ASSESSMENT OF THE GENETIC VARIABILITY AMONG RICE CULTIVARS REVEALED BY AMPLIFIED FRAGMENT LENGTH POLYMORPHISM (AFLP)

AFLP NA IDENTIFICAÇÃO DE VARIABILIDADE GENÉTICA EM CULTIVARES DE ARROZ

Gaspar Malone[1]; Paulo Dejalma Zimmer[2]; Mauricio Marini Kopp[1]; Luiz Anderson Teixeira de Mattos[1]; Fernando Irajá Félix de Carvalho[2]; Antônio Costa de Oliveira[2].

ABSTRACT

*Considerable amounts of information are available regarding genetic variation in rice (**Oryza sativa** L.). Such information is important for constructing mapping populations and targeting the collection and the use of germplasm. The objective of this study was to evaluate the molecular variability among a representative sample of rice genotypes. AFLP analysis was applied to 56 rice genotypes (Japanese, Philippines, and Brazilian) from the germplasm bank of CGF (Centro de Genômica e Fitomelhoramento), Universidade Federal de Pelotas, Pelotas/RS, Brazil. Six primer pairs were used and they amplified 249 bands, 205 (82.32%) of which were polymorphic. Three groups of genotypes were detected: one contained five out of seven lowland genotypes, the second contained almost all Brazilian and Philippine genotypes and the third group contained six Japanese genotypes. The remaining Japanese genotypes formed unique groups. Groups obtained by Principal Components Analysis were similar to groups obtained by UPGMA.*

*Key words: **Oryza sativa**, AFLP, lowland rice, upland rice, genetic variability.*

RESUMO

*Um volume considerável de informações acerca da variabilidade genética em arroz (**Oryza sativa** L.) é disponível atualmente. Tais informações estão sendo amplamente utilizadas para a escolha de genitores na construção de populações de mapeamento e na caracterização das coleções de germoplasma existentes. O objetivo deste trabalho foi acessar a variabilidade molecular de uma amostra representativa de 56 genótipos de arroz (Japoneses, Filipinos e Brasileiros), através da técnica de marcadores AFLP. Os acessos pertencem ao Banco de Germoplasma do Centro de Genômica e Fitomelhoramento (CGF), Universidade Federal de Pelotas, Pelotas/RS, Brasil. Seis pares de primers foram utilizados, revelando um total de 249 bandas, das quais 205 (82,32%) foram polimórficas. Três grupos de genótipos foram identificados: um primeiro contendo 5 dos 7 genótipos irrigados, um segundo grupo com quase a totalidade dos genótipos Brasileiros e Filipinos e um terceiro contendo seis genótipos Japoneses. O restante dos genótipos permaneceu formando grupos únicos, com apenas um genótipo. As agrupações de genótipos obtidas mediante a análise de componentes principais concordaram significativamente com as agrupações obtidas mediante agrupamento utilizando UPGMA.*

*Palavras-chave: **Oryza sativa**, AFLP, arroz irrigado, arroz de sequeiro, variabilidade genética.*

INTRODUCTION

Evaluation on diversity levels between adapted and elite germplasm can provide predictive estimates on genetic variation among segregating progenies for developing new pure-lines (MANJARREZ-SANDOVAL et al., 1997), and may help to estimate the degree of heterosis in progenies of some parental combinations (COX & MURPHY, 1990; BARBOSA-NETO et al., 1996). Rice is a self-pollinating and semi-aquatic plant adapted to survive the submergence for a certain period. It is classified in three groups depending on the requirement and tolerance of water in highland, lowland (irrigated and rain-fed), and deepwater (TAKAHASHI, 1984).

The narrow genetic basis in irrigated rice in Brazil has been reported (GUIDOLIN, 1993; RANGEL et al., 1996). The last authors verified that all irrigated rice cultivars used in Brazil belong to the *indica* group, and were obtained by crossings between introduced lines from *CIAT (Centro Internacional de Agricultura Tropical)* and IRRI (International Rice Research Institute) which, besides using methodologies favoring endogamy, use the same genotypes repeated times as parents in crossings. It has also been reported, that only ten ancestral contributed with 68% of the genes that now compose the gene pool of the irrigated rice in Brazil.

