a series of experiments involving the knockout and overexpression of SlMKK4 in tomato plants. They used RNA-seq analyses to determine the differential expression of genes in the stamens of genetically modified plants. The results showed significant changes in the expression of genes involved in auxin and sugar metabolism, which are crucial for pollen development.

Proteomics analyses using LC-MS were performed to identify differentially phosphorylated proteins between the SlMKK4 knockout and WT lines. The proteomics data revealed a set of differentially phosphorylated proteins, which were further analyzed to understand their roles in the signaling pathways regulated by SlMKK4 and SlMPK20.

The authors also measured the levels of soluble sugars and endogenous indole-3-acetic acid (IAA) in the plants. The findings indicated that knockout of SlMKK4 led to alterations in the levels of these metabolites, suggesting that SlMKK4 influences hormone and sugar metabolism during pollen development.

Overall, the article provides a comprehensive analysis of the molecular interactions between SlMKK4 and SlMPK20 and their impact on pollen development in tomato plants. The integration of RNA-seq, proteomics, and metabolic analyses offers a detailed understanding of the signaling pathways and metabolic processes regulated by these proteins.

Reference

Chen, L., Chen, L., Zhang, H., Xi, C., Fang, Y., Lai, Y., Pan, C., Lu, G., and Wu, Y. **SlMKK4 is responsible for pollen development in tomato**. *Plant Physiology and Biochemistry*. Volume 216, 10920. (**2024**).

5. Enhancing Phosphate Use Efficiency in Arabidopsis: Insights from the Symbiotic Interaction with Penicillium olsonii TLL1

This article explores the symbiotic interaction between the plant Arabidopsis thaliana and the fungus Penicillium olsonii TLL1, focusing on how this relationship enhances plant phosphate use efficiency (PUE). In particular, the molecular, genetic, and biochemical mechanisms underlying the beneficial interaction between Penicillium olsonii TLL1 and Arabidopsis and how Penicillium olsonii TLL1 enhances PUE in Arabidopsis were investigated.

Phosphate is a critical nutrient for plant growth, but its availability in the soil is often limited. Plants have developed various strategies to cope with phosphate scarcity, including altering root architecture and upregulating specific genes involved in phosphate acquisition and utilization.

The authors used a variety of experimental approaches, including proteomic and transcriptomic analyses, to identify changes in protein and gene expression levels in Arabidopsis when treated with Penicillium olsonii TLL1 under low-phosphate conditions. The results revealed significant alterations in the expression of genes related to root hair formation, phosphate transport, and phytohormone signaling pathways. For instance, genes such as ETC1, CPL3, and RHS2, which are associated with root hair development, showed enhanced expression, leading to increased root surface area and improved phosphate uptake.

Proteomic analysis using LC-MS experiments uncovered differentially expressed proteins in the roots and shoots of Arabidopsis treated with Penicillium olsonii TLL1. These proteins are involved in various biological processes, including stress

response, metabolism, and nutrient transport, suggesting that Penicillium olsonii TLL1 treatment modulates the plant's physiological and metabolic state to optimize phosphate acquisition and utilization.

The study also discusses the role of phytohormones, such as auxins and ethylene, in mediating the plant's response to phosphate deficiency. The interaction with Penicillium olsonii TLL1 appears to influence the levels and activity of these hormones, further enhancing the plant's adaptive responses.

In conclusion, the research provides comprehensive insights into the molecular mechanisms by which Penicillium olsonii TLL1 enhances PUE in Arabidopsis. This symbiotic relationship not only improves phosphate acquisition but also promotes overall plant health and resilience, potentially offering a sustainable solution for improving crop productivity in phosphate-limited soils. The findings underscore the importance of beneficial plant-microbe interactions in agricultural practices and pave the way for further studies to explore the application of symbiosis in other crops.

Reference

Agisha, V. N., Suraby, E. J., Dhandapani, S., Sng, Y. H., Lim, S. H., and Park, B. S. **Molecular Mechanisms of Phosphate Use Efficiency in Arabidopsis via Penicillium olsonii TLL1**. *Int J Mol Sci*. 25(23):12865. (**2024**).

6. Role of SsPtc3 in Regulating Protein Phosphorylation and Autophagy in Sclerotinia sclerotiorum

This article investigates the functional role of the protein phosphatase SsPtc3 in the growth and pathogenicity of the plant-pathogenic fungus Sclerotinia sclerotiorum. This research is important because it explores the molecular mechanisms by which SsPtc3 influences various biological processes, particularly through its role in protein phosphorylation, a critical post-translational modification (PTM) that regulates numerous cellular activities.

