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## Thesis abstracts

### *In vitro* morphogenesis and *Agrobacterium tumefaciens*-mediated transformation of eggplant (*Solanum melongena* L. cv. Embú)

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Eggplant explant responses were evaluated in the present study. Cotyledons and hypocotyls from 15-day *in vitro* grown plantlets were used as explants for somatic embryogenesis and organogenesis, respectively. The results showed that MS-based medium supplemented with 0.1 mg $L^{-1}$ IAA provided higher shoot regeneration frequencies and that 2.5 to 10 mg $L^{-1}$ NAA had similar effects over somatic embryogenesis. An average of 0.57 shoots and 80 embryos per explant were observed for organogenesis and embryogenesis, respectively. Regarding antibiotics, Timentin added to the medium led to better regeneration responses, in either shoots or embryos, compared to cefotaxime. On the other hand, cefotaxime supplementation in combination with NAA increased callus weight. Furthermore, a significant decrease in total embryo number and shoots was observed with the later. Kanamycin (50 mg $L^{-1}$) added to selective medium completely inhibited morphogenesis in hypocotyl and cotyledon explants, in spite of the high frequency of escapes in transformation experiments. Organogenic responses were suppressed by hygromicin at 7.5 mg $L^{-1}$, whereas some globular embryos were observed in the presence of 10 mg $L^{-1}$ of this antibiotic. But, as time progressed, the embryos that were differentiated oxidized without any further development. [Aiming the optimization] of the transformation protocol, Other factors that affect transformation efficiency were examined to optimize the transformation protocol, including the vector harboring *nptII* or *hpt* gene and cocultivation temperature. In transformation experiments organogenesis occurred only when kanamycin was the scorable marker, whereas somatic embryos regenerated on both kanamycin and hygromicin-supplemented medium. Although the number of explants harboring embryos was statistically non-significant, hygromicin was more efficient than kanamycin as shown by the higher frequencies of transformed plants among the regenerated ones. The *hpt* gene harboring agrobacteria strain C58C1 pRGG harboring *hpt* gene led to larger numbers of embryos compared to C58C1 pRGG neo5. Shoots regenerated only when explants were transformed with the latter. Co-cultivation temperatures ranging from 22 to 28 °C did not affect on the number of explants with embryos, although higher numbers of regenerants were obtained at 24 °C. The *Sw-5* gene transfer to eggplant 'Embú' was successfully accomplished. Segregation analysis of the transgene and resistance evaluation by means of tospovirus inoculation and DAS-ELISA were carried out with the R1 generation. PCR and inoculation data indicated that two or more copies were inserted in each transformation event. The plants that possessed at least one copy of the transgene presented hypersensitivity reaction, suggesting that the *Sw-5* gene was functionally active in the eggplant genome. Different types of lesions were observed in the inoculated transformed plants, which may be related with the number of transgene copies.

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### Aneuplody detection by FISH technique in bladder lesion interphase nuclei of the bladder lesions

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Bladder cancer prognosis has been related in particular to histological type and grade and to the clinical stage of era diagnostic. The predominant histological type of bladder cancer is transitional cell carcinoma (TCC). Some genetic studies have associated the occurence of chromosome alterations and in tumours supressor genes and oncogenes in tumours, such as *CDKN2, ARF, RB, TP53, BCL-2, c-ERBB-2* and the EGF-R receptor EGF-R to process the initiation and progression of bladder tumours. Tumour progression appears to occur jointly with the acquisition of chromosomal abnormalities such as deletion of 9p, 9q and 17p and gain of 1q, 5p, 7p, 11q, and 17q.

The present study was carried out because of a higher incidence of bladder tumours and the need to identify chromosome markers to help early diagnosis and risk of recurrence. the present study was carryed. Fluorescence in situ hybridization (FISH) was used to investigate numerical chromosome alterations with 7, 9 and 17 centromeric and classical satellite probes in fresh tissue interphase nuclei of fresh tissues. We analyzed fourteen bladder malignant tumours, two inflammation chronic and three normal urothelium biopsies from patients with TCC. Five bladder descamation cell samples and five lymphocyte specimens both from healthy people were used as a control. As the statstical analysis showed significative difference between both control samples we used the values obtained in bladder descamation cells. The cutoff criteria was defined by adding four standard deviations to the averages of the bladder descamation cell data.

FISH analysis of the bladder malignant tumours showed trisomy 7 and/or tetrasomy 7 in 85,7% (12/14) of the samples, monosomy 9 in 28,6% (4/14) and trisomy or tetrasomy 17 in 57,1% (8/14) of the samples. The two samples of cystitis from the patient with TCC recurrence showed trisomy 7 and monosomy 9 as the most important alterations. One of three normal bladder samples of the

normal bladder, also from the patient with TCC, showed the same numerical alterations (polisomy 7, 9 and 17) of the tumoral tissue. However, the other two samples showed only trisomy 7. The comparative analysis of low grade (I) with invasive (II until IV) TCC and undifferentiated anaplasic carcinoma showed higher number of the aneuploidy in higher grade tumours. These tumours showed increased frequency of the trisomy and tetrasomy 7, 9 and 17, characterizing polisomy and genetic instability. In TCC grade I and II Trisomy 7 was frequently observed associated with aneuploidy 9 in TCC grade I and II, thus suggesting the existence of a relationship between the tumour histopathology of the tumours and the numerical alterations.

This study indicated the participation of chromosomes 7, 9 e 17 in urothelial carcinogenesis: the alterations of chromosomes 7 and 9 were related to the initiation process and chromosome 17 to tumoral progression and recurrence. In these chromosomes are mapped The *ERBB, CDKN2* and *c-ERBB-2* genes are mapped in these chromosomes, respectively, whose products perform important functions in the regulation of the cell cicle. Therefore, genetic evaluation using these chromosomes together with molecular studies may be indicated for early diagnosis in risk patients of the risk and medical following of patients at risk of recurrence and metastasis.

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