Deciphering the role of abscisic acid on protein accumulation and phosphorylation driving sugarcane somatic embryogenesis

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Somatic embryogenesis has emerged as a valuable tool for the micropropagation and genetic transformation of sugarcane (*Saccharum* spp.), offering several advantages over traditional methods. Abscisic acid (ABA), a pivotal hormone in embryogenesis, plays a role in reserve accumulation and embryo development. Despite this, ABA-modulated molecular mechanisms in somatic embryogenesis are still elusive and could be useful for optimizing in vitro propagation and regeneration. This study aimed to investigate the role of ABA in regulating sugarcane somatic embryogenesis. Embryogenic calli were induced in Murashige and Skoog (MS) culture medium supplemented with 10 µM 2,4-dichlorophenoxyacetic acid (2,4-D). Following three cycles of 21-day dark multiplication, the calli were incubated in MS medium without 2,4-D, supplemented with 1, 10, 50, and 100 µM ABA or 0.1, 1, 10, and 50 µM fluridone (FDN, an ABA biosynthesis inhibitor). After 21 days, samples were collected for bottom-up phosphoproteomics and targeted metabolomics analysis. For embryo differentiation, the calli were transferred to maturation medium without any regulators, under light conditions for somatic embryo differentiation. The total number of somatic embryos was assessed after 42 days of maturation treatment. In all FDN treatments, a significant decrease in somatic embryo differentiation was observed and concentrations exceeding 0.1 µM led to reduced both callus growth and pigment accumulation. Furthermore, targeted metabolomic analysis showed a significant reduction in ABA accumulation in the FDN-treated callus. On the other hand, supplementation with exogenous ABA did not significantly alter somatic embryo production. The phosphoproteomics analysis revealed proteins such as Putative auxin efflux carrier component 3a, Auxin transport protein BIG, ABC transporter B family member 19, REL2 and brassinosteroid LRR receptor kinase involved in hormonal mechanisms, highlighting their importance during somatic embryogenesis. In addition, phosphorylation of proteins such as argonaute1, CTR9-like and Lysine-specific demethylase JMJ25 imply their involvement in the reprogramming of gene expression and stress responses. Therefore, maintenance of endogenous ABA levels in callus during somatic embryogenesis is an important factor driving accumulation and phosphorylation of proteins important for sugarcane somatic embryo differentiation and maturation. The protein-protein interaction networks found in this study reveal new players regulating plant development in response to ABA.

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