SsPtc3 is identified as a member of the protein phosphatase 2C (PP2C) family, which is known for its role in dephosphorylation. Dephosphorylation involves the removal of phosphate groups from proteins, a process that is essential for regulating the phosphorylation status of proteins and controlling the various signaling pathways in the cell. The study focuses on how SsPtc3 affects these processes, especially through its interaction with SsAtg1, a kinase involved in autophagy (a cellular degradation and recycling process).

Through a series of experiments, including yeast two-hybrid (Y2H) assays and co-immunoprecipitation (Co-IP) experiments, the researchers demonstrated that SsPtc3 physically interacts with SsAtg1. This interaction suggests that SsPtc3 may regulate autophagy by dephosphorylating SsAtg1, thereby influencing its activity.

To further elucidate the functional implications of SsPtc3, the researchers conducted phosphoproteomic LC-MS analyses to compare the phosphorylation profiles of WT S. sclerotiorum and a mutant strain lacking SsPtc3 (ΔSsptc3). The results revealed significant differences in the phosphorylation levels of various proteins between the two strains. Specifically, several peptides exhibited altered phosphorylation states, indicating that SsPtc3 plays a critical role in modulating phosphorylation-dependent signaling pathways.

Additionally, a conserved motif analysis of phosphorylation modifications provided insights into the specific amino acid sequences that are preferentially phosphorylated or dephosphorylated in the presence of SsPtc3. This analysis helped identify the conserved sequences that play a role in the phosphorylation process regulated by SsPtc3.

Overall, the study demonstrates that SsPtc3 is a pivotal regulator of protein phosphorylation in S. sclerotiorum, affecting various cellular processes, including autophagy. The findings suggest that SsPtc3's dephosphorylation activity is necessary for proper functioning of signaling pathways that control the pathogenicity and development of this fungal pathogen. Understanding these molecular mechanisms provides valuable insights into the biology of S. sclerotiorum and could inform strategies for managing the diseases it causes.

Reference

Jiao, W., Ma, D., Zuo, Q., Li, Y., Hu, J., , D., Zhang, Y., Liu, J., Zhang, X., and Pan, H. SsPtc3 Modulating SsSmk1-MAPK and Autophagy to Facilitate Growth and Pathogenicity in Sclerotinia sclerotiorum. *Mol Plant Pathol*. 25(12):e70037. (**2024**).

7. Unraveling Drought Resistance Mechanisms in Agropyron mongolicum: A Multi-Omics Approach

Drought is a major abiotic stress that significantly impairs plant growth and productivity. This article is a detailed study of the drought resistance mechanisms in Agropyron mongolicum, a perennial grass species adapted to arid environments and renowned for its superior drought tolerance. The research aims to elucidate the molecular basis of this resistance, which is crucial for developing crops with enhanced drought tolerance to improve agricultural sustainability in arid regions.

The study employs a multi-omics approach, integrating transcriptomic, proteomic, and metabolomic analyses to investigate the responses of A. mongolicum to drought stress. This comprehensive strategy allows for a detailed examination of the interactions between genes, proteins, and metabolites under stress conditions.

A. mongolicum seeds were sterilized, soaked, and germinated. The seedlings were then subjected to drought treatment, and samples were collected at various time points for analysis, enabling the researchers to capture the dynamic changes occurring in the plant in response to drought.

Transcriptomic analysis identified thousands of differentially expressed genes (DEGs) in both shoots and roots. These genes are involved in various biological processes, including metabolic pathways, redox enzyme activity, and hormone metabolism. Gene Ontology (GO) enrichment analysis highlighted significant associations with metabolic pathways, suggesting that these pathways play a key role in the plant's response to drought.

Proteomic analysis using LC-MS identified over 7,000 distinct proteins, with a subset showing differential expression under drought conditions. The differentially expressed proteins (DEPs) were enriched in pathways related to amino acid and carbohydrate metabolism, indicating their importance in drought resistance. Hierarchical clustering analysis revealed distinct protein expression patterns between shoots and roots, underscoring the tissue-specific responses to drought.

Metabolomic analysis showed significant changes in the levels of various metabolites under drought stress. The pathways related to amino acid metabolism, carbohydrate metabolism, and redox enzyme activity were prominently affected. These findings suggest that the accumulation of certain metabolites, such as water-soluble sugars, plays a crucial role in maintaining cellular function during drought stress.

