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AN OPTIMIZED PARAFFIN-EMBEDDING METHOD TO STUDY EMBRYO SAC DEVELOPMENT IN GRAPEVINE

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Pollination and fertilization are crucial stages in grape development affecting fruit set and, consequently, potential yield. The achievement of these two processes is linked to the successful completion of the events involved in the production of functional male (pollen) and female (embryo sac) gametophytes. This is particularly evident in seedless cultivars where defects during female gametophyte developmental processes have been observed through cytohistological analysis.

Plant histological processing includes a wide variety of methods and techniques that can be used in different combinations to achieve the desired result. Various methodologies can be found in literature to study the development of the grapevine female gametophyte, mainly based on resin- or paraffin-embedding techniques. Unlike resin-based techniques, paraffin-embedding approaches contain the costs and, despite that the achieved resolution is not so high, can be an efficient tool if there is no need to observe detailed structure of tissues, cells, organelles and macromolecular complexes. On the other hand, the protocols in the published papers are not always fully exhaustive, they report very long incubation times and also different stains, some of them requiring long ripening periods and/or not easy to handle.

Here we present a paraffin-embedding method for the examination of grapevine ovules at different phenological stages. The incubation times at each step (fixation, dehydration, clearing, infiltration, and staining) have been optimized in order to reduce the duration of the entire process. The staining combination used, as far as we know, has never been reported for grapevine's ovules. Good quality images with adequate contrast were obtained with this methodology allowing the observation of inner and outer integuments, nucellus and embryo sac structures.

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