Genetic variability in wheat (*Triticum aestivum L.*) germplasm revealed by RAPD markers

Fernanda Bered*[1], José Fernandes Barbosa Neto[2], Bibiana Mariano da Rocha[1] and Fernando Irajá Félix de Carvalho[3]

[1] Departamento de Genética, Instituto de Biociências, UFRGS, Av. Bento Gonçalves 9500, pr.43323, CEP 91501-970, Porto Alegre, RS, Brazil; [2] Departamento de Plantas de Lavoura, Faculdade de Agronomia, UFRGS, Av. Bento Gonçalves 7712, Porto Alegre, RS, Brazil; [3] Departamento de Fitotecnia, Faculdade de Agronomia "Eliseu Maciel", Pelotas, RS, Brazil. (* Corresponding Author. E-mail: fbered@portoweb.com.br)

ABSTRACT

The objective of this study was to assess and cluster genetic variability in wheat germplasm in order to generate useful information for crop breeders. Fifty-four wheat genotypes from different origins and eras were evaluated based on the use of RAPD markers. Once genetic variability is detected among breeding materials, appropriate crosses can be done, speeding up breeding process and decreasing costs. Of 100 primers screened, 20 produced scorable bands, which generated 91 bands. The average genetic similarity value among all genotype pairs was 0.88, showing large genetic relationships in the wheat germplasm evaluated. The dendogram clustered the genotypes into nine groups and showed efficiency in identifying genetic variability .

KEY WORDS: Genetic similarity, molecular markers, polymorphism.

INTRODUCTION

Classical breeding methods influence the outcome of genetic variability in breeding programs for wheat. Selection for traits increases the frequency of alleles or allelic combinations with favorable effects at the expense of other traits, many of which are eventually eliminated (Cao et al., 1998). Moreover, the foundation germplasm for most classes of wheat reflects a limited number of ancestral genotypes, thus restricting its genetic basis (Chen et al., 1994; Autrique et al., 1996). Consequently, methods for the identification of crop cultivars are particularly important for modern crops where morphological differences are sometimes inadequate to discriminate genotypes (Mailer et al., 1997). Monitoring the genetic variability within the narrow pool of elite breeding material can make crop improvement more efficient by allowing for the direct accumulation of favorable alleles (Plaschke et al., 1995). Since the choice of parents is crucial in plant breeding, the detection of genetic variability among superior breeding materials can then be used to guide the appropriate crosses (Martin et al., 1995; Martin Bohn et al., 1999). The selection of superior parents can speed up the breeding process and decrease the amount of material that needs to be screened to carry out such experiments (Plaschke et al., 1995).

Molecular markers provide the potential to efficiently identify genetic relatedness (Cao et al., 1999). The random amplified polymorphic DNA (RAPD) technique has been used in many species to determine genetic variability (Lawson et al., 1994; Gunter et al., 1996; Mailer et al., 1997; Cao et al., 1999). RAPD is fast, economical, and does not require radioactive material (Mailer et al., 1997). However, some limitations of RAPD markers should be realised, such problems with nonreproducible and nonhomologous similar-size fragments (Karp et al., 1996; Bartish et al., 1999). To overcome this problem, high-quality DNA has been used and PCR conditions have been optimizated (Bartish et al., 1999). Moreover, problems with nonhomologous fragments are limited when comparisons are made within and between closely related taxa (Bartish et al., 1999; Gallois et al., 1998; Chtourou-Ghourbel et al., 2001).

The objectives of this study were to determine genetic relationships of wheat germplasm, to assess genetic variability in wheat genotypes, and to provide information about genetic similarity among the genotypes that would be useful to wheat breeders.

MATERIAL AND METHODS

Fifty-four wheat genotypes from different origins and eras were evaluated based on their RAPD markers (Table 1). DNA was isolated in bulk from the fresh leaves of 10 plants using methods described by Heun

et al. (1991) and diluted in sterile water to a working concentration of 10 ng/ml .