The integration of the transcriptomic, proteomic, and metabolomic data collected in the study revealed that A. mongolicum's response to drought involves complex interactions between genes, proteins, and metabolites and impacts several important metabolic pathways:

Amino acid metabolism was significantly affected by drought stress. Upregulation of genes and proteins involved in this pathway suggests that amino acids play a protective role under drought conditions, possibly by acting as osmoprotectants or signaling molecules.

Carbohydrate metabolism also affected by drought stress. The accumulation of water-soluble sugars helps maintain cellular osmotic balance and provides energy during stress conditions.

Redox enzyme activity was notably altered, indicating the importance of maintaining redox homeostasis under drought stress. The upregulation of genes and proteins involved in this pathway suggests that the plant activates antioxidant defenses to mitigate oxidative damage.

Changes also occured in the expression of genes and proteins involved in cell wall synthesis. This suggests that the plant modifies its cell wall structure to enhance its mechanical strength and reduce water loss during drought.

In summary, the article presents a comprehensive multi-omics study of the drought resistance mechanisms in A. mongolicum. The integration of transcriptomic, proteomic, and metabolomic data provides a detailed understanding of the plant's response to drought stress, highlighting key pathways and processes that contribute to its drought tolerance. Understanding the molecular mechanisms of drought resistance is crucial for developing strategies to mitigate the impact of drought on agriculture. The insights gained from this study could contribute to the development of crops that are better adapted to arid environments, thereby enhancing food security in the face of climate change.

Reference

Ma, X., Liang, Q., Han, Y., Fan, L., Yi, D., Ma, L., Tang, J., and Wang, X. **Integrated transcriptomic, proteomic and metabolomic analyses revealing the roles of amino acid and sucrose metabolism in augmenting drought tolerance in Agropyron mongolicum**. *Front Plant Sci*. 15:1515944. (**2024**).

8. Enhancing Rice Grain Yield and Photosynthetic Efficiency through OsNOP2 Gene Knockout

The OsNOP2 gene is known to be involved in mRNA methylation, a critical process for regulating gene expression This study focuses on the role of the OsNOP2 gene in rice plants, specifically its impact on grain yield and plant physiology under normal, heat treatment, and saline soil conditions. The researchers employed various experimental techniques to manipulate and analyze the gene's function, including gene knockout (KO) experiments, protein analysis, and RNA studies.

Two knockout lines, KO1 and KO2, were created and then compared to WT plants. Detailed morphological analyses were conducted, including measurements of chloroplast size and number, protein expression levels related to photosynthesis, and overall plant growth under different nitrogen fertilization conditions.

The results showed significant differences between the KO lines and WT plants. The KO lines exhibited increased chloroplast size and number, leading to enhanced photosynthetic efficiency. This was further evidenced by higher levels of photosynthesis-related proteins and increased RubisCO content and activity in the KO plants.

Field studies provided crucial insights into the practical implications of these genetic modifications. The KO plants demonstrated improved grain yield per plant and per plot, increased tiller numbers, and higher grain weight and number per panicle. These findings suggest that knocking out the OsNOP2 gene positively influences rice productivity. To test whether OsNOP2 plays a similar role in other crops, OsNOP2 knockout lines of wheat and tomato were also studied. Similar significant increases in the yield were also observed, indicating that OsNOP2 affects plant growth and yield-related traits across many crops.

Additionally, the study investigated the molecular mechanisms underlying these phenotypic changes. RNA immunoprecipitation assays were conducted to investigate the interactions between OsNOP2 and its target RNAs. The data indicated that OsNOP2 plays a role in mRNA stability and translation, which are essential for proper plant development and stress responses.

Overall, the article presents a detailed account of how OsNOP2 knockout boosts rice yield by enhancing photosynthetic capacity and altering key physiological processes. The findings highlight the potential of targeting mRNA methylation pathways for crop improvement, offering promising avenues for future agricultural research and genetic engineering.

Reference

Gu, X., Li, X., Li, C., Wang, X., Yang, L., Guo, W., Mao, Y, Liu, H., Li, D., and Yan, S. **Elevating RNA m5C methylation provides a promising strategy for crop productivity**. *Research Square*. Preprint. (Accessed February 11, **2025**).

9. Comprehensive Characterization and Therapeutic Potential of Perilla-Derived Exosome-Like Nanoparticles

This article explores the characterization and potential therapeutic applications of exosome-like nanoparticles derived from the herb

Perilla frutescens, commonly known as perilla. In particular, the perilla-derived exosome-like nanoparticles (PELNs), were studied for their structural and compositional properties, as well as their biological activities.