The 1966 release of IR-8 cultivar by IRRI, being considered as the “green revolution" precursor for its novel agronomic traits such as low plant height, high tillering and mainly high grain production (PENG et al., 1999), changed deeply the rational behind breeding programs regarding the search for superior cultivars and for similar characters. Therefore, breeders started using in a massive way the IR-8 genotype and its derived lines as parents thus reducing in a drastic way the genetic variability of the populations. The excessive narrowing of the genetic basis takes the genetic uniformity and productivity to a plateau, which could bring serious problems to the Brazilian rice production, mainly the occurrence of diseases and pests.

Molecular markers can provide useful information to select divergent parents for developing both breeding and mapping populations. The AFLP (Amplified Fragment Length Polymorphism) (VOS et al. 1995) technique is an useful tool to study the relationships within members of various taxa and to determine genetic variability in many crop species as barley (RUSSELL et al., 1997; SCHUT et al., 1997), cassava (SANCHEZ et al., 1999), eucalyptus (LI, 2000), maize (LÜBBERSTEDT et al., 2000) and wheat (TYRKA, 2002). In rice, several studies on genetic variability using AFLP have been reported (CHO et al., 1997; FEDERICI et al., 2001). For all studies, AFLP detected polymorphism more efficiently than other DNA-based technologies. The measurement of genetic dissimilarity by the AFLP technique in an extensive population plays an important role regarding the choice of parents for genetic improvement.

This study was carried out to evaluate the genetic variability among 56 rice genotypes from different origins

[1] Graduate student of the PPGA / FAEM / UFPel. E-mail: gmalone@ufpel.edu.br
[2] Professor, Dr., Department of Crop Production, Faculdade de Agronomia Eliseu Maciel, Universidade Federal de Pelotas. Caixa Postal 354, CEP: 96001-970, Capão do Leão. Rio Grande do Sul – Brasil.

(Recebido para Publicação em 11/03/2005, Aprovado em 09/11/2005)

(Brazil, Philippines, and Japan), and two cultivation system (lowland and upland) by molecular characterization, using AFLP.

MATERIAL AND METHODS

Plant material

Fifty-six rice genotypes were used and are summarized in Table 1. Seeds were provided by the *CGF (Centro de Genômica e Fitomelhoramento*) from *Universidade Federal de Pelotas, RS – Brazil*. Plants were grown in field and genomic DNA was extracted in bulk (10 plants) from fresh young leaves using the CTAB method (SAGHAI-MAROOF et al., 1984).

AFLP analysis

The AFLP analysis was carried out according to the manufacturer's instruction manual (AFLP ANALYSIS SYSTEM I; INVITROGEN LIFE TECHNOLOGIES). Total DNA 100 ng was double digested with *Eco*RI and *Mse*I restriction enzymes. Six selective primer pairs were used (*M*-CAG/*E*-ACT, *M*-CAA/*E*-AGG, *M*-CTT/*E*-AAC, *M*-CAT/*E*-AGC, *M*-CTC/*E*-ACA, and *M*-CTG/*E*-AAC). Preamplification and selective amplification reactions were performed in PTC-100 termocycler in total reaction volume of 10μL. The amplification product was mixed with 6μL of loading buffer (98% formamide, 10mM EDTA, 0.025% Bromophenol Blue, 0.025% Xylene Cyanol), and denatured at 94°C for 6 minutes. The PCR product (6μL) was loaded on 6% polyacrilamide gel and the remaining was kept at minus 20°C. The electrophoresis was resolved in a manual sequencing apparatus at constant power (60W) using 1X TBE running buffer for 2h. AFLP bands were stained with Silver Nitrate according to BRIARD et al. (2000).

Table 1 – Identification number (ID), genotype name (GN), origin (O), and cultivation system (CS) for all genotypes.

