

LIPID MAPPING OF COWPEA PLANTS UNDER SALINITY: BIOCHEMICAL STRATEGIES FOR STRESS RESPONSE

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ABSTRACT: Salinity limits cowpea cultivation in semi-arid regions, requiring strategies to identify stress response mechanisms. In this study, conducted in the greenhouse and the Plant Physiology Laboratory of the Department of Biochemistry and Molecular Biology at the Federal University of Ceará (UFC), lipidomic analysis of cowpea leaves and roots at the V4 stage under salinity was performed, enabling the separation and identification of 36 metabolites, including fatty acids, fatty alcohols, alkanes, terpenes, steroids, and antioxidant compounds. PCA revealed a clear separation between treatments and tissues, demonstrating specific alterations in the lipid profile associated with salt stress. The accumulation of saturated fatty acids and alkanes suggests the strengthening of membranes and cuticular barriers, while terpenes, steroids, and alpha-tocopherol acetate reflect the activation of antioxidant and stress signaling mechanisms. These results reinforce the central role of lipid metabolism in cowpea salt stress response, highlighting lipidomics as a strategic tool to support management and the selection of more tolerant genotypes.

KEYWORDS: Lipidomics. *Vigna unguiculata*. Salt stress.

MAPEAMENTO DE LIPÍDIOS EM PLANTAS DE FEIJÃO-CAUPI SOB SALINIDADE: ESTRATÉGIAS BIOQUÍMICAS DE RESPOSTA AO ESTRESSE

RESUMO: A salinidade limita o cultivo do feijão-caupi em regiões semiáridas, exigindo estratégias para identificar mecanismos de resposta ao estresse. Neste estudo, realizado em casa de vegetação e laboratório de fisiologia vegetal do departamento de bioquímica e biologia molecular da Universidade Federal do Ceará (UFC), foi realizada a análise lipidômica de folhas e raízes de feijão-caupi no estágio V4 sob salinidade, permitindo a separação e identificação de

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36 metabólitos, incluindo ácidos graxos, álcoois graxos, alcanos, terpenos, esteroides e compostos antioxidantes. O PCA revelou uma clara separação entre tratamentos e tecidos, demonstrando alterações específicas no perfil lipídico associadas ao estresse salino. O acúmulo de ácidos graxos saturados e alcanos sugere o fortalecimento de membranas e barreiras cuticulares, enquanto terpenos, esteroides e acetato de alfa-tocoferol refletem a ativação de mecanismos antioxidantes e de sinalização de estresse. Esses resultados reforçam o papel central do metabolismo lipídico na resposta do feijão-caupi ao estresse salino, destacando a lipidômica como uma ferramenta estratégica para apoiar o manejo e a seleção de genótipos mais tolerantes.

PALAVRAS-CHAVE: Lipidômica. *Vigna unguiculata*. Estresse salino.

INTRODUCTION

In semi-arid areas, cowpea (*Vigna unguiculata*) plays a strategic role, being crucial for food security and the income of local farmers due to its ability to adapt to challenging environments and its nutritional value (Silva & Santos, 2021). However, soil salinity poses a major abiotic constraint, limiting cowpea development by impairing germination, growth, and productivity, which jeopardizes food production and the livelihood of small farmers (Munns & Tester, 2008; Silva & Santos, 2021). Salinity causes osmotic and ionic stress, leading to physiological and biochemical disturbances in plants, including oxidative damage and the disruption of cell membranes (Shabala & Pottosin, 2014).

Among the affected cellular components, lipid metabolism plays a central role in the stress response. Membrane lipids not only maintain cellular integrity but also actively participate in signaling pathways that regulate adaptation to osmotic and oxidative stress (Upchurch, 2008). Advances in lipidomics have enabled detailed mapping of lipid alterations under environmental stresses, revealing dynamic changes in lipid composition that are crucial for plant tolerance mechanisms (Wang et al., 2018). For example, in *Paspalum vaginatum*, salinity triggers specific lipid accumulation in roots, highlighting organ-specific responses to salt stress (Pan et al., 2023).

Despite the relevance of cowpea in agriculture in semi-arid regions, knowledge about how its lipidome changes under salinity is limited, especially in different organs such as leaves and roots. Understanding these biochemical adaptations is essential for identifying biomarkers

of salt tolerance and supporting breeding programs aimed at improving crop resilience (Nguyen et al., 2019).

Therefore, this study aims to analyze the lipidomic profile of cowpea leaves and roots under salt stress, identifying changes in lipid classes associated with the stress response and elucidating the biochemical strategies used by different plant organs to cope with salinity. This research will contribute to the knowledge needed to develop sustainable approaches to mitigate the impact of salinity on cowpea cultivation in vulnerable regions.