Polymerase chain reaction and electrophoresis

The primers used in this study were decamer oligonucleotides from Operon Technologies (Table 2). PCR was performed with a PTC-100 thermocycler (MJ Research) in a 25µl reaction volume containing 30 ng of genomic DNA, 2.5µl of 10x PCR buffer [75mM Tris HCl, 2mM $MgCl_2$, 50mM KCl, 20mM $(NH_4)_2SO_4$, 0.001% BSA], 0.5µl of 10mM dNTP mixture, 30ng of primer, one unit of *taq* polymerase (GIBCO) and milliQ water. The reaction was carried out for 3 min at 94°C, followed by 45 cycles of 1 min at 94°C (denaturation), 1 min at 36°C (annealing), 2 min at 72°C (elongation) and a final stage of 7 min at 72°C. The reaction product was mixed with 2.5µl of a stop buffer solution.

The RAPD products were separated by electrophoresis using 1.6% agarose gel in 1X TBE buffer, stained with ethidium bromide, and then visualized and photographed under UV light. Sizes of amplified products were estimated by comparing with a 100 bp DNA ladder.

Selection of primers

Initially, 100 primers were screened, using DNA from 4 cultivars. The primers that resulted in bands capable of being scored were tested against the remaining genotypes. Although faint bands were produced, only bright bands were used in this study.

Data analysis

Data were scored according to the presence or absence of amplification products. Bands were scored as 0 for absent and 1 for present. The resulting data matrix was then used to generate similarity indices between the genotypes. The SIMQUAL program of NTSYS-pc was used to calculate Jaccard's similarity coefficient: $SJ = \left(\frac{a}{n-d} \right)$ where,*a* is the number of positive matches, *n* is the sample size, and *d* is the number of negative matches. A graphic phenogram of the genetic relatedness among the 54 genotypes was produced by means of UPGMA cluster analysis. A 99% confidence interval was estimated using the normal approximation technique according Barbosa-Neto et al. (1997) in order to estimate how many

Table 1. Origin and year of release of evaluated hexaploid wheat.

Genotype	Year	Origin	Genotype	Year	Origin
Alondra 4546 (ALO)	1980	Mexico	Embrapa 40 (EMB40)	1995	Brazil
Anahuac 75 (ANA)	1981	Mexico	Frontana (FRO)	1943	Brazil
Bezoataja (BEZ)	1967	Rússia	IAC5	1966	Brazil
BH1146	1955	Brazil	IAS20	1963	Brazil
Bonaerense (BONA)	1987	Argentina	IAS54	1970	Brazil
BR14	1985	Brazil	Jacuí (JAC)	1973	Brazil
BR15	1985	Brazil	Las Rosas Inta (LRI)	1983	Argentina
BR18	1986	Brazil	Nobre (NOB)	1969	Brazil
BR23	1987	Brazil	OC16	1989	Brazil
BR32	1988	Brazil	OC18	1990	Brazil
BR34	1989	Brazil	OC22	1992	Brazil
BR35	1989	Brazil	OC8148	1984	Brazil
BR37	1990	Brazil	OC935	[1/]	Brazil
BR38	1990	Brazil	OC9511	[1/]	Brazil
BR43	1991	Brazil	OC952	[1/]	Brazil
Cajeme 71 (CAJ)	1971	Mexico	OC953	[1/]	Brazil
Caldwell (CAL)	1981	U.S.A	OC958	[1/]	Brazil
CEP 14	1985	Brazil	OC9941	[1/]	Brazil
CEP11	1984	Brazil	PEL	1978	Brazil
CEP24	1992	Brazil	PF79547	1981	Brazil
CEP27	1995	Brazil	Prointa Isla Verde (PIV)	1988	Argentina
CNT10	1977	Brazil	Prointa Oásis (PRO)	-	Argentina
Cotiporã (COT)	1965	Brazil	RS1	1984	Brazil
E7414	1967	Brazil	RS8	1991	Brazil
Embrapa 15 (EMB15)	1992	Brazil	S8020	1984	Brazil
Embrapa 16 (EMB16)	1992	Brazil	Sonora 64 (SON)	1975	Mexico
Embrapa 24 (EMB24)	1993	Brazil	Trintecinco (TRI)	1936	Brazil

[1/] inbred lines obtained in 1995.

Table 2. Sequence of primers evaluated, number of scorable bands and size range of scorable bands.

