Development of an optimized protocol for nuclear protein extraction of young sugarcane (*Saccharum spp*) leaves

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Sugarcane is an economically important grass. It is cultivated for its stalks, which accumulate sucrose, and constitutes the base for products like sugar and bioethanol. The subproteome improves the sensibility and resolution of the protein set studied, reducing the complexity of the proteome analyzed. The comprehension of the nuclear proteome is essential for understanding genome regulation and function, and provides important clues to the molecular function of novel proteins. In this study, we report the isolation of nuclear proteins from young sugarcane leaves. The nuclei were isolated from fresh tissue of one-month-old sugarcane leaves, using the Folta and Kaufman (2000) protocol, with modifications. The preparation of nuclei with percoll gradients was chosen, once it allows the recovery of a higher amount of nucleus. The integrity of the isolated nuclei was evaluated by staining with 1% acetolactic orcein. The suspension was placed on a slide and covered with a cover glass. The results reveal the nuclei as uniform spheres with little presence of cellular debris. The nuclear proteins were then isolated with the utilization of TRI Reagent (Sigma), and quantified by Bradford method to further identification. For the analysis of sugarcane nuclear proteome, 100 µg of isolated proteins were loaded onto a 1-D SDS-PAGE. The gel was stained with Comassie Brilliant Blue and divided into 21 sections, which were digested and purified. The proteins were identified by mass spectrometry (LC-MS/MS) and analyzed by Mascot Daemon. So far, nuclear proteins such as ribosomal proteins, RNA polymerase IV, transposon protein, histones H4, H3 and H2B.1 have been identified, but some contaminants like Rubisco have also been found. Thus, the protocol has been optimized and the proteins are being identified.

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