MATERIALS AND METHODS

Plant material and growth conditions

Cowpea (*Vigna unguiculata*) seeds, obtained from the Active Germplasm Bank (BAG) of the Department of Plant Science, Federal University of Ceará (UFC), were initially germinated in vermiculite. Subsequently, seedlings were transferred to a hydroponic system containing Hoagland's nutrient solution at one-quarter of the original concentration. The plants were grown in a greenhouse, associated with the Plant Physiology Laboratory of the Department of Biochemistry and Molecular Biology, UFC, until reaching the V4 developmental stage.

Salt stress treatment and sample collection

At the V4 stage, plants were divided into two distinct groups: one group remained in the standard nutrient solution (control), while the other was transferred to a nutrient solution supplemented with 75 mM NaCl to induce salt stress. Leaf and root samples were collected 14 days after the onset of treatment. The leaf and root tissues were harvested and immediately frozen in liquid nitrogen for subsequent lipid profile analysis.

Lipid extraction and derivatization

Lipid extraction followed the protocol described by Lisek et al. (2006), using 300 mg of frozen plant tissue. Samples underwent sequential extractions with chloroform, methanol, and ultrapure water. An aliquot of 200 μ L from the lower chloroform phase (non-polar fraction) was dried in a vacuum concentrator at room temperature. For derivatization, the dried extracts were treated with N-methyl-N-(trimethylsilyl)-trifluoroacetamide (MSTFA) and incubated with shaking at 37 °C for 30 minutes.

GC-MS analysis and data processing

The derivatized samples were analyzed by gas chromatography coupled to mass spectrometry (GC-MS; QP-PLUS 2010, Shimadzu, Japan). Chromatogram and mass spectra data were processed using Xcalibur™ 2.1 software and compared with a reference mass spectral library for lipid identification. Relative lipid concentrations in cowpea leaves and roots under control and salt stress conditions were uploaded to the MetaboAnalyst 6.0 platform (<http://www.metaboanalyst.ca>) for additional statistical and multivariate analyses.

RESULTS AND DISCUSSION

Across all treatments and plant tissues, we identified and classified 36 metabolites related to lipid metabolism, including 13 fatty acids, 7 fatty alcohols and saturated alkanes, 5 terpenes, 3 steroids, sphingosine, vitamin, and 6 other compounds involved in lipid biosynthesis. This diversity of metabolites underscores the complex biochemistry underlying cowpea's response to salt stress. Fatty acids, as essential components of cell membranes, are crucial for maintaining membrane integrity and modulating lipid fluidity—an essential feature under osmotic and ionic stress conditions caused by salinity (Shabala & Pottosin, 2014). In addition, the terpenes and steroids identified here play recognized roles in antioxidant defense and hormonal regulation, both fundamental for mitigating oxidative damage and maintaining cellular homeostasis (Cao et al., 2017). The detection of sphingosine and vitamin compounds further highlights the involvement of bioactive and antioxidant lipids in adaptive metabolic pathways during stress (Hou et al., 2016). These results are consistent with previous studies reporting significant metabolic shifts in the lipid profile of plants exposed to environmental stresses, including salinity (Wang et al., 2018).

Principal Component Analysis (PCA) also revealed a clear separation between treatments and tissues, with PC1 and PC2 explaining 64.5% and 23.8% of the total variation, respectively (Figure 1). This strong separation denotes distinct lipidomic profiles influenced both by organ type and salt exposure, reflecting the specific biochemical strategies each organ employs to mitigate the effects of stress.