Primer	Sequence (5'-3')	N° of scorable bands	Size range of scorable bands (bp)
OPA-10	GTGATCGCAG	6	506-2036
OPA-12	TCGGCGATAG	3	506-1018
OPA-15	TTCCGAACCC	4	500-1500
OPF-1	ACGGATCCTG	7	100-1500
OPF-7	CCGATATCCC	6	350-2652
OPF-12	ACGGTACCAG	4	298-1018
OPF-14	TGCTGCAGGT	7	350-2652
OPG-4	AGCGTGTCTG	5	396-1018
OPG-6	GTGCCTAACC	7	396-1636
OPG-11	TGCCCGTCGT	4	298-1018
OPI-4	CCGCCTAGTC	3	1018-1636
OPI-8	TTTGCCCGGT	2	1018-1636
OPI-11	ACATGCCGTG	4	344-1018
OPI-13	CTGGGGCTGA	4	450-1000
OPI-15	TCATCCGAGG	4	506-1018
OPI-16	TCTCCGCCCT	1	506-1018
OPI-17	GGTGGTGATG	7	344-1018
OPI-18	TGCCCAGCCT	6	506-1636
OPI-19	AATGCGGGAG	4	506-2036
OPI-20	AAAGTGCGGG	4	396-1018
Media		**4,6**	

markers have to be used to estimate distance between different genotypes.

RESULTS

A total of 100 primers were initially evaluated, and 20 were used to generate our data matrix (Tables 2 and 3). A total of 91 bands were scored, of which 37 (40.7%) varied among genotypes. The maximum number of polymorphic bands per primer was 6 with primer OPF14 (Table 4). Examples representative of polymorphic bands are shown in Figure 1. The presence or absence of some bands were specific for genotypes (Table 5). Of 37 informative amplification products, 12 were informative in only one genotype.

Table 3. Results obtained from screening of primers.

Primers	Number
Evaluated	20
Produced faint bands	48
Did not produce amplifications	32
Total	**100**

Genetic similarity and cluster analysis

The 91 amplification products were used to calculate the genetic similarity (GS) among the 54 genotypes. The average of GS value was 0.88 for all possible pairs of genotypes and ranged from 0.68 (CEP24 and BH1146) to 1.00 (OC9511 and BR38). The confidence interval estimated for the genetic similarity value was ±0.08.

A dendrogram based on GS values grouped the genotypes into nine distinct groups using the average GS as a cut-off point (Figure 2). Genotype CNT10 grouped separately from all other genotypes, constituting Group 1; Group 2 and 3 consisted of CEP24 and Embrapa15 respectively; Group 4 included genotypes Las Rosas Inta and OC22; the composition of Groups 5 and 6 was highly heterogeneous, consisting of cultivars from different origins and eras; Group 7 clustered Bezostaya and BR23; Group 8 consisted of 3 cultivars from Embrapa and Group 9 clustered 3 genotypes from Mexico.

DISCUSSION

The narrowness of genetic basis in the modern improved wheat cultivars is widely accepted and

