TRANSFORMATION OF RICE WITH INSECTICIDAL GENES BY BIOLISTIC METHOD AND REGENERATION OF TRANSGENIC PLANTS

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Introduction

Pest damage has been a very important limiting factor for rice production not only in China but also over the world. The potential of crop breeding by plant genetic manipulation to protect crop from insect has been widely recognized. It has been confirmed that the cowpea trypsin inhibitor (CpTI) was antimetabolic to a wide range of insects (1). In previous work, we have obtained CpTI transgenic tobacco plants which showed resistance to *Heliothis armigera* (2, 3). In this research, we anticipated to further increase of rice resistance to insect pests by introducing CpTI gene and potato proteinase inhibitor II (PI-II) into rice.

Material and Methods

The plasmids pBCA-Hpt and pBA-Hpt used in transformation were constructed in our laboratory, the former carried CpTI gene and the later carried PI-II gene, both under the control of rice *act1* gene promoter (4), and hygromycin phosphotransferase (Hpt) gene was used as plant selective marker.

Immature embryos and embryogenic calli were used as transformation recipients, which derived from 3 varieties of japonica rice ZH-8, ZH-10 and ZZ-321. The recipients were bombarded *via* Biolistic Particle Delivery System (PDS-1000/He, Bio-Rad) and were incubated on high osmotic NMB mediu m (5, 6) with 0.5-0.7 M mannitol for 4 hrs before bombardment and for another 24 hrs after bombardment, then were transferred to NMB callus inducing medium(2.4-D 2 mg/l) for 3-5 days. Selection was performed on NMB selective medium with 30 mg/l hyg B, the hyg^r calli were transferred on the same medium but with 40 mg/l hyg B at interval of 3-4 weeks. After 3 circles of subculture, the hyg^r calli were transferred on NMB regenerating medium (6-BA 0.5 mg/l,KT 2.5 mg/l or TDZ 0.5 mg/l) with 50 mg/l hyg B, when regenerated shoots growing to 5 cm high, they were transferred to NMB medium (without hormone but with 50 mg/l hyg B) for further developing of roots and were finally transferred to greenhouse.

The contents of CpTI of hgy^r rice plants were determined by ELISA procedure. Southern blot and PCR assays of transgenic plants were going on according to standard procedure (7). Hpt activity was detected according to method previously reported (6). Bioassay of transgenic rice plants were carried out using stalk borer (*Chilo partellus*) in our collaboration laboratory (Bioassay laboratory, Tieling Institute of Agricultural Sciences, Liaoning Academy of Agricultural Sciences, Liaoning Province, China).

Results

After 3 selective circles, the yield of hyg^r calli of ZH8, ZH10 and ZZ321 were 4.4 %, 30.3 % and 25.5 % respectively, while untransformed calli as control always died after 2 or 3 selective circles. One month later after regeneration, green pots appeared on calli, for another 15-20 days, the green shoots were regenerated, the regeneration frequencies were 6-20 %. Currently more than four hundred hyg^r plantlets have been obtained and have been transferred to greenhouse, F1 generation seeds have obtained from some of them.

CpTI and PI-II could be detected in some of hyg^r rice plants at a high concentration (more than 1ug/mg dissolvable protein), but in most plants at low level even could not be detected. Southern blot, PCR and enzyme activity assays confirmed that these Hyg^r-plants were transgenic plants. The preliminary results of bioassay showed resistance of transgenic rice to stalk-borer.

Discussion

It has been confirmed by previous experiments that the three rice genotypes used in this research were more easily to be regenerated by tissue culture *in vitro* no matter from immature embryo or from embryogenic callus derived from immature embryo. I has been suggested by Professor Tian,W.Z. that a high osmotic treatment of recipients before and after bombardment which might extremely enhance transformation frequencies (personal communication). More than half of transgenic plants were sterile, our opinion trended towards that it was caused by tissue culture but not by expression of foreign gene, because the same phenomena exist in plants regenerated from untransformed calli. It was previously expected only a few transgenic plants in which foreign gene could express at a higher level, for it has been reported that the chromosomal location of insertion (position effect) greatly affects the expression level of inserted genes (8).

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