ID	GN	O	CS*	ID	GN	O	CS
01	Pelota	Brazil	L	29	Catetão	Brazil	U
02	417	Brazil	L	30	Mun 1	Philippines	U
03	Firmeza	Brazil	L	31	IAC 47	Brazil	U
04	Chui	Brazil	L	32	Rusip	Philippines	U
05	El passo	Brazil	L	33	Khao xiou khay	Philippines	U
06	Taim	Brazil	L	34	Jaguari esav IAMG 19	Brazil	U
07	Taipei	Brazil	L	35	Khao sim	Philippines	U
08	Birigui	Brazil	U	36	Ketan Tlasih	Philippines	U
09	1/52/4	Brazil	U	37	khao Khane	Philippines	U
10	IPSL 462	Brazil	U	38	Khao kangkaynoi	Philippines	U
11	Amarelho Iamg 2	Brazil	U	39	Khao sin	Philippines	U
12	H 10/v7	Brazil	U	40	Seion uruchi	Japan	U
13	12-V-17	Brazil	U	41	Nakahara mochi	Japan	U
14	E-Nawm	Philippines	U	42	Toukyo hirayama	Japan	U
15	Patnai 6	Brazil	U	43	Susono mochi	Japan	U
16	Ketebei	Philippines	U	44	Miyako	Japan	U
17	Mayorly	Philippines	U	45	Aichi rikutou 1	Japan	U
18	Lakun	Philippines	U	46	Nourin 16	Japan	U
19	Bacaba	Brazil	U	47	Mino mochi	Japan	U
20	Sibakas	Philippines	U	48	Col/miyazaki/1963	Japan	U
21	IAC 5544	Brazil	U	49	Yonaoshi	Japan	U
22	Gbegbete	Philippines	U	50	Matsuyama	Japan	U
23	2/52/4	Brazil	U	51	Hiderishirazu	Japan	U
24	Dawn dawn	Philippines	U	52	Igisu mochi	Japan	U
25	IAC 1246	Brazil	U	53	Saiban	Japan	U
26	13-V-13	Brazil	U	54	Gose yonkoku	Japan	U
27	Sawak	Philippines	U	55	Shizuoka	Japan	U
28	Sew gland dong	Philippines	U	56	Oota tamasari 10 erabi 33	Japan	U

* L - Lowland cultivation system; U- Upland cultivation system.

Data collection and analyses

All polymorphic (PB) and monomorphic bands (MB) ranging between 200 bp and 1,000 bp were evaluated for each primer pair (PP). Although monomorphic bands were excluded from statistical analyses regarding the statistical test used. Presence or absence of each single fragment was coded as one or zero, respectively, in a binary data matrix. Dice coefficient (SNEATH & SOKAL, 1973) was selected to construct the similarity matrix. Cluster analysis by the UPGMA method, Cophenetic Analysis (ROHLF & SOKAL, 1981) between dendrogram and similarity matrices, and Principal Components Analysis were performed using the NTSYS pc v. 2.1 computer program (ROHLF, 2000).

RESULTS AND DISCUSSION

In the AFLP analysis (Table 2), 249 bands were scored on the 56 genotypes using six Primer pair (PP), resulting on an average of 41.5 per PP.

The total number of bands per PP varied from 28 (*M*-CTT/*E*-ACC pair) to 53 (*M*-CAT/*E*-AGC pair). The number of Polymorphic Bands (PB) varied from 22 (*M*-CTT/*E*-AAC pair) to

43 (*M*-CTC/*E*-ACA pair). Out of 249, 205 (82.4%) were polymorphic on an average of 34.1 per PP (Table 2).

Table 2 - Primer Pair (PP), Polymorphic Bands (PB), Monomorphic Bands (MB), and Total Bands (TB) for the AFLP analysis.

PP	PB	MB	TB
M-CAG/*E*-ACT	26	4	30
M-CAA/*E*-AGG	42	3	45
M-CTT/*E*-AAC	22	6	28
M-CAT/*E*-AGC	39	14	53
M-CTC/*E*-ACA	43	9	52
M-CTG/*E*-AAC	33	8	41
General Total	205	44	249
(General Total PB /TB)	82.4%		
TB/PP – General Mean			41.5
PB /PP – General Mean	34.1		

Genetic similarities among the genotypes, using Dice coefficient, ranged from 0.105 to 0.957. The cophenetic matrix value (r) (ROHLF & SOKAL, 1981) from the comparison between the dendrogram and similarity matrix was 0.95. This value, considered very representative, allows us to choose genotypes directly in the dendrogram. The average genetic similarity between all genotypes (0.570) was used to establish a cut off value for cluster formation.

The genotypes El Paso and Taim, both irrigated, showed the maximum genetic similarity (0.957) while the genotypes Oota tamasari (Japanese) and E-nawm (Philippine) showed the minimum genetic similarity (0.105). Clustering by UPGMA at Dice coefficient indicated that 16 clusters were obtained, 13 consisting of single genotypes and 3 clusters, A, B and C, containing more than one genotype (Figure 1).

