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truxillensis). Stigmas and anthers of the late developmental stages were prepared for light and electron (scanning and transmission) microscopies. Tests to detect oil, polysaccharides and proteins were also performed. The pollen grains were middle size (25-35μm), amb subtriangular to triangular, 3-colporate, mesocolpium perforated and apocolpium psilate. Plastids containing oil, starch grains and protein bodies occurred in the vegetative cell cytoplasm of all the species analyzed, although in a different quantity in each species. Pollenkit was not observed. Stigma of pre-anthesis flower presented simple trichomes and a thin cuticle covering a round stigmatic surface. The stigmatic surface is formed by secretory cells. During the anthesis, the simple trichomes retracted exposing the stigmatic surface, and secretory cells produced lipophilic and hydrophilic substances. The secreted substances were retained in the subcuticular and intercellular spaces. This type of stigma, made of secretory cells and covered by a cuticle, is considered as semidry. Controlled pollinations (bagged inflorescence) indicated that automatic pollination did not occur; also, visits by stingless bees Trigona fuscipennis Friese 1908 were observed. These informations, added of lack of pollenkit and presence of stigmatic secretion, allowed us to conclude that pollen germination depends on pollinator. The flower would be tripped by the stingless bee, which would provoke the cuticle disruption, permitting the contact of pollen with the stigmatic fluid; consequently, the pollen would rehydrate and germinate. Melittophilos species usually have oily pollen grain. In Indigofera pollen grain contained starch grain and protein bodies besides oil droplets. Then, reserve substances might not be associated with pollinator in this group. It should be mentioned that this is the first report of semidry stigmas in Indigofera, the third largest genus in the Leguminosae (FAPESP).

Molecular characterization and polymorphism of superoxide dismutase (SOD) in olive (Olea europaea L.) pollen. Putative roles in the interaction pollen-stigma

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Reactive Oxygen and Nitrogen Species (ROS and RNS) offer a dual perspective in physiological systems. Despite the fact that their accumulation may cause important damages to plant tissues, they have been described to play important roles in stress, defence against pathogens and signal
transduction in plants. ROS and particularly NO are recently emerging as signalling molecules in pollen-stigma interactions (Hiscock and Allen, 2008). Therefore, ROS and RNS production, accumulation and transformation into other metabolites have to be tightly controlled. Different enzymes integrate the antioxidant controlling panel of plant tissues. Among these, peroxidases have been shown to be widely present in Angiosperm stigmas. The activity of these enzymes like NOX (NADPH oxidase) have been described in pollen tubes (Potocky et al., 2007).

The enzyme superoxide dismutase (SOD) catalyzes the disproportionation of superoxide radicals in biological systems. This enzyme family is composed of three major forms depending on their prosthetic group (Mn-SOD, Cu,Zn-SOD y Fe-SOD) (Alché et al., 1998).

In the present study, Cu,Zn-SODs were cloned from mature pollens of a large number of olive cultivars. Over 70 sequences were obtained from either cDNA or gDNA extracted from 11 olive cultivars. Bioinformatic analysis showed that all sequences displayed a high degree of conservation. However, sequence microheterogeneities were frequently observed. They did not affect key amino acids (i.e. Cys residues involved in the formation of intra-molecular bridges, or His residues concerned in the interaction with the Cu atom). Some of the substitutions affected protein motifs liable to post-translational modifications and likely involved in regulation. A deletion of 8 amino acids was detected in a low proportion of the sequences analyzed.

Protein extracts from mature pollen were used to determine SOD activity both by spectroscopic measurement and in native gels. Differences in the level of activity were detected depending on the cultivar analyzed. Four to five reactive bands were observed in the gels, also depending on the cultivar analyzed. The use on specific inhibitors allowed us to determine the presence of a relevant band corresponding to Mn-SOD in a large proportion of the cultivars, and 4-5 bands characterized like Cu, Zn-SOD. Immunoblotting analysis showed several immunoreactive bands in the extract, which may correspond to different isoforms of the enzyme, including the presence of polymeric forms.

Finally, a system to microscopically detect the presence of ROS and particularly SOD activity in vivo by generating superoxide radicals was assayed in several samples of olive pollen. SOD activity in the mature pollen was highly dependant on pollen viability. The presence of such a complex antioxidant system in the olive pollen and the occurrence of intercultivar differences points out to a putative involvement of the system in the compatibility behaviour of this species. The olive tree is considered to be preferentially allogenous (this is, preferentially cross-fertilized by pollen from a different olive cultivar). Several models as regard to the
putative role of this antioxidant system in the interaction pollen-pistil in the olive are presented and discussed.

References


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