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Poster Abstract – F.04

COMPARATIVE HISTOLOGICAL, BIOCHEMICAL AND MOLECULAR APPROACHES TO STUDY CITRUS REPRODUCTIVE BIOLOGY

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In contrast to what is known in other fruit tree species, the self-incompatibility reaction in citrus has not been fully investigated. It is assumed to be of gametophytic system and genetically controlled by the S-locus, which in other species encodes for glycoproteins showing ribonuclease (S-RNases) activity, regulated by several enzymes such as transglutaminase (TGase).

In order to investigate pollen-pistil interaction, the growth of pollen tubes of two clementine (*Citrus clementina* Hort. ex Tan.) varieties, 'Comune' (self-incompatible) and 'Monreal' (a 'Comune' self-compatible mutation) has been analyzed in self- and cross-pollination conditions. The same analysis was carried out on pummelo (*Citrus grandis* L. Osbeck), a self-incompatible ancestor species. We sequentially examined by histological assays, along two weeks after self and cross pollination, the number and morphology of pollen tubes at various pistil levels and TGase activity in the style.

To identify genes involved in pollen-pistil interaction and in self-incompatibility response, a research based on cDNA-AFLP technique was carried out to compare transcript profiles between the two clementine varieties and search for differentially expressed genes. RNA was extracted from emasculated non-pollinated pistils and from self-pollinated pistils four days after pollination. cDNAs of the two varieties were amplified using 43 *Eco*RI/ *Mse*I primer combinations, and more than a hundred differentially expressed or polymorphic Transcript Derived Fragments (TDFs) were successfully cloned and sequenced.

Pollen tubes in self-pollinated 'Comune' arrested their development in the upper part of the style, while in self-pollinated 'Monreal' they grew down to the ovary. TGase activity appeared strongly involved in pollen tube growth inhibition confirming the presence of S-RNase-based gametophytic self-incompatibility. Among the TDF showing homology with known genes, BLASTX search identified several genes related to reproductive biology such as calcium modulated proteins, receptor-like kinases, thioredoxins, a histone deacetylase gene, a putative cleavage and polyadenylation specificity factor, transcription factors and genes related to hormone metabolism and signaling. Moreover, a high percentage of active retrotransposons were identified among the TDFs, which may indicate their possible role in the regulation of self-incompatibility genes as already reported for other species. Some of the TDFs were analysed through RT-PCR and showed differences in their expression patterns.