The Cluster C was formed with only 6 out of 17 Japanese genotypes. Except Nakahara-mochi, all Japanese genotypes stayed outside of the former groups, indicating that they were differently enough to not be included either into cluster A or B. The remaining genotypes formed isolated groups (Figure 1).

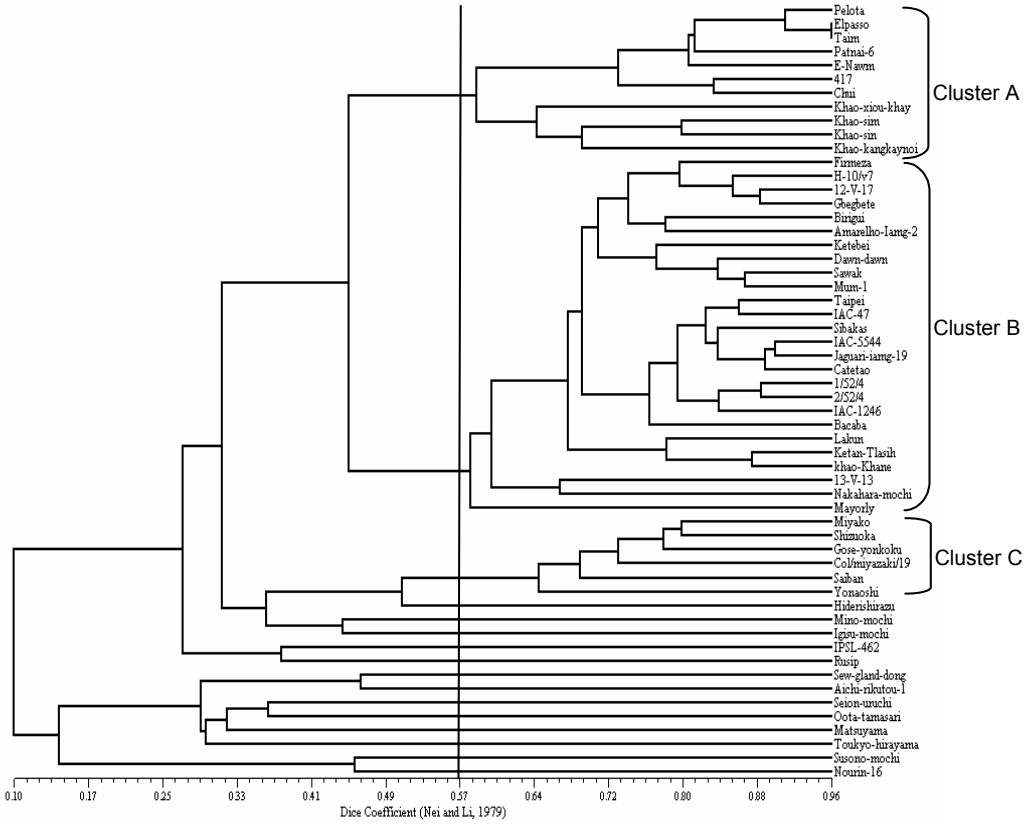

Figure 1 - Clustering analysis using the Unweighted Pair-Group Method with arithmetic Averages (UPGMA) for 56 rice genotypes included in this study. The vertical line shows the average genetic similarity among whole genotypes.

A Principal Component Analysis based on genetic distance matrix show similar cluster formation to UPGMA method (Figure 2). The first two components explained 63.9% of the total variation (data not shown). It revealed that Cluster A remained apart from the remaining clusters, while Cluster B and Cluster C showed some overlapping (Figure 2), indicating the presence of intermediate genotypes between both clusters (Cluster B - Brazilian and Philippines), (Cluster C - Japanese).

The genetic similarity detected among the Brazilian and Philippine genotypes in group A is probably due to the fact that upland rice varieties cultivated in Brazil have their genetic constitution composed of both *indica* and *japonica* sub-especies, being these traits acquired from crossings between traditional cultivars of upland rice (japonica) and lowland rice cultivars (indica).