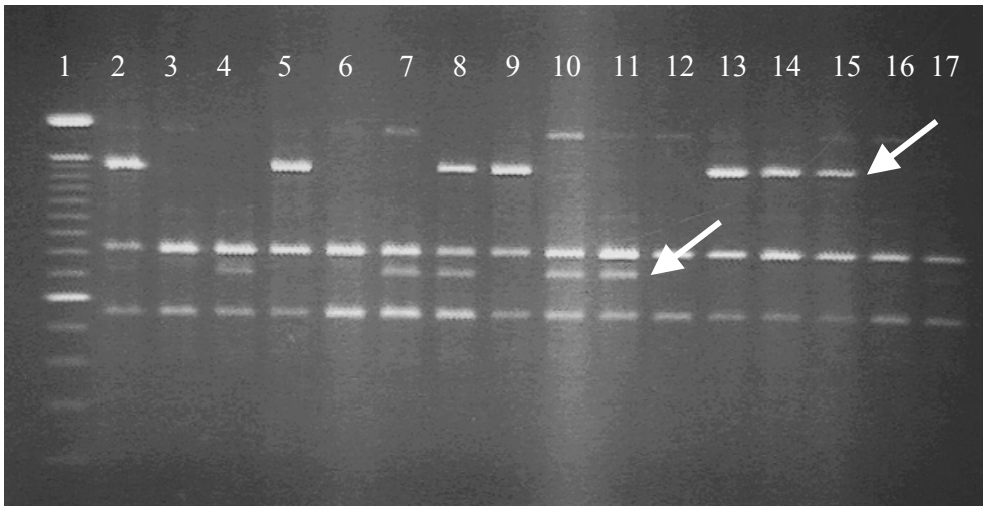

Figure 1. An example of the polymorphism detected by RAPDs using primer OPA15. Lane 1: 100 bp DNA Ladder. Lane 2: ANA. Lane 3: ALO. Lane 4: BEZ. Lane 5: BH1146. Lane 6: BON . Lane 7: BR14. Lane 8: BR15. Lane 9: BR18, Lane 10: BR23. Lane 11: BR32. Lane 12: BR35. Lane 13: BR37. Lane 14: BR43. Lane 15: CAJ. Lane 16: CAL. Lane 17: CEP11. Arrows: polymorphic fragments.

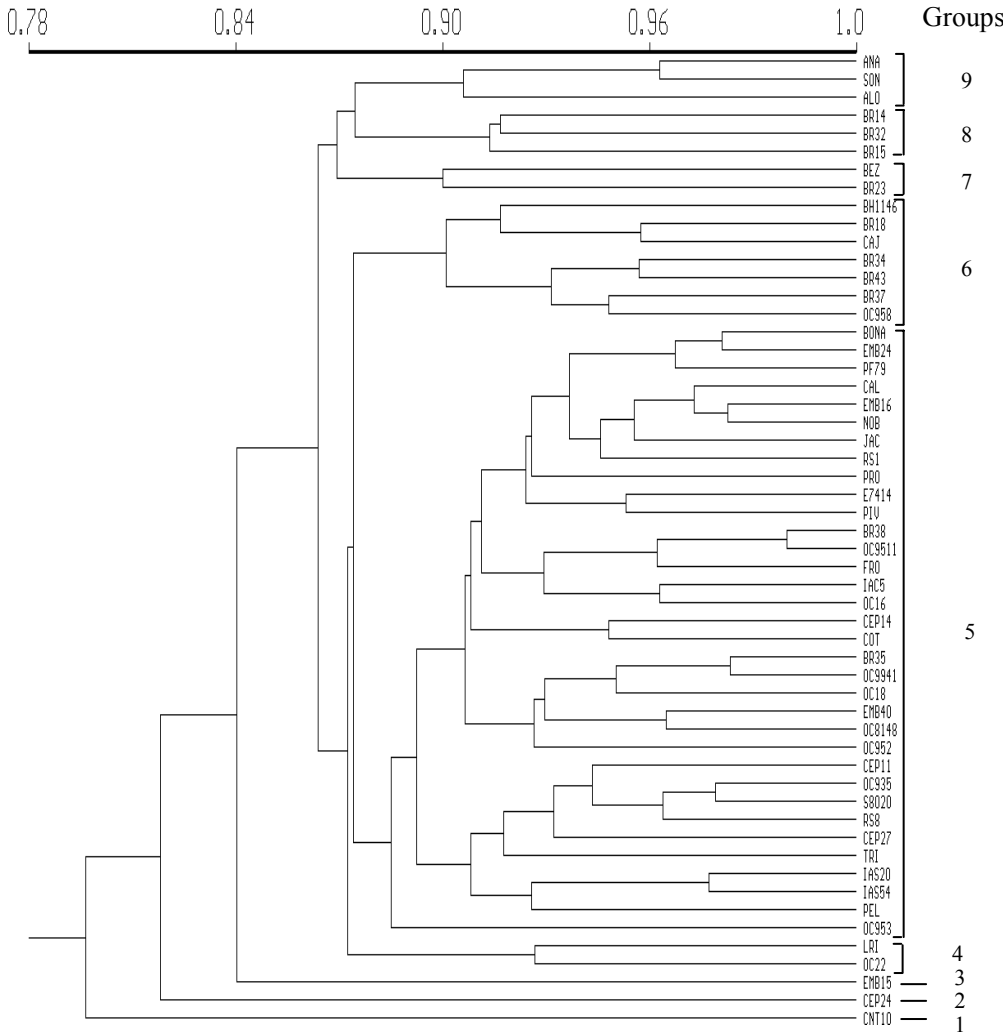

Figure 2. Dendogram obtained from similarity matrix based on Jaccard's coefficient considering RAPD markers

Table 4. Percentage of polimorphic and monomorphic bands obtained from 20 primers evaluated.

