

crosscopy analysis of endosperm and cotyledon showed the presence of large dense proteins bodies (PBs), surrounded by numerous oil bodies (OBs) filling up the cytoplasm. Water intake by the seed during imbibition produced PBs swelling. The most noteworthy feature after 3 days of *in vitro* germination was the presence of large PBs originated by fusion of smaller PBs. Close spatial proximity between PBs and OBs was also observed. Between 3-12 days germination, PBs experimented important changes leading to the formation of a large vacuolar compartment and a decrease in the number of PBs and OBs. These changes were accompanied by plastids biogenesis. After 26 days germination, the cellular organization became typical for a mesoderm leaf cell, with well differentiated chloroplasts surrounding a large central vacuole. All these cellular changes are the result of a gradual mobilization of SSPs and lipids.

S08.213

Assessment of the Allergenic Content of Olive Pollen from Portuguese Cultivars

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Olive pollen contains allergenic proteins able to elicit respiratory allergy. Up to date, 10 allergens have been characterized in this material, referred as Ole e 1 to Ole e 10. The presence of variability in their level of expression and in the number and molecular characteristics of the expressed forms has been reported. These differences are intrinsic to the genetics of each cultivar and have been analyzed in a number of Spanish cultivars. In the present study, we identify and quantify the presence of four allergens (Ole e 1, Ole e 2, Ole e 5 and Ole e 9) in the pollen of twelve Portuguese olive cultivars. Ole e 1 allergen is considered the olive pollen major allergen, playing an important role in olive pollen hydration and germination. Ole e 2 is a pollen profilin which binds to actin and is also involved in signal transduction. Ole e 5 is a protein exercising Cu, Zn superoxide dismutase activity, whereas Ole e 9 corresponds to a 1,3 beta-glucanase. In order to determine the level of expression of these proteins, we carried out SDS-PAGE by using pollen protein extracts. Protein profiles were analyzed by Coomassie staining. Equivalent gels were transferred to membranes and probed with antibodies to the four allergens studied. Different isoforms of these allergens were detected, with differential distribution in the pollen cultivars, both in number and in the level of expression. Immunoblots were also probed with sera from allergic patients. The reported differences are likely involved not only in the allergenic properties of this pollen, but also in the physiology of the olive reproductive system, including pollen dynamics, ability to adapt to different conditions, capacity to fertilize etc.

S08.214

A Histological Evaluation of Adventitious Root Formation in Olive (*Olea europaea* L. cv. Galega Vulgar) Microshoots Cultured *in vitro*

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The main Portuguese olive cultivar, 'Galega vulgar', presents a recalcitrant behaviour regarding its propagation by semi-hardwood cuttings, with average rooting rates between 5-20%. The *in vitro* culture techniques recently allowed increasing these rooting rates to 60-75% creating for the first time conditions to perform anatomical studies on adventitious root origin on this olive cultivar. After a 15s quick-deep treatment in a 14.700µM IBA sterile solution, microshoots of 'Galega vulgar' were *in vitro* rooted using semi-solid OM culture media devoid of growth

regulators. Samples from the basal portion of the *in vitro* cultured microshoots were taken at established times. The samples were fixed in FAA, impregnated in liquefied paraffin and, after being sectioned in a rotary microtome, stained with Safranin O + Aniline Blue and observed in an optical microscope. After 5-7 days in culture, some cells from the cortex and also from the sub-epidermal tissue revealed a dense cytoplasm and presented high mitosis rates. The first morphogenetic root fields were observed in the former tissues and also in the parenchymatic calli after 12-16 days in culture. Root primordial, already presenting a connection to the plant vascular system, became visible at epidermal surface after 20 days. Parenchymatic calli vascularisation as revealed by the presence of xylem traqueids was also observed. No root morphogenesis arising from the cambial region was reported contrasting to what is common in stem cuttings from easy to root plant species.

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Interaction of Crop Load and Water Status on Growth of Olive Fruit Tissues and Mesocarp Cells

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Field-grown olive trees (*Olea europaea* L. cv. Leccino) were hand-thinned at 5-6 or 2-3 weeks after full bloom (AFB) over two consecutive growing seasons to establish heavy or low crop loads, and then were subjected to three irrigation regimes: a) FI, fully-irrigated with pre-dawn leaf water potential (LPWP) greater than -1.1 MPa; b) DI, deficit irrigated (LPWP between -1 and -3.3 MPa); c) SI, water stressed (LPWP higher than -4.2 MPa). Fruits were sampled at pit hardening and harvest, the mesocarp separated from the endocarp and their fresh weight, dry weight determined. Additional fruits were fixed in FAE for anatomical studies, their mesocarp and endocarp area measured on transverse slices with an image analysis system connected to a stereo microscope. Mesocarp cell size and number were then determined by microscope after standard paraffin processing. The effect of crop load on growth of fruit tissues depended on the irrigation regime. Within the FI or DI treatments the mesocarp dry weight was higher at low than at high crop load. In the SI treatment, mesocarp dry and fresh weights were 180 and 200% respectively those for trees bearing high crop loads. The pulp-pit ratio was decreased by high cropping for both DI and SI treatments, but was unaffected for the fully-irrigated trees. High crop load significantly decreased fruit oil content (% dw) of FI trees. Comparing trees with high crop loads, DI showed higher fruit oil content than FI. Overall, there was a notable interaction of crop load and water status on fruit growth processes at multiple levels, including the absolute and relative growth of pulp and pit, mesocarp cell division and enlargement, and oil accumulation.

S08.216

Vegetative Growth and Ecophysiological Aspects in Young Olive Plants Inoculated with Olive Leaf Yellowing Associated Virus (OLYaV)

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An investigation started some years ago in the Sicilian olive culture, to ascertain the sanitary status of trees, revealed that large part of the plants were affected by Olive Leaf Yellowing associated Virus (OLYaV), although the symptoms of the above mentioned diseases were not exhibit. In order to organize and manage an olive nursery under the European rules (Conformitas Agricola Communitatis) it is important to ascertain if an asymptomatic diseases can affects morphological and eco-physiological traits of young, potted, olive trees. Researches were carried out using two years old virus free plants of cv. Frantoio grafted on seedling, grown in pots (32 l) and compared with the same plants previously inoculated with OLYaV by chip budding. Data on vegetative growth, within tree dry matter partitioning, gas exchange and stem water potential at the end of the second year were collected in both, virus free and virus affected trees. Data related to trunk diameter, shoots