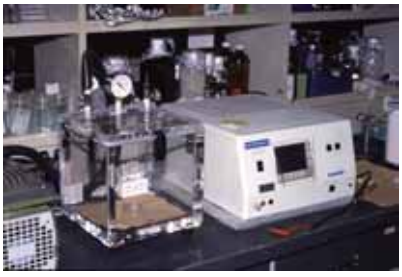
National Institute of Agrobiological Resources
Takshi Hagio Ph.D
2-1-2 Kannondai Tsukuba Ibaraki Japan 305-8602
TEL:+81-298-38-8372 FAX:+81-298-38-8397
Mailto:hagio94@nias.affrc.go.jp

A novel system for the introduction of foreign genes into plant mature seeds

We have developed a novel system that can directly deliver foreign genes into plant mature seeds using electroporation. Mature seeds of rice and wheat were soaked in water overnight. Then the seeds were incubated in the electroporation buffer containing plasmid DNAs for several hours under the condition of reduced air pressure. DNAs are considered to be transported into cells across cell walls with the help of depressurization treatment. Electroporation was carried out with an electroporation device in a 10 mm wide cuvette containing 1.0 ml of electroporation buffer. After the electroporation the seeds were incubated in water for two days. To determine the optimal conditions of gene transfer, GUS (β-glucuronidase) gene expression assay were carried out. To obtain stably transformed plants, the seeds were transferred to selection medium. After the plant regeneration putative transgenic chimeras and sexual offspring were analyzed. Both in rice and wheat, fertile transgenic plants were regenerated and self-fertilized seeds were obtained. Transgene presence was confirmed by Southern hybridization. Transmission of the transgene into the next generation (T_1) was indicated by PCR analysis. This method is simpler and more rapid than conventional techniques, and it can be applied to a wide range of commercial rice and wheat varieties. The transformation procedure presented here does not require the establishment of genotype-dependent tissue culture system. We also observed transient gene expression in seeds of soybean, tomato and *Brassica*.

Electroporation device and GUS gene expression in wheat mature seeds



Vacuum chamber and Electroporator

From left to right:
a) vacuum treatment and electroporation,
b) only vacuum treatment,
c) control GUS gene expression is visibly observed in blue area