Primer	Polimorphic Bands	Monomorphic Bands	Total
OPA-10	4	2	6
OPA-12	0	3	3
OPA-15	3	1	4
OPF-1	5	2	7
OPF-7	0	6	6
OPF-12	2	2	4
OPF-14	6	1	7
OPG-4	0	5	5
OPG-6	5	2	7
OPG-11	0	4	4
OPI-4	2	1	3
OPI-8	0	2	2
OPI-11	1	2	3
OPI-13	3	1	4
OPI-15	3	1	4
OPI-16	0	1	1
OPI-17	0	7	7
OPI-18	2	4	6
OPI-19	1	3	4
OPI-20	0	4	4
Total	**37**	**54**	**91**
%	**40.7**	**59.3**	**100**

demonstrated by molecular analysis (Chen et al., 1994; Martin et al., 1995; Barbosa-Neto et al., 1996; Sun et al., 1998), studying morphological traits (Spagnoletti-Zeuli and Qualset, 1987; Souza and Sorrells, 1991; van Beuningen and Busch, 1997a) and estimating coefficient of parentage (Martin et al., 1991; Barbosa-Neto et al., 1996; Mercado et al., 1996; van Beuningen and Busch, 1997b; Burkhamer et al., 1998). RAPD analysis has been used to study di-, tetra- and hexaploid wheats of several origins, and the GS values obtained range around 0.60-0.90, which agree with our data (Vierling and Nguyen, 1992; Sun et al., 1998; Fahima et al., 1999). Molecular data frequently are highly correlated with those obtained form morphologic traits, and often poorly correlated with coefficient of parentage data. (Autrique *et al*, 1996; Barbosa-Neto et al., 1996).

In the present study, an average GS value of 0.88 was detected for all pairs of genotypes. Several authors have reported a large genetic similarity in wheat (Chen et al., 1994; Martin et al., 1995; Barbosa-Neto et al., 1996; Martin Bohn et al., 1999). The narrowness of genetic base in the modern cultivars can be demonstrated in many crops and must be related with intensive plant breeding (Chen et al., 1994). Melchinger et al. (1994) estimated a mean GS of 0.79 for unrelated pairs of barley cultivars. The high genetic similarity within switchgrass ecotypes was demonstrated by average similarity value of 81.4%, showing the lack of variability in switchgrass populations (Gunter et al., 1996).

Of 100 primers tested, only 20 yielded amplified fragments that could be scored. Joshi and Nguyen (1993) obtained 27.5% of primers that produced faint bands in tetraploid wheat. Fahima et al. (1999), studying RAPD polymorphism in *Triticum dicoccoides*, reported that the RAPD primers amplified between 5 and 15 bands per genotype, however only 5-6 of them could be scored. Problems with reproducibility of RAPD can be solved optimizing running conditions, screening primers, and discarding primers that generate inconsistent banding patterns. In addition, only strongly reproducible bands should be used in the analysis, while variable faint

Table 5. Wheat genotypes differentiated from specific fragments.

Fragmet	Size (bp)	Genotype present	Genotype absent	Total of genotypes
A10-01	1200	Embrapa 15	-	49
A10-02	980	-	Embrapa 15	49
A10-05	680	Embrapa 15	-	49
A10-06	600	-	Embrapa 15	49
A15-02	800	-	OC952	48
F01-03	1600	BH1146	-	48
F14-03	750	-	OC953	48
G06-03	900	-	CNT10	46
G06-04	810	-	CNT10	46
G06-07	430	-	CNT10	46
I13-04	450	-	BEZ	53
I18-06	600	OC952	-	46

bands must be excluded (Chowdari et al., 1998).

In this study 40.7% of polymorphic bands were found. Vierling and Nguyen (1992) reported that 40% of fragments of cultivated diploid wheat genotypes were polymorphic. According to Lawson et al. (1994), 84.2% of the total number of amplified fragments in sunflower were polymorphic. This indicates that the genetic basis of cultivated sunflower is wide and molecular markers are powerful tools for the detection of polymorphisms between varieties.

The genotype-specific fragments detected in this study can be useful in fingerprinting the varieties. The RAPD-based analysis may enhance the efficiency of searching for genotypes with unique variants from germplasm collections (Menkir et al., 1997).

