CLONING OF SUPEROXIDE DISMUTASE FROM A NEMATODE-RESISTANT COTTON GENOTYPE FOR OVEREXPRESSION IN TOBACCO

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The cultivation of cotton (*Gossypium hirsutum* L.) is of high importance to Brazil and its production has been increasing in recent years. However, this crop is severely affected by different pests and diseases, which cause significant yield losses. *Meloidogyne incognita* is one of the most important pests that affect cotton productivity. This nematode is sedentary and causes damage to the plant roots. Due to the action of proteins released by the parasite, the plant cells increase their cytoplasmic content without dividing, resulting in the generation of giant cells. In a preliminary study, we found that the gene encoding the protein superoxide dismutase is differentially expressed in roots of a nematode-resistant cotton genotype, when compared to a susceptible one. The objective of the present work was to isolate the gene encoding superoxide dismutase (SOD) for future transformation of the model plant *Nicotiana tabacum*, which is naturally susceptible to the nematode *M. incognita*. Initially, the gene was amplified by RT-PCR from roots of the cotton variety IAC 21. The primers were designed containing the recombination sites of the Gateway cloning system (Invitrogen). After amplification, the PCR fragment was purified and the recombination reaction with the vector pDONR 221 was prepared. After this step, the recombined product was introduced via electroporation into competent cells of *Escherichia coli* strain Omnimax 2R T1. The amplified fragment was approximately 500 bp corresponding to the size expected for the SOD gene. The next step will be the recombination of the gene into the binary vector pK7WGF2 for future tobacco transformation via Agrobacterium

BIOSAFETY OF TRANSGENIC COMMON BEANS RESISTANT TO *BEAN GOLDEN MOSAIC VIRUS*

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Bean golden mosaic virus (BGMV) is an important *Begomovirus* distributed in most of the bean growing areas in Brazil. Disease losses can reach up to 100% in a singly early affected field. We recently showed that transgenic common bean Olathe 5.1 expressing a small interfering RNA derived from a fragment of the *rep* gene of BGMV successfully prevents plants from infection by BGMV both under high inoculation pressure in the greenhouse or in the field. According to the Brazilian law, the biosafety of Genetically Modified Organisms (GMOs) shall be based on the Molecular Characterization (including protein biosafety), Agronomic and Environmental evaluation, and Substantial Equivalence. The transgene is present as two copies in a single locus, and was inherited as a single gene in a Mendelian way. Up to 30% of heterozygous plants may be infected by BGMV thus showing gene dosage effect. However, homozygous plants are completely resistant. Agronomic evaluation was carried out in three locations at differing geographic regions during two years. Yield as well as its components evaluated did not show differences between the transgenic and the conventional parental genotype. There were no visual differences between the two plant types. The same experiments were used to evaluate both insect pests and regular visiting insects throughout the growing cycle, as well as the soil prevailing or inhabiting microorganisms. Except for resistance to BGMV the transgenic line Olathe 5.1 was essentially like its counterpart Olathe Pinto in all of the risk assessment

undertaken. The present work is an example of a public sector effort to build on useful traits such as disease resistance in an orphan crop grown by many small land holders. We estimated that about 200.000 ha may return to bean production, which would yield enough for about 20 million adults.

HUMAN EMBRYONIC STEM CELLS (HESC)

Juliana Georges

Pluripotent human embryonic stem (hES) cells are an important experimental tool for understanding differentiation and development, and its application not only in basic but also in applied research make them a potential source of different tissues for transplantation. However, one important challenge for the clinical use of these cells in therapy is the issue of immunecompatibility, which may be dealt with by the establishment of hES cell banks to attend different populations.

The essential components of the derivation process are the embryos, embryo manipulation methods, feeder cells, culture media and general culture conditions.

In 2008, according to Biosafety Law (Law No. 11.105) of March 24th, 2005, Pereira's group generated the first hESC Brazilian lineage, named BR-1 (46, XY), which was fundamental to the consolidation of this research area in our country. Embryos were donated from *in vitro* Fertilization (IVF) clinics after parents consent, and cultured until reach blastocyst stage. Whole blastocysts or mechanically dissected inner cell masses (ICM) were seeded in hES-certified matrigel-coated dishes, and cultured in mTeSR1 defined medium. Surviving outgrows were monitored daily until stable ES-like growth was observed. One outgrowth, named BR-1, survived and was continuously passaged mechanically. Colonies exhibited the characteristic morphology of hES cell and expressed pluripotency markers (OCT-4, TRA-1-81, TRA-1-60, SSEA-4), as observed after immunocytochemistry assays using appropriate antibodies. BR-1 cells formed embryoid bodies (EB) when cultured in suspension and absence of FGF; in adherent plates, EB cells could differentiate in endoderm, ectoderm and mesoderm, as verified by immunstaining. Approximately 150 clumps of BR-1-undifferentiated colonies were injected subcutaneously in immunodeficient mice and could efficiently form teratomas. To our knowledge, this is the first reported line of hES cells derived in South America. In contrast to the other hES cell lines established in defined medium, BR-1 maintained a stable normal karyotype as determined by genomic array analysis after 6 months in continuous culture.

In 2010, our group generated a second hESC Brazilian lineage, named BR-2 (69, XXX), derived through a 3PN embryo (unviable embryo), and although this lineage is not suitable for cell therapy, it will be interesting as an *in vitro* model of human polyploidy. The technique used for the derivation and characterization of this line was the same of BR-1. The characterization obtained an abnormal karyotype, with the result triploid (69, XXX) were strongly positive for markers of undifferentiated pluripotent cells such as OCT-4, TRA-1-60 and TRA-1-81. In HLA typing was observed in the presence of three specific in the typing of HLA-A and HLA-B and in the typing of HLA-C was observed the presence of more than two specificities, but it was not possible to determine them. With the EBs formation, we observed the differentiation of lineage in neural cells, cardiac, adipose, muscle and others. So we are continuing studies with immunocytochemistry using SOX17, HNF3β, α-ACTININ, MYOD, NF200 and MAP2 markers to comprove the three germ layers and analyzing the teratoma formation.

In conclusion, it can be affirm that studies with different types of hESC must be accompanied with enthusiasm and caution. It is inherent in every research area in advances development and setbacks, and still do not know what kind of cell therapy and fulfill the promise will be most appropriate to treat the diseases. Still, hESC should be viewed not only as a therapeutic agent, but as a research model where we can study the mechanisms behind the cellular differentiation, embryonic development, cancer and epigenetic changes, among others. On the other hand, this knowledge of basic biology may lead to an improved quality of human life.