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Mobilization of seed storage proteins of the 11S-type in the cotyledon during olive seed germination *in vitro*

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It is well known that plants accumulate storage proteins (SSPs) during seed development, predominantly in specialized storage tissues like the cotyledon or the endosperm. Storage proteins are mobilized during seed germination and when seedlings start to grow [1, 2]. The 11S proteins are the major storage proteins in most legumes and in many other dicots (i.e. species from the Brassicaceae and Cucurbitaceae families) and some cereals such as oats and rice [3]. They are stored as large complexes in protein bodies. We have used an antiserum raised against the SSPs of the 11S type present in the olive seeds [4], to investigate both the mobilization of these proteins and their localization in the differentiating cotyledon during the olive seed germination in vitro. For this purpose, immunocytochemical and Western-blotting techniques were used. A progressive decrease in the amount of the 11S proteins present in the cotyledons was observed starting from mature and imbibed seeds until reaching a minimum 650 hours after the beginning of the germination. These results strongly indicate that continuous hydrolysis of the examined protein occurs, and confirm the utilization of storage proteins during seed germination. Immunocytochemical localization of the 11S SSPs by fluorescence and transmission electron microscopy showed significant changes in both their amount and localization throughout the differentiating steps of the cotyledon cells. At early stages of seed germination (before the formation of a central vacuole in the cotyledon cells), the 11S SSPs were specifically and highly accumulated in protein bodies. After 100 hours of seed germination, the 11S SSPs were detected in a significantly lower amount. They presented a uniform distribution in the vacuolar area, and also were detected concentrated in dark inclusions which were observed inside the vacuole. At the ultrastructural level, the strongest signal indicating 11S proteins was observed associated to electron-dense inclusions inside the vacuole, whereas the remaining intravacuolar areas showed very low labeling. Signal in the cytoplasm of the cotyledon cells during the later steps of in vitro germination was also shown. The obtained results indicate that 11S SSPs are continuously hydrolyzed in protein bodies/vacuoles. Moreover, its concentration in the intravacuolar inclusions may indicate that the proteolysis may occur there. Alternatively the inclusions may function as a shuttle for SSPs and/or products of their degradation into the cytoplasm, where they serve as a source of amino acids.

- [1] Herman et al. (1999) Plant Cell 11: 601.
- [2] Müntz et al. (2001) J. Exp. Bot. 52: 1741.
- [3] Shewry et al. (1995) Plant Cell 7: 945.
- [4] Alché et al. (2006) J. Agric. Food Chem. 54: 5562.

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