The results obtained in the present study indicate the efficiency of RAPD for grouping genotypes. However, the high genetic relationship of cultivars resulted in clusters of heterogeneous groups (e.g. Groups 5 and 6) with genotypes having different origins and eras. Moreover, the molecular markers used had unknown distribution in the genome, which could have caused random concentrations in certain areas, at the expenses of others. The small confidence intervals indicate that the experimental estimates had a satisfactory level of precision.

CONCLUSIONS

The wheat genotypes evaluated had a high degree of genetic relationship considering the RAPD markers amplified.

RESUMO

Variabilidade genética em trigo (*Triticum aestivum* L.) com base em marcadores RAPD

Cinqüenta e quatro genótipos de trigo de diferentes origens e épocas foram avaliados a partir de marcadores moleculares do tipo RAPD. O objetivo desse estudo foi avaliar e agrupar a variabilidade genética existente em trigo visando gerar informações úteis aos melhoristas. Uma vez detectada variabilidade genética entre os genótipos, podem ser realizados cruzamentos mais apropriados, acelerando o processo de melhoramento e baixando custos. Dos 100 primers testados, 20 produziram bandas passíveis de escore, no total de 91. O grau de similaridade médio entre os genótipos foi de 0,88, revelando elevado relacionamento genético entre o germoplasma de trigo disponível. O dendograma agrupou os genótipos em nove grupos, demonstrando eficiência em caracterizar a variabilidade genética.

REFERENCES

Autrique, E.; Nachit, M.M.; Monneveux, P.; Tanksley, S.D. and Sorrells, M.E. 1996. Genetic diversity in durum wheat based on RFLPs, morphological traits, and coefficient of parentage. Crop Science. 36:735-742.

Barbosa-Neto, J.F.; Sorrells, M.E. and Cisar, G. 1996. Prediction of heterosis in wheat using coefficient of parentage and RFLP-based estimates of genetic relationship. Genome. 39:1142-1149.

Barbosa-Neto, J.F.; Hernández, C.M.; O'Donoughue, L.S. and Sorrells, M.E. 1997. Precision of genetic relationship estimates based on molecular markers. Euphytica. 98:59-67.

Bartish, I.V.; Jeppsson, N. and Nybom, H. 1999. Population genetic structure in the dioecious pioneer plant species *Hippophae rhamnoides* investigated by random amplified polymorphic DNA (RAPD) markers. Molecular Ecology. 8:791-802.

Burkhamer, R.L.; Lanning S.P.; Martens, R.J.; Martin, J.M. and Talbert, L.E. 1998. Predicting progeny variance from parental divergence in Hard Red Spring wheat. Crop Science. 38:243-248.

Cao, W.; Hucl, P.; Scoles, G. and Chibbar, R.N. 1998.Genetic diversity within spelta and macha wheats based on RAPD analysis. Euphytica. 104:181-189.

Cao, W.; Scoles, G.; Hucl, P. and Chibbar, R.N. 1999. The use of RAPD analysis to classify *Triticum* accessions. Theoretical and Applied Genetic. 98:602-607.

Chen, H.B.; Martin, J.M.; Lavin, M. and Talbert, L.E. 1994. Genetic Diversity in Hard Red Spring Wheat Based on Sequence-Tagged-Site PCR Markers. Crop Science. 34:1628-1632.

Chowdari, K.V.; Davierwala, A.P.; Gupta, V.S.; Ranjekar, P.K. and Govila, O.P. 1998. Genotype identification and assessment of genetic relationships in pearl millet [*Pennisetum glaucum* (L.) r. Br] using microsatellites and RAPDs. Theoretical and Applied Genetic. 97:154-162.

Chtourou-Ghorbel, N.; Lauga, B.; Combes, D. and Marrakchi, M. 2001. Comparative genetic diversity studies in the genus *Lathyrus* using RFLP and RAPD

markers. Lathyrus Lathyrism Newsletters. 2:62-68.

Fahima, T.; Sun, G.L.; Beharav, A.; Krugman, T.; Beiles, A. and Nevo, E. 1999. RAPD polymorphism of wild emmer wheat populations, *Triticum dicoccoides*, in Israel. Theoretical and Applied Genetic. 98:434-447.

