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Preparation of fresh and dry plant tissue samples for Synchrotron-based XRF analysis

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Synchrotron-based X-ray fluorescence spectroscopy techniques are useful for understanding the ionome of biological materials, including plants, at the tissue or cellular level. Nevertheless, sample preparation is crucial and challenging as both tissue structure and elemental composition need to be preserved. In this study, we explored the Synchrotron Laboratory to establish suitable cryofixation methods for high-resolution XRF analysis of plant tissues. Leaves from coffee (Coffea arabica), soybean (Glycine max (L.) Merrill), and tomato (Solanum lycopersicum) plants were detached, and 5 x 5 mm pieces were immersed in optimal cutting temperature (OCT) medium and frozen through rapid-plunging in supercooled isopentane. The resulting blocks were fixed on an adhesive cellophane film (Fitar, Brazil) and cut using a cryostat at -25℃ to yield 30-µm thick cross sections. We also evaluated the preservation of tomato fruit tissue structure, where analyzing fresh tissue was unnecessary. The samples were cut with a single-blade razor (carbon steel), and the sample slides were placed on the XRF cup sample holder between two layers of 6 µm thick polypropylene XRF film. The samples were stored in the freezer for 12 hours, followed by the freezing-dry process. The results showed that the structure of coffee, maize, and tomato leaves was preserved during the cryofixation process, indicating that the procedures employed are suitable for the fresh leaf tissues of different species. Furthermore, no evidence of radiation damage, sample dehydration, or structural collapse within the measurement timeframe was observed, suggesting that these analyses might be accomplished without the use of a cryojet. Similar results were obtained for tomato fruit, which is suitable for measuring low mobile nutrients regarding their redistribution as Ca.

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