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CYTOLOGICAL AND MOLECULAR EVIDENCES SUPPORT A SPOROPHYTIC SELF-INCOMPATIBILITY SYSTEM IN OLIVE

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Olea europaea L. is one of the oldest agricultural tree crop species and, in spite of the great economical and cultural impact, a few studies have been carried out on its reproductive barriers. The aim of this research was to elucidate the self-incompatibility system in olive from cyto-histological and bio-molecular standpoints. Self-incompatibility is one of the most effective systems adopted by flowering plants to prevent inbreeding, maintaining so diversity within the species. Olive is actually classified as a gametophytic self-incompatible (GSI) plant because of distinctive morphological traits, as wet-type pistil and bi-nucleated pollen. However, detailed cytological analyses of more than 34,000 pollen grains performed using pistils of self-compatible and self-incompatible cultivars under self-pollination and open-pollination conditions, were not in agreement with GSI. In fact, only 4-10% of the total pollen grains, varying with the cultivars, were germinated and none of the emerging pollen tubes was able to penetrate the stigma surface of self-incompatible cultivars (Leccino, Moraiolo and Dolce Agogia) after self-pollination. It is worth noting that GSI reaction normally occurs in the transmitting tissue of the style, being controlled by specific RNases. Furthermore, no results were achieved by molecular analyses aimed at cloning genes involved in the GSI system. Vice versa, our cytological observations were in agreement with a sporophytic self-incompatibility (SSI). The molecular attempts to isolate candidates for SSI led us to the cloning of two *OeSRK* (S-locus Receptor Kinase, the female determinant) and two *OeSLG* (S-locus Glycoprotein, an enhancer of the incompatibility response) genes. As far as the male determinant is concerned, we were not able to isolate any candidate by routine molecular approaches, such as PCR with degenerated primers and RACE experiments, because of its high intra-specific nucleotide variability. However, a screening of about 465,000 ESTs, belonging to olive flower-specific libraries, allowed us the definition of the SCR-like (S-locus Cysteine Protein, the male determinant) pattern and the isolation of 28 contigs showing SCR-like features. Then, quantitative Real-Time PCR assays enabled the identification of one *OeSCR*-like gene, showing a strong anther-specific expression at the time of pollen dispersal. Moreover, quantitative Real-Time PCR assays, replicated using different subdomain-specific primer combinations, revealed an antagonist transcriptional expression pattern in flowers of cultivars Leccino and Frantoio (the latter being self-compatible) for the genes *OeSRK* and *OeSLG*. All the genes related to a putative SSI system are currently tested by means of *in situ* hybridization in flowers of both self-incompatible and self-compatible cultivars to study their spatial gene expression patterns and domains. Yeast-

two-hybrid screenings are also in progress to test the protein-protein interaction between our male and female candidates. On the whole, a new hypothesis for the genetic system controlling the self-incompatibility reaction can be postulated for olive.