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Progress Towards Developing Striped Stem Borer *(Chilo suppressalis)* Resistant, Transgenic Mediterranean Rice at CIRAD

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I – Gene transfer to rice: the state of the art

Gene transfer to rice has so far relied on either PEG (Zhang and Wu, 1988) or electroporation (Toriyama *et al.*, 1988), mediated direct DNA uptake into protoplasts, particle bombardment of organized tissues (Christou *et al.*), calluses (Li *et al.*, 1993) and cell suspensions (Cao *et al.*, 1992) or electroporation of intact tissues and calluses (Plant Genetic System, unpublished). Successfully and repeatedly transformed varieties belong to temperate japonicas (Isozyme group VI) (Tapei 309, Yamahoushi, Nipponbare, Norin 8) (Zhang *et al.*, 1988, Zhang et Wu, 1988, Toriyama *et al.*, 1988, Shimamoto *et al.*, 1989, Hayashimoto *et al.*, 1990, Tada *et al.*, 1990, Battraw *et al.*, 1992, Li *et al.*, 1992, Rathore *et al.*, 1993), aus-type (Chinsurah Boro II) (Isoyme group II) (Datta *et al.*, 1992), or indicas (isozyme group I) (IR54, IR72) (Peng *et al.* 1992, Datta *et al.*, 1992). Production of transgenic Mediterranean rices has been reported in cultivars Labelle (Li *et al.*, 1992b), Gulfmont (Christou *et al.*, 1991) and Radon (Rathore *et al.*, 1993).

Short-term breeding objectives for rice transformation are transfer of plant or bacterial genes conferring resistance to insects (endotoxins, lectins and protease inhibitors), viruses (Coat protein, antisense RNA, satellite DNA), bacteria (phytoalexins, antimicrobial agents, detoxifying genes), fungi (glucanase, chitiniase, PAL and HMGR genes), and herbicides (overproducing or detoxifying genes). Rice plant having integrated synthetic CryIA (b) Bacillus thurengiensis toxin gene (Fujimoto *et al.*, 1993), snowdrop lectin gene (Boutter *et al.*, 1993), cowpea and potato protease inhibitor genes (Duan *et al.*, 1993), Tungo bacilliform (Qu *et al.*, 1993) and stripe (Hayakawa *et al.*, 1992) virus coat protein genes, chitintase and PAL genes (Zhu *et al.*, 1993), phosphinothricin (Toki *et al.*, 1992, Datta *et al.*, 1992, Cao *et al.*, 1992, Rathore *et al.*, 1993) and sulfonylurea (Li *et al.*, 1992a) resistance genes have already been produced. Medium- to long-term researches aim at improving grain quality and enhancing tolerance to abiotic stresses (anosia, drought, salt, cold) (Toenniessen, 1991).

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II – Work carried out at CIRAD

Developing a gene transfer method for Mediterranean rice

CIRAD-CA undertook a Mediterranean and tropical upland rice transformation project early in 1990. Work focused on direct gene transfer to protoplasts, which was the only reliable method producing transgenic rices at that time, and on Mediterranean varieties, which are more amenable to tissue culture procedures than upland varieties. In 1991, an efficient method for regenerating plants from protoplasts of temperate and tropical *Japonica* varieties was set up (Guiderdoni and Chaïr, 1992) and variation among

progenies of regenerated protoclones has been assessed using molecular markers—in collaboration with ORSTOM—(Mezencev *et al.*, 1993b) and field evaluation (Mezencev *et al.*, 1994a). In 1993, putative transgenic rice plants have been regenerated from geneticin-resistant calluses deriving from protoplasts treated with PRG or electroporation. These plants are being evaluated using PCR and Southern blotting, in collaboration with ORSTOM. Various plant promoters are being tested in both transient and stable transformation assays. Once an efficient transformation system is set up, transfer of a construct bearing both CryIA (c) and CryIB *Bacillus thuringiensis* toxin genes, active against the stem borer *Chilo suppressalis*, will be attempted.

Identifying new *Bacillus Thuringiensis* (B.t.) strains and toxin active against striped stem borer (SSB), *Chilo suppressalis.*

Five strains of B.t., isolated from diseased lepidoptera larvae were assayed on *Chilo suppressalis* larvae. Two out of the five were active against SSB with an LC-50 of 0.16 and 0.35 µg/ml respectively (Fiuza *et al.*, 1993a). These strains might be valuable as microbial agent or as a source of gene for SSB-resistant transgenic rice.

Binding of CryIA(b) toxin was immunocytochemically analyzed on tissues sections of SSB larvae. Measurement and localisation of fluorescence was assessed using laser scanning flow cytometry. Preliminary results indicate that CryIA(b) toxin bound to the microvilli of the midgut epithelial cells and that the binding sites are not entirely localized on the whole length of the brush border membrane. Moreover the fluorescence seems to be emitted only from particular area of the insect midgut (Fiuza *et al.*, 1993b).

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