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Improvements in cytological preparations for fluorescent *in situ* hybridization in *Passiflora*

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ABSTRACT. Cytological preparations for the fluorescent *in situ* hybridization (FISH) technique require cytoplasm-free metaphases, with well-spread chromosomes, for the localization of DNA sequences and chromosome mapping. We tested various procedures for FISH analysis of *Passiflora cacaoensis*, *P. gardneri* and hybrid F_1 progeny of *P. gardneri* x *P. gibertii*. Two treatments with four enzymes and three incubation times were compared. The material was treated with 1.0 M HCl before enzymatic digestion. The following criteria were used to determine the quality of the metaphases: a) lack or presence of cytoplasm; b) well-spread chromosomes or with overlap; c) complete or incomplete chromosome number (2*n*). The enzyme Pectinex® SP ULTRA gave the best performance, with the shortest incubation time. The best results were observed after 30 min of incubation; more than 70% of the metaphases did not have large amounts of cytoplasm or overlapping chromosomes, and about 75% maintained the chromosome number. FISH was carried out using a 45S rDNA probe (*p*Ta71) labeled with biotin and detected with fluorescein isothiocyanate. Sites with strong staining and without

nonspecific signals were observed. Our methodological adaptations allowed the preparation of metaphase slides of high quality for the FISH technique, with less time required for the preparation of samples.

Key words: Passion flower; Cytogenetic; Pectinex; FISH