Although Japanese genotypes did not show genetic relationship with the remaining genotypes, the genotypes Miyako, Shizuoka, Gose-yonkoku, Col/miyasaki/1963, Saiban and Yonaoshi formed a cluster within Cluster B, showing higher relationship with Brazilian and Philippine genotypes on these cluster (Figure 2).

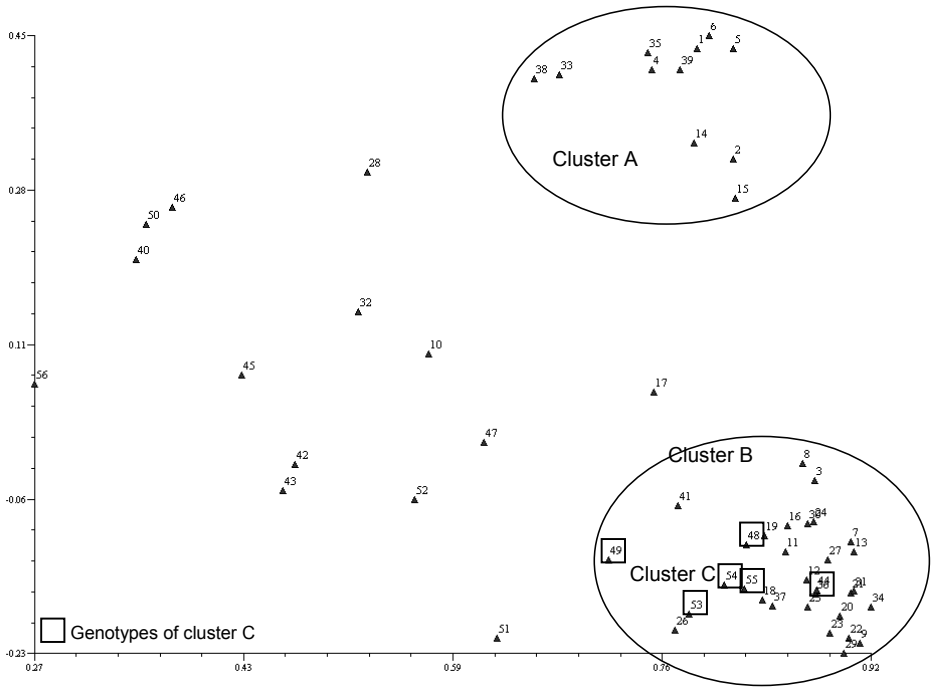

Figure 2 - Bi-dimensional classification of the genotypes by analysis of principal components obtained from the similarity matrix based on the coefficient of Nei & Li.

The results of this study showed that a narrow genetic basis is found on the lowland rice germplasm, suggesting that rice breeders have preferred convergent crosses with limited transgressive segregation, but high genetic potential. The presence of lowland genotypes in the intermediary (B) group indicates the possibility of finding genotypes with lowland profile, but more genetically distant. These may be used in planned crosses, if one wants to increase the genetic basis of Brazilian lowland germplasm.

CONCLUSION

Lowland rice germplasm in Brazil has a narrower genetic basis than upland and a collection of Japanese genotypes, indicating the recurrent use of the same parents in breeding programs.

ACKNOWLEDGMENTS

We thank to *Conselho Nacional de Pesquisa e Desenvolvimento* (CNPq), *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior* (Capes), *Universidade Federal de Pelotas* (UFPel) and FAO/IAEA for support and fellowships.

REFERENCES

AFLP Analysis System I – Cat N° 10544-013, AFLP Starter Primer Kit – Cat N° 10483-014. Life Technologies - Gibco®.

BARBOSA-NETO, J.F.; SORRELLS, M.E.; CISAR, G. Prediction of heterosis in wheat using coefficient of parentage and RFLP-based estimates of genetic relationship. **Genome**, Ottawa, v.39, p.1142–1149. 1996.

BRIARD, M.; CLERA, V.; GNZEBELUS, D. et al. Modified Protocols for rapid carrot genomic DNA extraction and AFLP™ analysis using silver stain on radioisotopes. **Plant Molecular Biology Reporter**, Athens, v.18, p.235–241. 2000.

CHO, Y.C.; GREGORIO, G.B.; KANG, K.H. et al. Fingerprinting of Korean commercial *japonica* rice cultivars based on AFLP analysis. **Rice Genetics Newsletter**, v.14, n.27, 1997.