The researchers utilized transmission electron microscopy to visualize the PELNs, revealing their size and morphology. The particle size distribution was analyzed, showing a consistent range that is typical for nanovesicles. Compositional analysis identified various microRNAs (miRNAs) in the PELNs, which are small non-coding RNA molecules involved in the regulation of gene expression. The miRNAs' gene targets were subjected to Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses to understand the biological processes and pathways they might influence.

A significant part of the study focused on the proteomic analysis of PELNs using LC-MS. The team identified a total of 983 proteins in the PELNs, which were then annotated using the UniProt database, providing a comprehensive overview of the protein composition. Molecular weight distribution analysis showed that 91% of the identified proteins fell within the 10–100 kDa range, aligning with the size range typically associated with extracellular vesicle (EV) proteins. Domain analysis of these proteins revealed their involvement in a broad spectrum of cellular processes. Notable domains included the P-loop-containing nucleoside triphosphate hydrolase, NAD(P)-binding domain superfamily, protein kinase-like domain superfamily, protein kinase domain, and serine/threonine-protein kinase. The list of involved domains suggests that the proteins in PELNs play crucial roles in energy metabolism, signal transduction, and other cellular activities.

In addition to proteomics analyses, the researchers also performed lipidomic and metabolomic analyses. These analyses identified various lipids and metabolites in the PELNs, which added to the understanding of their potential roles in cellular communication and metabolic regulation.

The biological activities and therapeutic potential of PELNs were investigated in zebrafish as a model system, focusing on their toxicity, biocompatibility, antioxidant properties, and ability to modulate immune responses. These studies indicated that perilla PELNs are not toxic and instead have potentially beneficial bioactive properties including potent anti-inflammatory and antioxidant effects.. The PELNs inhibited neutrophil (a type of white blood cell that is released to fight infections and inflammatory processes) migration to zebrafish wounds, attenuated the expression of immune marker genes, and modulated inflammatory biochemical pathways. The PELNs also enhanced enzymatic and non-enzymatic antioxidants and reduced reactive oxygen species levels in zebrafish subjected to stress.

The study findings highlight the potential of PELNs as a natural, plant-derived therapeutic agents with antioxidant and immune-modulating properties. The comprehensive characterization of PELNs paves the way for future research into their applications in medicine, particularly in the treatment of oxidative stress-related diseases and immune system disorders.

Reference

Huanga, J., Chen, L., Wenhua Li, W., and Chang, C. J. **Anti-Inflammatory and Antioxidative Effects of Perilla Frutescens-Derived Extracellular Vesicles: Insights from Zebrafish Models**. *SSRN*. Preprint .(Accessed February 11, **2025**).

Summary

Recent research using the Orbitrap Astral mass spectrometer has advanced plant proteomics, providing deeper insights into plant physiology and stress responses. By integrating proteomics data with genomics, transcriptomics, and metabolomics data, researchers can build comprehensive models of plant biology, paving the way for advancements in agriculture, environmental management, and biotechnology.

The research highlighted in this ebook points to the numerous applications of plant proteomics, including the discovery of biomarkers for plant diseases, understanding stress responses, improving crop quality and yield, and elucidating the mechanisms of plant-microbe interactions. For example, proteomics research focusing on soybean nodule formation and differentiation helped to identify the molecular changes associated with symbiotic nodule development and effective nitrogen fixation. In a study of chive seedlings, proteomics analysis using the Orbitrap Astral instrument enabled quantification of over 7,000 protein changes related to drought stress. The results underscored significant shifts in metabolic processes, particularly those related to polysaccharide and glucan metabolism, both of which are essential for drought response. Other research exploring the interaction between the plant Arabidopsis thaliana and the fungus Penicillium olsonii TLL1 highlights how this symbiotic relationship enhances plant phosphate use efficiency. Proteomics analysis under low-phosphate conditions identified numerous differentially expressed proteins involved in stress response, metabolism, and nutrient transport.

The Orbitrap Astral mass spectrometer is a powerful tool for plant proteomics due to its proven ability to enable high-throughput, detailed protein analyses that are driving understanding of plant biology and responses to environmental challenges.

Resources

Brochure

The Orbitrap Astral Mass Spectrometer unlocks new possibilities across a wide range of applications. Discover more about its advanced capabilities. Read here,

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