Pak. J. Bot., 42(1): 89-96, 2010.

GENETIC DIVERSITY IN WHEAT GERMPLASM COLLECTIONS FROM BALOCHISTAN PROVINCE OF PAKISTAN

ASIF ALI KHAN*, AHSAN IQBAL, FAISAL SAEED AWAN AND IFTIKHAR A. KHAN

Department of Plant Breeding and Genetics/ Centre of Agricultural Biochemistry and Biotechnology, University of Agriculture, Faisalabad, Pakistan

Abstract

Productivity of wheat varieties being bred for the last many years is stagnant in Pakistan, apparently because of the narrowed genetic base of their parental lines. As a part of the national wheat germplasm characterization programme, we examined genetic diversity among 75 accessions of wheat using RAPD markers and assessed the relationship and genetic distance between them. The accessions surveyed were comprised of landrace populations of *Triticum aestivum* L., collected from various districts of the Balochistan province of Pakistan, which is considered a reservoir of genetic diversity, particularly for wheat. The genetic similarity revealed by RAPD markers among the wheat accessions was medium to high. The accessions collected from Sibi and Pishin districts had the greatest similarity. The polymorphism revealed in the wheat accessions, appeared to be distributed with the location of collections. The high degree of similarity even among the presumably landrace material emphasises the need for the expansion of germpalsm resources and development of wheat varieties with diverse genetic background, which could substantiate the wheat breeding programmes to increase its productivity.

Introduction

Assessment of genetic variability within crop accessions has important implications in breeding and conservation of genetic resources. It is useful in the characterization of individual accessions and cultivars and, therefore, it is important to have this information for germplasm collections, to determine the range of diversity in accessions and during long-term maintenance of collections. Molecular marker system is one of the most effective methods for DNA profiling of crop genotypes and assessing genetic diversity and relatedness among them. In the breeding process, parental lines are selected from the available gene pool of contemporary varieties, and in some cases wide relatives or exotic germplasm are used to introduce a new trait, which could be tracked reliably through molecular markers (Keller *et al*., 1999; Seyfarth *et al*., 1999; Martin *et al*., 2000). Thus in addition to help assess the genetic diversity, molecular markers facilitate the identification of genes responsible for target traits and effective management of segregating and back cross populations. Depending upon the lab facilities, an array of molecular markers has been used in various crop species. Among the DNA markers, Random Amplified Polymorphic DNA (RAPD) markers have been shown to demonstrate a reasonable level of polymorphism at an affordable cost even in less equipped Labs and skimpy expertise (Rahman *et al*., 2001; Zenglu *et al*., 2001; Lawson *et al*., 2006). Therefore, the technique has a reasonable merit with practical plant breeders, who can use the technique to substantiate their phenotypic observations. To cut down the field testing cost and time, RAPD markers can be used to assess diversity in the available

*E-mail: asifpbg@hotmail.com

germplasm, to identify genes of interest and to develop a set of markers for the screening of progenies (Karp *et al*., 1998). DNA marker technology is particularly valuable for analysis of crops such as wheat with relatively low levels of genetic diversity (Roder *et al*., 1995; Korzun *et al*., 1997; Chalmers *et al*., 2001, Bai *et al*., 2003). The study presented in this paper is a part of the national wheat germplasm characterization programme, carried out under Agricultural Linkage Programme in the Department of Plant Breeding and Genetics and Centre of Agricultural Biochemistry and Biotechnology, University of Agriculture, Faisalabad, Pakistan. In the project studies, RAPD markers were used for molecular characterisation and the estimation of genetic diversity in the wheat germplasm collections from the Balochistan province of Pakistan.

Materials and Methods

Collection and multiplication of germplasm: The landrace material collected from remote areas of various districts of Balochistan consisting of 75 accessions of wheat belonging to *Triticum aestivum* L. (Table 1.) was assessed for genetic diversity. The collected germplasm was multiplied under field conditions in the Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad as the seed samples collected were too small in size.

