Activities of rice biotechnology in Hungary

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Activities of Rice Biotechnology in Hungary, 1994

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N.B.: This paper was discussed with rice biotechnology project leaders: Prof. L.E. Heszky, B. Jenes and rice breeder Dr. I. K. Simon.

In Hungary the rice biotechnology research is determined by two important facts: grant (national/international) for rice biotechnology research and economic situation of rice breeding/cultivation.

Financial background. There are two state offices (National Committee for Technological Development and National Found for Scientific Research) to support the research via competition of research programmes. So far, rice biotechnologists have successfully obtained grants for research in each period (e.g., Dama new rice variety, the first plant variety of biotechnology origin in Hungary was released through the financial assistance of NCTD; the construction of a home-designed particle gun in ABC was supported by NCTD, etc.).

After the economic change in Hungary, the economic situation of rice cultivation is worse than before as the low quality rice imported is more profitable than the domestic cultivation (lack of protective tariff). Hungarian rice consumers traditionally pay little attention to rice quality. The impact on rice breeding results in less seed business and lower inputs for research and breeding.

Rice biotechnology research is carried out at three different places in Hungary. The most important results and contributions to rice biotechnology improvement are summarized below according to research institutes.

I – University of Agricultural Sciences, Gödöllö; Department of Genetics and Plant Breeding (Head of Department and Rice Project: Prof. L. E. Heszkv Ph.D., D.Sc.)

❑ **Improvement of rice tissue culture system.** It was possible to reduce genotypic differences in rice plant regeneration using plumule meristems as the explant. The frequency of plant regeneration was increased from 37% to 83% by changing the hormone concentration.

Prolonged regeneration was achieved in cell suspension and callus by continuous subculture (1 year) on medium containing 0.5-1% NaCl. The ratio of green plants was increased by a low X-ray dose in long-term cell suspension.

❑ **Breeding.** The pollen haploid soma clone breeding method (PHS-method) was elaborated. The theory of the PHS method was established using somatic tissues and cells of androgenic haploid plants. Significant variation was achieved in some agronomical characters by the PHS method in comparison with soma clones of seed and meristem callus of zygotic origin. The PHS soma clones were introduced in breeding and the new soma clone-oriented variety Dama was released in 1992. This variety is the most resistant to *Piricularia*, and has the best grain profile and cooking quality in Hungary. Its yield potential is 6-7 tons/hectare.

In Hungary, Dama is the first plant variety developed by the use of a plant biotechnology method. In our opinion, the PHS-method of improvement appears to be suitable for successful application by breeders of other plant species.

II – Agricultural Biotechnology Centre, Gödöllö, Institute for Plant Sciences (Project: Tissue Culture and Gene Transfer Systems in Monocots. Project Leader: Barnabas Jenes M.Sc.) (This institute was visited by the Rice Biotechnology Working Group in February.)

❑ **Establishment of an efficient, repeatable Plant-protoplast-plant system in Japonica rice genotypes as a base for gene transfer into rice**. Several gene delivery methods have been compared—PEG treatment, electroporation and ultra sound effect (sonication) in relation to protoplast viability and regeneration efficiency after the treatments. The work is being carried out with various genotypes at the diploid and haploid level.

❑ **Construction of a home designed particle gun and its use for transgenic rice production.** The home made particle gun was used for gene delivery into rice ('HB-42' Japonica genotype) cultured tissue. The microprojectiles were accelerated by controlled high pressure nitrogen gas. Plasmid DNA carrying the Act1 promoter from rice linked with gusA gene and the Hph gene were coated on the surface of Tungsten microprojectiles (0.7-1.1 um diameter). Shooting distance, nitrogen pressure were optimized, as well as the size of microprojectiles for callus tissue.

Transgenic plants have been regenerated from the Hygromycyn resistant calli. Histochemical staining indicated the expression of gusA in the root and leaf tissues of transformed plants. Southern-blotting and PCR results showed the presence of the introduced gusA gene in T and T plants.

T and T plants are analysed for the inheritance of the foreign genes in the rice plant. The technique is now routinely in use, and seems to be applicable to other important monocot crops, such as wheat, barley and maize.

❑ **Transformation methods** are being applied for production of insect resistant transgenic rice plants carrying genes of wild monocot species. It is hoped that these genes will provide resistance against all the sucking insects of cultivated rice.

III – Cereal Research Institute, Szeed; Cell and Tissue Culture Laboratory (Head: János Pauk Ph.D.)

❑ **Extension of genotype background of protoplast studies to agronomically important rice genotypes.** Development of cell suspensions, protoplast culture and plant regeneration can be extended to different agronomically important varieties, using the following conditions: (1) strict callus selection on proline-containing medium for suspension culture initiation; (2) use of the same macro- and micro elements in cell suspension and protoplast culture media; (3) induction of embryogenic callus on the surface of ABA and Dicamba containing media.

❑ **Production of Transgenic fertile rice using Particle gun delivery** (UNESCO Fellowship in Braunschweig, Technische Universitat, Germany). Strictly selected immature embryo-derived callus was used for the development of suspension cultures. In the transformation experiment, fine embryogenic suspensions was plated on filter paper over selection medium and then bombarded. Gold micro carriers coated with plasmid DNA (pGSGLUC1, pRT99GUS) were accelerated at high velocity using the biolistic particle delivery system (PDS-1000). For selection after bombardment, agar medium at each stage was supplemented with 75-100 mg/l of G-418. Isolated calli were tested for NPT II and only NPT II positive calli were used in the regeneration experiment. 31 NPT II positive plants were grown in the greenhouse, and 16 of them were fertile. The presence and expression of the introduced genes in R transgenic plants and their offspring were confirmed using the NPT II test, southern analysis and fluorometric GUS assays.

The results carried out in the laboratories of the above listed Institutes, are integrated into rice breeding at the Research Institute for Irrigation, Szarvas; Rice Breeding Section (Head: Ibolya K. Simon, M.Sc., Ph.D.).