Optimization of strategies for improved immunolocalization and morphology for correlative light and electron microscopy in plant tissues

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Immunolocalization is a versatile approach used to reveal the distribution of diverse components in tissues (immunohistochemistry) and cells (immunocytochemistry), making it an essential technique for a broad array of biological systems. Moreover, a wide selection of commercially available antibodies helps drive their potential applications in basic and applied research. The performance of immunolocalization relies on many factors, including the purity and specificity of the antibody, as well as the in situ antigen epitope availability. Despite extensive sample processing, the preservation and/or recovery of sample antigenicity, while maintaining good sample morphology, is highly desirable for most immunolocalization experiments.

The detection of diverse epitopes can be a challenging task, especially when combining light (LM) and electron microscopy (EM) for correlative studies. Multiple factors must be considered including, but not limited to, sample fixation, buffers, en bloc staining with heavy metals, resin formulations and polymerization (e.g., UV or heat), to name a few. Failure to preserve an epitope could happen at any of these stages making it complex to troubleshoot the exact reason for a failed outcome. Therefore, combining efficient and reliable pre- and post-immunolabeling methods in a way that delivers reproducible results in correlative LM and EM would be advantageous for the research community.

Our efforts focused on plant materials with abundant antigens present throughout the target tissues. We evaluated several embedment/de-embedment methods using epoxy and acrylic resins that were compatible with LM and EM immunolocalization. Also, we applied different pre- and post-embedding chemical treatments to preserve and/or reveal the antigen for immunolabeling compatible with LM or EM imaging. In particular, our goal was to develop versatile and reliable resin-based immunolocalization protocols for correlative light and electron microscopy with robust immunolocalization and improved ultrastructural morphology.

REFERENCES: