COMPLEXES D'ESPÈCES, FLUX DE GÈNES ET RESSOURCES GÉNÉTIQUES DES PLANTES

Actes du colloque international, Paris 8-10 janvier 1992
organisé en hommage à Jean Pernès
Professeur à l'Université de Paris-XI

1992
Diffuseur :
Lavoisier - Technique et Documentation
14, rue de Provigny, F-94236 Cachan Cedex

OCHKO A., KIEFER M.C., CORDESSE F., REDDY A.S. and DELSENY .., 1991 — Distribution and organization of a tandemly repeated 352 bp sequence .n the *Oryzae* family. *Theor. Appl. Genet.*, 82 : 57-64.

ELCASSO-TREMOUSAYGUE D., GRELLET F., PANABIERES F., ANANIEV E.D. and DELSENY M., 1988 — Structural and transcriptional characterization of the external spacer of a ribosomal RNA nuclear gene from a higher plant. *Eur. J. Biochem.*, 172 : 767-776.

EBHARDT C., BLOMENDAHL C., SCHACHSTABEL U., DEBENER T., SALAMINI F. and RITTER E., 1989 — Identification of 2 n breeding lines and 4 n varieties of potato (*Solanum tuberosum* ssp. *tuberosum*) with RFLP finger prints. *Theor. Appl. Genet.*, 78 : 16-22.

LASZMANN J.C., 1987 — Isozymes and classification of Asian rice varieties. *Theor. Appl. Genet.*, 74 : 21-30.

RELLET F., DELCASSO D., PANABIERES F. and DELSENY M., 1986 — Organisation and evolution of a higher plant alphoïd-like satellite DNA sequence. *J. Mol. Biol.*, 187 : 495-507.

:NA K.K. and KHUSH G.S., 1989 — Monosomic alien addition lines of rice : production, morphology, cytology and breeding behavior. *Genome*, 32 : 449-455.

ANIATIS T., FRITSCH E.F. and SAMBROOK J., 1982 — Molecular cloning : a laboratory manual. Cold Spring Harbor Laboratory Press.

lcCOUCH S.R., KOCHERT G., YU Z.H., WANG Z.W., KHUSH G.S., COFFMAN W.R., TANKSLEY S.D., 1988 — Molecular mapping of rice chromosomes. *Theor. Appl. Genet.*, 76 : 815-829.

KA H.I., 1974 — Experimental studies on the origin of cultivated rice. *Genetics*, 78 : 475-486.

LMEDILLA A., DELCASSO D. and DELSENY M., 1984 — Methylation pattern of nuclear ribosomal DNA genes from rice (*Oryza sativa*). *Plant Sci. Lett.*, 37 : 123-127.

EDDY A.S., KIEFER-MEYER M.C., CORDESSE F., SECOND G. and DELSENY M., 1992 — Characterization of new variants of a satellite DNA from *Oryza officinalis* specific for the CC genome of rice (en préparation).

ANO Y. and SANO T., 1990 — Variation of the intergenic spacer region of ribosomal DNA in cultivated and wild rice species. *Genome*, 33 : 209-218.

ECOND D., 1982 — Origin of the genic diversity of cultivated rice (*Oryza* ssp) : study of the polymorphism scored at 40 isozyme loci. *Jap. J. Genet.*, 57 : 25-57.

ECOND D., 1985 — Evolutionary relationships in the *sativa* group of *Oryza* based on isozyme data. *Genet. Select. Evol.*, 17 : 89-114.

AKAIWA F., OONO K. and SUGIURA M., 1984 — The complete nucleotide sequence of a rice 17S rDNA gene. *Nucl. Acid Res.*, 12 : 5441-5448.

AKAIWA F., KIKUCHI S. and OONO K., 1990 — The complete nucleotide sequence of the intergenic spacer between 25S ans 17S rDNAs in rice. *Plant Mol. Biol.*, 15 : 933-935.

SUNODA S. and TAKAHASHI N., 1984 — *Biology of rice. Development in Crop Science*, 7. Japan Scientific Societies Press, Tokyo.