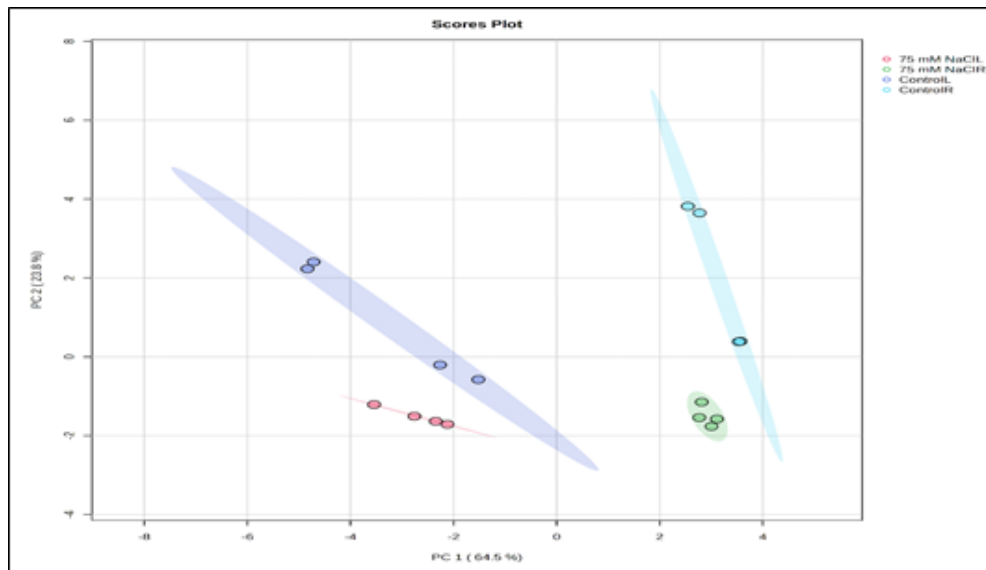


Figure 1. Principal Component analysis (PCA) of metabolic profiling of leaf and root. V4 stage cowpea plants were grown in control and 75 mM NaCl. The ellipses indicate the 95 % confidence interval of the groups.

To identify potential biomarkers of the response to salt stress, volcano plot analysis revealed that tetradecane, heneicosane, heptadecanoic acid, and α -tocopherol acetate were significantly elevated in leaves under salinity (Figure 2). This finding is consistent with previous studies reporting metabolic changes in the lipid profiles of plants under environmental stress, including salinity (Wang et al., 2018), suggesting that these metabolites may act as biochemical markers of stress adaptation. However, it is important to note that while GC-MS is effective for volatile and semi-volatile compounds, it may limit the detection of non-volatile or highly polar lipids, potentially overlooking other relevant metabolites.

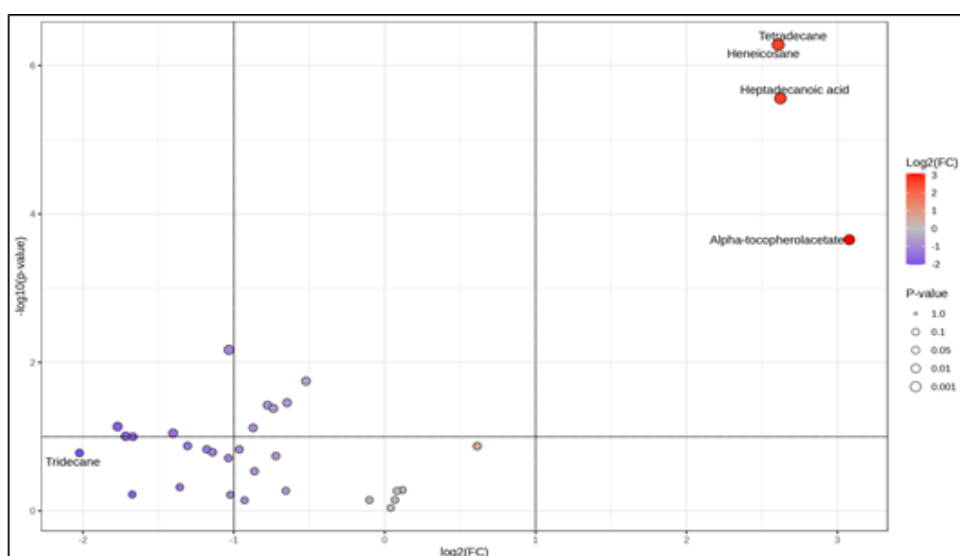


Figure 2. Volcano plot showing potential biomarkers in cowpea leaves. The plot highlights metabolites with significant changes in abundance between experimental conditions. Red points indicate metabolites with statistically significant differences. Points to the right and left of the vertical lines indicate upregulated and downregulated metabolites, respectively.

In roots, significant increases were observed for heptadecanoic acid, all-trans-squalene, tetradecane, β -sitosterol, and cembrene under salt stress (Figure 3), indicating a robust biochemical response possibly associated with membrane stabilization and signaling.

