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Trends in rice researches: Novel use of biotechnology

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Résumé. *En moins d'une décennie, la biotechnologie du riz est passée d'une situation où elle représentait une activité marginale à celle où le riz est considéré comme le support, le modèle des recherches en biotechnologie pour les céréales. Il est significatif que les fonds accordés pour les recherches en biotechnologie du riz proviennent de la Fondation Rockefeller en faveur des riz* Indica. *Mais, pour autant, les riz* Japonica *qui intéressent la région méditerranéenne n'ont pas été négligés. Les techniques de culture des tissus sur le riz (comprenant les riz haploïdes) et de production de riz hybrides par les voies somatiques ou transgéniques ont été perfectionnées et des utilisations variées de la biotechnologie ont été identifiées. Elles ont été orientées vers l'amélioration de la résistance aux maladies et aux insectes (riz transgénique), vers la production de riz hybride tolérant au sel (hybridation somatique) et vers l'amélioration de la rapidité et de l'efficacité d'incorporation de nouveaux gènes désirables par l'utilisation de marqueurs moléculaires sélectionnables permettant d'identifier les "*quantitative trait loci". *Des découvertes récentes sur la conservation de la structure des génomes entre riz et blé sont particulièrement utiles dans ces domaines et peuvent expliquer pourquoi il a été possible d'établir une interaction entre azorhizobia à la fois avec le riz et le blé pour la fixation endophyte de l'azote, laquelle permettrait de réduire l'apport d'azote sans réduire les rendements (avec réduction consécutive de la pollution). La principale question que posent les investigations en biotechnologie des céréales, du riz en particulier, est l'identification des besoins de la part des sélectionneurs et des producteurs. Quelles sont les priorités pour les recherches en biotechnologie du riz sous climat méditerranéen ?*

Mots clés. *Climat méditerranéen – riz – priorités en biotechnologie*

Abstract. In less than a decade rice biotechnology has moved from a position of neglect to a position where rice is now regarded as a model plant for cereal research and biotechnological developments. It is noteworthy that most recent funding and impetus for rice biotechnology has come from the Rockefeller Foundation for Asian indica varieties, but japonica varieties, widely adaptable to Mediterranean Climate regions, have not been neglected. Procedures for rice tissue culture (including haploid rices) and for transgenic and somatic hybrid rice production have been perfected, and various intended uses of biotechnology have been identified. These have included improving disease and insect resistance (transgenic rice) and salinity tolerance and hybrid rice production (somatic hybridisation), and the improvement of the speed and efficiency of incorporation of desirable new genes by utilization of closely linked, selectable molecular markers and the utilization of molecular markers to identify important quantitative trait loci. Recent evidence for the conservation of genome structure between rice and wheat is particularly pertinent in these respects, and may also explain why it has been possible to establish an endophytic nitrogen fixing interaction between *azorhizobia* in both rice and wheat which shows promise to reduce nitrogenous fertiliser inputs without yield reductions (with associated pollution advantages). A central challenging question for these biotechnological investigations is the identification of needs of rice breeders and rice producers for such biotechnological investigations. What are the priorities for the novel use of biotechnology for Mediterranean Climate rices?

Key words. Mediterranean climate – rices – biotechnology priorities

I – Introduction

In the field crops industry, including rice, the application of biotechnology can be divided into two major areas. One is biotechnology in aid of plant breeding for traditional goals and the other is in aid of the production of industrial products in plants. Plant breeders are increasingly attempting to use biotechnology as a tool to increase the efficiency of traditional methods. The utilization of modern biotechnology for rice improvement is still however in its initial stages. During the past decade, the production of transgenic plants has emerged as an additional tool for the rice breeder. In transformation only specific cloned genes of interest are introduced into the recipient plants, eliminating the need for repeated backcrosses normally associated with conventional breeding to remove undesirable, co-transferred genes.

Dealing with polygenetic traits such as salt tolerance, yield and grain quality will be conceivable if dissection into several single genes is possible. At present, however, sexual and somatic hybridisations are preferred for dealing with polygenic traits. An attempt will be made in this review to identify the priorities for the novel use of biotechnology for Mediterranean Climate rices by surveying studies undertaken on this topic in the FAO Mediterranean Climate Rice Research Network Biotechnology Working Group since its inception.