VU T.Y. and WU R., 1987 — A new rice repetitive DNA shows sequence homology to both 5S RNA and tRNA. *Nucl. Acids Res.*, 15 : 5913-5923.

VU H.K., CHUNG M.C., WU T., NING C-N. and WU R., 1991 — Localization of specific repetitive DNA sequences in individual rice. *Chromosoma*, 100 : 330-338.

:HAO X., WU T., XIE T. and WU R., 1989 — Genome specific repetitive sequences in the genes *Oryza*. *Theor. Appl. Genet.*, 78 : 201-209.

Restriction fragment length polymorphism in pearl millet, *Pennisetum glaucum*

Chunji LIU *, John WITCOMBE **, T.S. PITTAWAY *, M. NASH *, C.T. HASH ***, and Mike GALE *

Résumé : L'analyse de 19 génotypes différents de mil a l'aide de 200 sondes d'ADN génomique à simple ou nombre réduit de copies a permis de mettre en évidence l'existence d'un polymorphisme important au sein de l'espèce, mais aussi le rôle prépondérant des phenomenes de mutation de paires de bases par rapport aux processus de délétion insertion, dans la présence de RFLP.

La comparaison intervarietale souligne, avant même l'élaboration d'une carte génétique, l'intérêt d'une utilisation des marqueurs moleculaires pour répondre à certaines interrogations fondamentales du sélectionneur. Ainsi la comparaison des cultivars ICMB 841 et 81B (ICMB 1) a révélé l'inexactitude des pedigrees publies. De meme, la comparaison de quatre hybrides F1 avec leur parents respectifs a montre que le niveau d'hetérozygotie peut être un predicteur de la performance des hybrides de mil particuliérement utile.

Mots-clés : *Pennisetum*, RFLP, sélection.

Abstract : Analysis of 19 diverse pearl millet genotypes with 200 single or low copy genomic DNA probes showed the species to be extremely polymorphic and that RFLP was due, in large part, to base-pair mutations rather than deletion insertion events.

The intervarietal comparisons indicated that, even before a genetic map is constructed, the molecular markers can be employed to resolve questions of importance for breeders. For example, comparisons involving ICMB 841 and 81B (ICMB 1) showed clearly that their published pedigrees are incorrect, and comparisons among four F_1 hybrids and their parents indicated that heterozygosity level may be a useful predictor of performance in hybrid millet.

Key-words : *Pennisetum*, RFLP, plant breeding.

* Cambridge Laboratory, Colney Lane, Norwich NR4 7UJ, UK.
** Centre for Arid Zone Studies, Bangor, Gwynedd LL57 2UW, Wales, UK.
*** ICRISAT, Patancheru, Andhra Pradesh 502 324, India.

Introduction

The utility of DNA markers to construct detailed genetic maps has been demonstrated in many crop species. The application of these maps and DNA probes in breeding research has been demonstrated for parental evaluation in F1 hybrid maize breeding (Walton and Helentjaris, 1987), analysis of genetic control of quantitative characters in tomato (Paterson *et al.*, 1988), and the provision of selection 'tags' for important qualitative genes in rice (Yu *et al.*, 1991). The new dense genetic maps have also found application in the resolution of some of the outstanding questions from classical genetic studies, such as demonstrating intergenomic relationships between maize and sorghum (Hulbert *et al.*, 1990), and between tomato and potato (Bonierbale *et al.*, 1988 ; Gebhardt *et al.*, 1991), the quantification of sex differences in recombination rates in tomato (De Vicente and Tanksley, 1991) and the description of evolutionary translocations in wheat and rye (Liu *et al.*, 1992). The use of maps in gene isolation, by providing landmarks for DNA walking, will soon be demonstrated in crop plants as has already been achieved with mammalian genes (Orkin, 1986).

