Comparative quantitative proteome and phosphoproteome analysis of castor bean (Ricinus communis L.) seed endosperm at different imbibition stages

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Introduction: Castor bean (*Ricinus communis* L.) stands out as one of the world\'s major oilseed crops, recognized for its role in studying fatty acid metabolism with biotechnological applications. Its seeds not only yield oil rich in triricinoleate, valuable across various industrial sectors and in health, but they also store significant amounts of allergenic proteins and harmful toxins. Given the relevance of its characteristics, investigating seed development and germination is essential, as these processes are dynamic and influenced by post-translational modifications (PTMs). The study aims to characterize the proteome and phosphoproteome of castor oil seed endosperm throughout imbibition and germination processes.

Materials and methods: Seeds endosperm collected at four imbibition stages (3 hours after the onset of imbibition – HAI – 6 HAI, 36 HAI and 62 HAI) and mature stage were dissected. Proteins were extracted with a solution containing phenol and tris buffer pH 7.9, and digested with trypsin (1:50, enzyme: substrate ratio). Phosphorylated peptides were enriched in batch using titanium dioxide 1.2 mg and 0.6 mg, and eluted with 1.5% ammonium hydroxide solution (pH 11). The unretained and enriched fractions were then subjected to a liquid chromatography system coupled to an Orbitrap Exploris^{TM} 480.

Results and discussion: In the analysis of the unretained fractions, 9676 peptides and 1743 proteins were identified. The groups M, 3, 6, 36, and 62 have 807, 820, 787, 741 and 941 proteins, respectively. PCA and statistical analysis underscored differences between the imbibition stages and the germinated seed (62 HAI). The qualitative analysis revealed a 166 down-abundant proteins compared to 97 up-abundant proteins, including ribosomal proteins involved in protein synthesis. The breaking of seed quiescence begins between 6 and 36 HAI, by becoming metabolically active after 36 HAI with a higher number of positively regulated proteasomal, heat shock, and ribosomal proteins. In the phosphoproteomic analysis, 2613 phosphopeptides and 634 phosphoproteins were identified. The data highlighted 55 differentially abundant phosphopeptides, with an overrepresentation of SP-type motif, primarily phosphorylated by kinases from the MAPKs, CDKs, and CDKs-like families. Comparing all datasets of differentially abundant proteins, peptides, phosphoproteins, and phosphopeptides, six proteins are potentially regulated by phosphorylation, including splicing factors that regulate seed germination in response to ABA levels. Phosphorylated motif analysis revealed 13 motifs overrepresented, including 3 exclusives to castor bean seed germination, with distinct abundance trends among stages.

Conclusion: In conclusion, the comparative analysis of early castor bean seed germination highlights protein synthesis as a crucial process, along with identifying the phases determining quiescence breaking. Proteins regulated by phosphorylation were identified exhibiting variations in abundance when phosphorylated, including allergenic and toxic proteins. Overrepresented motifs display distinct patterns during imbibition, including those exclusive to castor bean seeds. These results highlight the impact of phosphorylation throughout proteins in seeds germination, as well as a more comprehensive understanding of the seed imbibition process.

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