II – The use of rice cell and tissue culture

As discussed by Heszky and Simon-Kiss (1992), before 1980 plant breeders could increase the genetic variability of rice in two ways, by crossing or by mutation. The production of haploid rice through the culture of anthers and microspores had important applications in rice breeding. Doubling the chromosome complement of haploid material was a rapid method of inducing homozygosity, thereby shortening the time for the development of new varieties and enabling recessive genes expressed in haploids to become fixed when the chromosome number is doubled. Additionally, it was observed that genetic variation occurred in plants regenerated from cultured cells constituting the basis of somaclonal variation (Larkin P.J., Scowcroft W.R., 1981). Commonly observed variation in rice cell and tissue culture derived plants includes the number of tillers per plant, plant height, flag-leaf length, heading date, panicle length, fertility and the number of seeds produced; positive variation could be selectively exploited for rice crop improvement (Abdullah R., Thompson J.A., Khush G.S., Kaushik R.P., Cocking E.C., 1989). Particularly pertinent in this respect was the production of 'Dama', the first rice variety of biotechnological origin produced and registered in Hungary in 1992 (Heszky L.E., Simon-Kiss I., 1992). Double haploid (DH) somaclones of haploid origin were produced from Hungarian rice varieties. Breeding work with these double haploid somaclone plants enabled the selection of new individuals of earlier maturity, better seed profile and increased blast resistance with a yielding capacity and agronomical traits identical with those of the original Hungarian rice varieties. The registered Dama variety from these DH somaclones had good resistance to blast and the best seed profile and cooking quality (Heszky L.E., Simon-Kiss I., 1992).

III – Useful genes for rice biotechnology

The application of biotechnology for rice improvement is still at an early stage of development. An attempt has been made to rank the priorities for biotechnological thrusts utilising cell and tissue culture, transgenic and somatic hybrid plants for rice improvement world-wide based on the judgement of knowledgeable scientists (Herdt R.W., 1991). In priority order these improvements are in resistance to tungro virus, bacterial blight, sheath blight, ragged stunt virus and blast, also soil factors (coastal saline/acid), iron deficiency, temperature and water, physiological opportunities (greater lodging resistance, cytoplasmic male sterility, apomixis) and insects (brown plant hopper, yellow stem borer).

These biotechnological objectives must be put in the perspective that many of these objectives are already being attempted, often with significant success, by breeding procedures utilising a range of hybridisations, including the use of the genetic diversity of wild rice species in wide hybridisations. For biotechnological applications linked to the production of transgenic plants, research strategies are aimed at identifying and isolating the required genes. Progress is often slow because either the genes have not been identified and isolated, or because many genes are involved making the production of transgenic plants difficult, or with our current knowledge presently impossible.

Within the FAO Biotechnology Mediterranean Climate Network methods are being perfected for the development of robust procedures for transgenic rice production from a range of rice varieties using protoplasts, biolistic and *Agrobacterium* delivery systems (Ayres N.M., Park D., 1994). *Agrobacterium*-mediated transgenic rice production appears increasingly efficient for transgenic rice production for a wide range of rice varieties (Gosal S.S., Zhang J., Azhakanandam K., Fernando S., Power J.B, Lowe K.C., Davey M.R., Cocking E.C., 1996). Work at CIRAD has been focused on the transfer into rice of a construct bearing both CryI A(c) and CryI B *Bacillus thuringiensis* toxin genes, active against the rice stem borer, *Chilo suppressalis* and at CNRS (in collaboration with the Scripps Institute, USA) on the transfer into rice of tungro virus coat protein genes for enhanced tungro virus resistance.

Worldwide we are presently in the early stages of the development and testing of transgenic rice plants containing novel genes capable of conferring beneficial agronomic traits (Hall T.C., Xu Y., Huntley C.C., Yu H., Seay J., Connell J.C., Lepetit M., Dong J., Wallace D., Way M.O., Bucholz W.G., 1993). Only a few detailed studies have been undertaken on the inheritance of genes in transgenic rice plants; these have highlighted problems likely to be encountered in the control of gene expression in transgenic seed progeny (Schuh W., Nelson M.R., Bigelow D.M., Orum T.V., Orth C.E., Lynch P.T., Eyles P.S., Blackhall N.W., Jones J., Cocking E.C., Davey M.R., 1993).