The strategy for rapid development of a new map of maximum utility in breeding depends on the degree and nature of the polymorphism available. For example, a relative lack of polymorphism among tomato varieties necessitated the use of an interspecific cross (Helentjaris *et al.*, 1986 ; Bernatzky and Tanksley, 1986), while the high levels of RFLP available in maize allowed a choice of mapping populations of direct utility to breeders (Helentjaris, 1987). The predominant source of RFLP, either base-pair mutations such as in common bean (Chase *et al.*, 1991) or deletion/insertion such as in rice (McCouch *et al.*, 1988) will also determine screening strategies during map development. The experiments described below were carried out to determine these statistics in pearl millet, *Pennisetum glaucum*

Pearl millet is of major importance in low rainfall areas in Asia and Africa and there are many breeding and research applications awaiting the development of maps and polymorphic genetic marker systems.

Materials and methods

Genetic materials

A diverse range of 19 genotypes were studied, including (a) five maintainer lines, all with Tift 23B (registered as Tift 23DB$_1$, Burton 1969) in their pedigrees : Tift 23DB (registered as Tift 23 DB$_1$, Burton 1969) is a backcross derivative of Tift 23B ; 5141B, a backcross of Tift 23B with a downy mildew resistant source (Pokhriyal *et al.*, 1976 ; Dave, 1987) ; ICMB 841, reported to be a selection from variability within 5141B (Singh *et al.*, 1990) ; 81B (= ICMB 1), reported to be a mutation of Tift 23DB (registered as ICMB-1, Kumar *et al.*, 1984) ; and 843B (= ICMB 2), derived from crossing Tift 23DB and [illegible] 185642 (ICRISAT, 1985) ; (b) five restorer lines : BJ 104, H 77/833-2, ICMP 423, ICMP 451, and K 560 ; (c) four hybrids : ICMH 423 from parents ICMA 841 and ICMP 423, ICMH 451 from parents 81A (= ICMA-1) and ICMP 451, BK 560 from parents 5141A and K 560 ; and HHB 67 from parents 843A and H 77 833-2 ; and (e) five other genotypes : 700651-1, P7-3, 7042(S)-1, ICMP 85410, and (LGDI-B-10)-1 (abbreviated to LGD-1 below), selected as potential parents for mapping populations and to assess the level of polymorphism in very diverse genotypes.

Probes

A genomic library of 1000 clones was constructed with total plant DNA extracted from leaves of the pearl millet genotype 7042(S)-11. 50 µg DNA was digested with 100 U PstI to completion and subjected to electrophoresis on 1 % agarose gel. The DNA fragments in the size range of 500-3000 bp were collected using DEAE membrane. The purified fragments were ligated into the PstI site of PUC18 plasmid vectors. The *E. coli* bacterial strain DH5a was transformed and plated out on Xgal IPTG ampicillin LB plates.

Individual colonies were picked out and grown in 5 ml LB containing 10 µg ml carbenicillin. Those clones containing highly repeated sequences were identified by transferring PstI digested plasmid mini-preparations from 1 % agarose gels of Hybond N$^+$ nylon membranes, and hybridizing these with HaeIII restricted total genomic DNA. The remaining putative low copy inserts were PCR-amplified directly.

RFLP procedures

Methods for plant DNA isolation, restriction enzyme reactions, gel electrophoresis, Southern transfer, probe labelling and filter hybridization were as described by Sharp *et al.* (1988), with the modification that Hybond N$^+$ nylon membranes were used. After hybridization the membranes were first washed twice in 2xSSC and 1 % SDS for 15 min each and then followed by two washes in 0.2xSSC and 1 % SDS for 15 min each at 65 °C.

Results and discussions

Genomic clones

The use of PstI to generate the genomic DNA fragments for cloning follows the demonstration that CG-rich methylation sensitive sites are particularly effective in enriching libraries for low copy clones in several species, e.g. PstI in maize (Burr *et al.*, 1988) and rice (McCouch *et al.*, 1988) and HpaII in wheat (Cheung *et al.*, 1992). In pearl millet this strategy is particularly effective since only 11 % of the clones give significant signal when hybridized with total genomic DNA. The remaining clones, of which 200 were analyzed, detected single copy sequences (83 %), 2 to 4 copy se-