Gallois, A.; Audran, J.C. and Burrus, M. 1998. Assessment of genetic relationships and population discrimination among *Fagus sylvatica* L. by RAPD. Theoretical and Applied Genetic. 97:211-219.

Gunter, L.E.; Tuskan, G.A. and Wullschleger, S.D. 1996. Diversity among populations of switchgrass based on RAPD markers. Crop Science. 36:1017-1022.

Heun, M.; Kennedy, A.; Anderson, J.A.; Lapitan, N.L.V.; Sorrells, M.E. and Tanksley, S.D. 1991. Construction of a restriction fragment lenght polymorphism map for barley (*Hordeum vulgare*). Genome.34:437-447.

Joshi, C.P. and Nguyen, H.T.1993. Application of the ramdom amplified polymorphic DNA technique for the detection of polymorphism among wild and cultivated tetraploid wheats. Genome. 36:602-609.

Karp, A.; Seberg, O. and Buiatti, M. 1996. Molecular techniques in the assessment of botanical diversity. Annals of Botany. 78:143-149.

Lawson, W.R; Henry, R.J.; Kochman, J.K. and Kong, G.A. 1994. Genetic diversity in sunflower (*Helianthus annuus* L.) as revealed by random amplified polymorphic DNA analysis. Australian. Journal of Agriculture Research. 45:1319-1327.

Mailer, R.J.; Wratten, N. and Vonarx, M. 1997. Genetic diversity amongst australian canola cultivars determined by randomly amplified polymorphic DNA. Australian Journal of Experimental Agriculture. 37:793-800.

Martin, J.M.; Blake, T.K. and Hockett, E.A. 1991. Diversity among North American Spring Barley cultivars based on coefficient of parentage. Crop Science. 31:1131-1137.

Martin, J.M.; Talbert, L.E.; Lanning, S.P. and Blake, N.K. 1995. Hybrid performance in wheat as related to parental diversity. Crop Science. 35:104-108.

Martin Bohn, H.; Utz, F. and Melchinger, A.E. 1999. Genetic similarities among winter wheat cultivars determined on the basis of RFLPs, AFLPs, and SSRs and their use for predicting progeny variance. Crop Science. 39:228-237.

Melchinger, A.E.; Granner, A.; Mahendra, S. and Messmer, M.M. 1994. Relationships among european barley germplasm: I. Genetic diversity among winter and spring cultivars revealed by RFLPs. Crop Sci. 34:1191-1199.

Menkir, A .; Goldsbrough, P. and Ejeta, G. 1997. RAPD based assessment of genetic diversity in cultivated races of sorghum. Crop Science. 37:564-569.

Mercado, L.A.; Souza, E. and Kephart, K.D. 1996. Origin and diversity of North American hard spring wheats. Theoretical and Applied Genetic. 93:593-599.

Plaschke, J.; Ganal, M.W. and Roder, M.S. 1995. Detection of genetic diversity in closely related bread wheat using microsatellite markers. Theoretical and Applied Genetic. 91:1001-1007.

Souza, E. and Sorrells, M.E. 1991. Relationships among 70 North American oat germplasms: I.Cluster analysis using quantitative characters. Crop Science. 31:599-605.

Spagnoletti-Zeuli, P.L.S. and Qualset, C.O. 1987. Geografical diversity for quantitative spike characters in a world collection of durum wheat. Crop Science. 27:235-241.

Sun, Q.; Ni, Z.; Liu, Z.; Gao, J. and Huang, T. 1998. Genetic relationships and diversity among Tibetan wheat, common wheat and European spelt wheat revealed by RAPD markers. Euphytica. 99:205-211.

Van Beuningen, L.T. and Busch, R.H. 1997a. Genetic diversity among North American spring wheat cultivars: III. Cluster analysis based on quantitative morphological traits. Crop Science. 37:981-988.

Van Beuningen, L.T. and Busch, R.H. 1997b. Genetic diversity among North American spring wheat cultivars: I. Analysis of the coefficient of parentage matrix. Crop Science. 37:570-579.

Vierling, R.A. and Nguyen, H.T. 1992. Use of RAPD markers to determine the genetic diversity of diploid, wheat genotypes. Theoretical and Applied Genetic. 84:835-838.

Received: June 10, 2002;

Accepted: October 28, 2002.