IV – The use of molecular markers

Work in this area in the FAO Mediterranean Climate Network has involved improvement of the speed and efficiency of incorporation of desirable new genes by utilisation of closely linked, selectable molecular markers and the utilisation of molecular markers to identify important quantitative trait loci. In this general area work at CIRAD has involved the mapping of blast and rice yellow mottle virus resistance genes in collaboration with ORSTOM and Cornell University, USA and mapping genes conferring grain aroma in rice through RFLP and HPLC analyses. At CNRS investigations have involved the identification, mapping and cloning of *Magnaporthe grisea* avirulence genes and the mapping of rice yellow mottle virus resistance genes in rice. The recent evidence for synteny between rice and wheat, indicating that many rice chromosomes contain homeologous genes and genomic DNA fragments in a similar order to that found on wheat chromosomes (Kurata N., Moore G., Nagamura Y., Foote T., Yano M., Minobe Y., Gale M., 1994) is particularly pertinent in these respects.

V – Useful wide crosses

The genus *Oryza* has about twenty species, two of which are cultivated, *O. sativa* the common cultured rice which is grown world wide and *O. glaberrima*, the cultivated African rice which is grown in West Africa. The others are wild rice species most of which can be successfully sexually crossed, including the use of embryo rescue procedures, for the introgression of a wide range of useful genes. Of particular interest is the use of protoplast fusion procedures, coupled with the regeneration of plants from the products of the fusion, for the introgression of both nuclear and cytoplasmic genes (Schuh W., Nelson M.R., Bigelow D.M., Orum T.V., Orth C.E., Lynch P.T., Eyles P.S., Blackhall N.W., Jones J., Cocking E.C., Davey M.R., 1993). At the University of Nottingham within the FAO Network somatic hybrid plants have been produced from the heterokaryons resulting from fusing rice cell suspension-culture protoplasts and mesophyll protoplasts isolated from the salt-tolerant wild rice species, *Porteresia coarctata* (Kurata N., Moore G., Nagamura Y., Foote T., Yano M., Minobe Y., Gale M., 1994). This work opens up the possibility of obtaining cultivated rice with improved salinity tolerance. Protoplast fusion technology is also enabling the range of cytoplasmic male sterile (CMS) rice lines for hybrid rice production to be significantly increased. For instance, the transfer of CMS from Chinsurah Boro into the Japonica variety Nipponbare (Kumar A., Cocking E.C., 1987).

VI – Inoculating rice with azorhizobia for endophytic nitrogen fixation

It is generally recognised that the introduction of endophytic symbiotic biological nitrogen fixation into rice, and other major non-legume crops, would be one of the most significant contributions that biotechnology could make to agriculture (Finch R.P., Slamet I.H., Cocking E.C., 1990). It is also generally recognised that novel uses of biotechnology should emphasise that the resultant improvements should be sustainable and environmentally benign and friendly. Work at the University of Nottingham in the FAO Mediterranean Climate Network has shown that rhizobia such as *Azorhizobium caulinodans* which forms root and stem nodules on the tropical legume, *Sesbania rostrata* enter rice, maize, wheat and oilseed rape plants at points of emergence of lateral roots by crack entry. This work has shown that *A. caulinodans* can colonize the cracks at the points of emergence of lateral roots of rice and can subsequently colonise adjacent cortical tissue, forming large intercellular pockets of rhizobia. We have also discovered that specific flavonoids, such as naringenin, stimulate crack entry invasion and act as signals in this respect (Kyozuka J., Kaneda T., Shimamoto K., 1989).

The ultimate aim of this investigation of establishing a stable endophytic interaction between diazotrophs (such as *Azorhizobium caulinodans*) and non-legumes (such as rice) is that the diazotroph should fix nitrogen and transfer this fixed nitrogen to the plant. Ongoing collaborative work and strategic planning within the FAO Mediterranean Climate Network could enable this aim to be achieved, since it is now possible in rice to clearly define the various genetic and physiological factors which are limiting, both for rhizobial colonization and for endophytic nitrogen fixation. One of the outcomes of this work should be, as for legumes, an ability to reduce the input of nitrogenous fertilisers for rice production without reductions in yield.