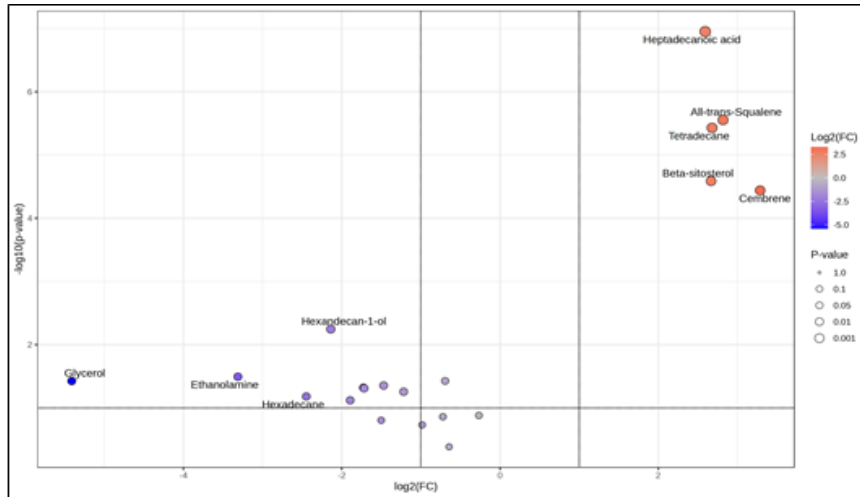


Figure 3. Volcano plot showing potential biomarkers in cowpea roots. The plot highlights metabolites with significant changes in abundance between experimental conditions. Red points indicate metabolites with statistically significant differences. Points to the right and left of the vertical lines indicate upregulated and downregulated metabolites, respectively.

Interestingly, the increase in certain metabolites in control plants (Figure 4) may represent the basal metabolic state, where the absence of stress signals results in stable membrane lipid profiles without the need for remodeling. This observation emphasizes the dynamic nature of lipid metabolism as a responsive mechanism rather than a static feature.

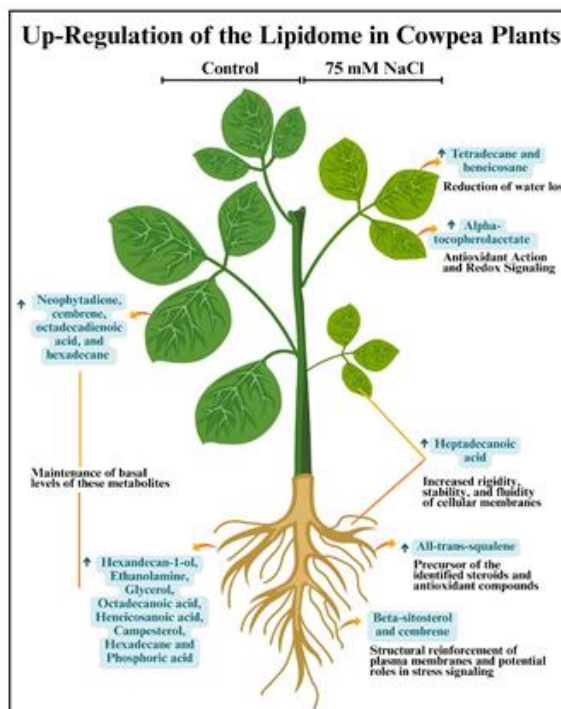


Figure 4. Summary of lipidomic adjustments in cowpea plants under salinity.

The accumulation of long-chain alkanes such as tetradecane and heneicosane in leaves likely contributes to strengthening cuticular barriers, thereby reducing water loss through transpiration and enhancing tolerance to osmotic stress (Yu et al., 2020; Sharma et al., 2023). Similarly, the increase in saturated fatty acids such as heptadecanoic acid, in both leaves and roots, may enhance membrane rigidity and resilience, counteracting the fluidizing effects of salt-induced stress (Li et al., 2021; Khan et al., 2022). The accumulation of α -tocopherol acetate, a potent antioxidant, underscores the activation of oxidative stress mitigation pathways, protecting membrane lipids from peroxidation and preserving cellular function under salinity (Chaudhary et al., 2022; Wu et al., 2020).

Furthermore, the modulation of phytosterols such as β -sitosterol and diterpenes such as cembrene in roots suggests roles in strengthening the plasma membrane structure and potentially mediating stress signaling cascades (Chaudhary et al., 2022; Wu et al., 2020; Sharma et al., 2023). The presence of all-trans-squalene, a precursor in steroid biosynthesis, may indicate upregulated synthetic pathways essential for producing protective compounds under stress conditions (Yu et al., 2020).

CONCLUSION

This study reveals that lipid metabolism acts as a critical hub in the plant's response to stress, orchestrating dynamic adjustments in structural and biochemical processes that are essential for preserving cellular integrity. The clear separation observed in the PCA analyses, along with the identification of key metabolites through volcano plots, demonstrates that lipidomics is an effective tool for uncovering the adaptive strategies of cowpea under salinity.

Furthermore, the research highlighted specific changes in lipid types that are vital for membrane stability and antioxidant systems, as well as distinct responses between leaves and roots, depending on the organ. These findings provide valuable insights for the management and selection of more resilient genotypes, thereby advancing genetic improvement and agricultural practices for cowpea cultivation in salinity-affected areas.

ACKNOWLEDGMENTS

We would like to thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Fundação Cearense de Apoio ao Desenvolvimento Científico e Tecnológico (FUNCAP), Instituto Nacional de Ciência e Tecnologia em Agricultura Sustentável no Semiárido Tropical (INCTAgriS) for their support.

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