COX, T.S; MURPHY, J.P. The effect of parental divergence on F_2 heterosis in winter wheat crosses. **Theoretical and Applied Genetics**, Berlin, v.79, p.241–250, 1990.

FEDERECI, M.T; DUNCAN, V; NORIHIKO, T; et al. Analysis of Uruguayan weedy rice genetic diversity using AFLP molecular markers. **Electronic Journal of Biotechnology.** Universidad Católica de Valparaiso – Chile, v.4, n.3, 2001.

GUIDOLIN, A. F. **Caracterização de genótipos de arroz irrigado por técnicas eletroforéticas**. Pelotas, 1993. 92p. Dissertação (Mestrado em Agronomia) - Faculdade de Agronomia "Eliseu Maciel", Universidade Federal de Pelotas.

LI, C. Estimation of genetic diversity in *Eucalyptus microtheca* populations with dominant AFLP markers. **Journal of Tropical Forest Science**, Kuala Lumpur, v.12, n.2, p.456-487. 2000.

LÜBBERSTEDT, T.; MELCHINGER, A.E.; DUBLE, C. et al. Relationships among Early European Maize Inbreds. **Crop Science**, Madison, v.40, p.783-791. 2000.

MANJARREZ-SANDOVAL, P.; CARTER, T.E.; WEBB, D.M. et al. RFLP genetic similarity estimates and coefficient of parentage as genetic variance predictors for soybean yield. **Crop Science**, Madison, v.37, p.698-703, 1997.

PENG, S.; CASSMAN, K.G.; VIRMANI, S.S. et al. Yield potential trends of tropical rice since the release of IR8 and the challenge of increasing rice yield potential. **Crop Science**, Madison, v.39, p.1552-1559. 1999.

RANGEL, P.H.N.; GUIMARÃES, E.P.; NEVES P.C.F. Base Genética das Cultivares de Arroz (*Oryza sativa* L.) irrigado do Brasil. **Pesquisa Agropecuaria Brasileira**, Brasília, v.31, n.5, p.349-357. 1996.

ROHLF, F.J. NTSYS-pc: Numerical taxonomy and multivariate **analysis system.** Version 2.1. Exeter Publications, New York, USA. 2000.

ROHLF, F.J.; SOKAL, R.R. Comparing numerical taxonomic studies. **Systematyc Zoology,** Washington, v.30, p.459-490. 1981.

RUSSELL, J. R.; FULLER, J. D.; MACAULAY, M. et al., Direct comparison of levels of genetic variation among barley accessions detected by RFLPs, AFLPs, AARs and RAPDs. **Theoretical and Applied Genetics**, Berlin, v.95, p.714–722, 1997.

SAGHAI-MAROOF, M. A.; SOLIMAN, K.M.; JORGENSEN, R.A. et al. Ribosomal DNA spacer length polymorphism in barley: Mendelian inheritance, chromosome location and population dynamics. **Proceedings of the National Academic of Science**, New York, v.89, p.1477-1481. 1984.

SANCHEZ, G; RESTREPO, S.; DUQUE, M.C. et al. AFLP assessment of genetic variability in cassava accessions (Manihot esculenta) resistant and susceptible to the cassava bacterial blight (CBB). **Genome**, Ottawav, v.42, n.2, p.163-172. 1999.

SCHUT, J. W.; QI. X; STAM, P. Association between relationship measures based on AFLP markers, pedigree data and morphological traits in barley. **Theoretical and Applied Genetics**, Berlin, v.95, p. 1161–1168. 1997.

SNEATH, P.H.A.; SOKAL, R.R. **Numerical Taxonomy- the principles and practice of numerical classification.** W. H. Freeman, San Francisco, 1973.

TAKAHASHI, N. Differentiation of ecotypes in *Oryza sativa* L. *In:* TSUNODA, S; TAKAHASHI, N. **Biology of rice**. Japan Scientific Societies Press, Tokyo, 1984,p.31–67.

TYRKA, M. A simplified AFLP method for fingerprinting of common wheat (*Triticum aestivum* L.) cultivars. **Journal of Applied Genetics**, Poznañ, v.43, n.2, p.131-143. 2002.

VOS, P.; HOGERS, R; BLEEKER, M. et al. AFLP: a new technique for DNA fingerprinting. **Nucleic Acids Research**, Oxford, v.23, p.4407–4414, 1995.