VII – Priorities for the novel use of biotechnology

What should be the priorities for the novel use of biotechnology for the FAO Mediterranean Climate Rice Research Network? Hopefully, with the background information surveyed in this review, it will now be possible to establish priorities to enable better progress to be made.

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References

• **Abdullah R., Thompson J.A., Khush G.S., Kaushik R.P., Cocking E.C.** (1989). Protoclonal variation in the seed progeny of plants regenerated from rice protoplasts. *Plant Sci.* 65:97-101.

• **Ayres N.M., Park D.** (1994). Genetic transformation of rice. *Crit. Rev. Plant Sci.* 13(3):219-239.

• **Finch R.P., Slamet I.H., Cocking E.C.** (1990). Production of heterokaryons by the fusion of mesophyll protoplasts of *Porteresia coarctata* and suspension culture-derived protoplasts of *Oryza sativa*: a new approach to somatic hybridisation in rice. *J. Plant Physiol.* 136:592-598.

• **Gosal S.S., Zhang J., Azhakanandam K., Fernando S., Power J.B, Lowe K.C., Davey M.R., Cocking E.C.** (1996). Agrobacteria for transgenic rice production. Agro-Food-Industry Hi-Tech (submitted).

• **Gough C., Webster G., Vasse J., Galera C., Batchelor C.A., O'Callaghan K.J., Davey M.R, Kothari S.L., Dénarié J., Cocking E.C.** (1996). Specific flavonoids stimulate intercellular colonization of non-Legumes by *Azorhizobium caulinodans. Proc. 8th Intnatl. Cong. Molecular Plant-Microbe Interactions, Knoxville, USA, 14-19 July, 1996.*

• **Hall T.C., Xu Y., Huntley C.C., Yu H., Seay J., Connell J.C., Lepetit M., Dong J., Wallace D., Way M.O., Bucholz W.G.** (1993). Transgene expression and agronomic improvement of rice. *Phil Trans. R. Soc. Lond.B.* 342:197-201.

• **Herdt R.W.** (1991). Research priorities for rice biotechnology. In: *Rice Biotechnology*. Biotechnology in Agriculture No. 6 (eds.) G.S. Khush and G.H. Toenniessen. International Rice Research Institute - CAB International, Wallingford, Oxon, U.K. pp. 19-54.

• **Heszky L.E., Simon-Kiss I.** (1992). 'Dama', the first plant variety of biotechnology origin in Hungary, registered in 1992. *Hungarian Agricultural Research,* Vol. 1, No. 1, pp. 30-32.

• **Kumar A., Cocking E.C.** (1987). Protoplast fusion: A novel approach to organelle genetics in higher plants. *Amer. J. Bot.* 74:1289-1303.

• **Kurata N., Moore G., Nagamura Y., Foote T., Yano M., Minobe Y., Gale M.** (1994). Conservation of genome structure between rice and wheat. *Bio/Technol.* 12:276-278.

• **Kyozuka J., Kaneda T., Shimamoto K.** (1989). Production of cytoplasmic male sterile rice (*Oryza sativa* L.) by cell fusion. *Bio/Technol.* 7:1171-1174.

• **Larkin P.J., Scowcroft W.R.** (1981). Somaclonal variation - a novel source of variability from cell culture for plant improvement. *Theor. Appl. Genet.* 60:197-214.

• **Schuh W., Nelson M.R., Bigelow D.M., Orum T.V., Orth C.E., Lynch P.T., Eyles P.S., Blackhall N.W., Jones J., Cocking E.C., Davey M.R.** (1993). The phenotypic characterisation of R2 generation transgenic rice plants under field conditions. *Plant Sci.* 89:69-79.

• **Toenniessen G.H.** (1991). Potentially Useful Genes for Rice Genetic Engineering. In: *Rice Biotechnology*. Biotechnology in Agriculture No. 6 (eds.) G S Khush and G H Toenniessen. International Rice Research Institute - CAB International, Wallingford, Oxon, U.K. pp. 253-280.