DNA extraction: The multiplied seed of the wheat accessions was harvested and stored separately for molecular marker studies. The seed of the 75 accessions was sown in plastic pots (250 ml) and after two weeks of growth, DNA was extracted from them following the modified DNA extraction procedure developed in our laboratory (Khan *et al*., 2004). Briefly, after cutting into small pieces the leaf tissues were weighed and transferred immediately into zipper plastic bags containing 1.5ml CTAB solution. The leaf material in the bags was completely homogenized with a hand roller. After incubation, at 65°C for 30 minutes the homogenized leaf tissues (0.75 ml) were transferred into two 1.5 ml micro tubes. Equal volume (0.75ml) of chloroform: isoamylalcohol (24:1) was added and the tubes were inverted vertically 5-10 times followed by spinning at 13000rpm for 10 minutes. After centrifugation, 800ul of supernatant was transferred from both tubes into another 1.5ml micro tube. Then, approximately 700µl (0.9 volume) of isopropanol was added in the supernatant and mixed by inverting the tube about 10 times. The DNA was pelleted, washed and resuspended in 150ul of 0.1X TE. Finally the concentration of DNA was measured at 260nm in a spectrophotometer. The quality of DNA was checked by running 5µl DNA on 0.8% agarose gel prepared in 0.5X TBE buffer. The DNA samples giving smear in the gel were rejected and extracted again.

Amplification and analysis: The PCR amplifications were performed in 25 µl reactions containing 15 ng genomic DNA, 1 unit *Taq* polymerase, 3 mM $MgCl_2$, 100 mM dNTPs and 0.2 mM decamer RAPD primers. The PCR was carried out on a DNA thermocycler (Eppendorf) programmed as (95°C/5 min)1, (95°C/1 min, 36°C/1 min, 72°C/2 min)40, (72°C/10 min)1. The PCR products were separated on 1.2% agarose gel in TBE buffer with added ethidium bromide (10ng/100 ml) and agarose gels were photographed with UV light. All amplifications were repeated and only reproducible bands were scored for analysis. The wheat accessions screened for RAPD primers were scored for presence (1) and absence (0) of bands from top to the bottom of each lane. The RAPD data on the number of bands for the 75 wheat accessions thus collected was subjected to Popgene software (Version 1.44) using Unweighted Paired Group of Arithmetic Means (UPGMA) and similarity matrix Nei and Li's (1979).

Table 1. The wheat accessions collected from various districts of Balochistan.

Sr. no.	Acc. no.	Genus	Species	L. name	O. name	District name
1.	011146	*Triticum*	*aestivum*	WHEAT	PARC/SVP	Unknown
2.	011153	*Triticum*	*aestivum*		PARC/SVP	Quetta
3.	011154	*Triticum*	*aestivum*		PARC/SVP	Quetta
4.	011155	*Triticum*	*aestivum*		PARC/SVP	Unknown
5.	011160	*Triticum*	*aestivum*		PARC/SVP	Mastung
6.	011167	*Triticum*	*aestivum*		PARC/SVP	Kalat
7.	011226	*Triticum*	*aestivum*		PARC/SVP	Loralai
8.	011235	*Triticum*	*aestivum*		PARC/SVP	Quetta
9.	011240	*Triticum*	*aestivum*		PARC/SVP	Sibi
10.	011241	*Triticum*	*aestivum*		PARC/SVP	Sibi
11.	011242	*Triticum*	*aestivum*		PARC/SVP	Sibi
12.	011285	*Triticum*	*aestivum*	AABI	PARC/SVP	Pishin
13.	011286	*Triticum*	*aestivum*	SARA	PARC/SVP	Pishin
14.	011317	*Triticum*	*aestivum*	DANDAN	PARC/SVP	Noshki
15.	011318	*Triticum*	*aestivum*	DAYAK	PARC/SVP	Noshki
16.	011319	*Triticum*	*aestivum*	KHANI	PARC/SVP	Noshki
17.	011514	*Triticum*	*aestivum*		ARI-QUETTA	Quetta
18.	011516	*Triticum*	*aestivum*		ARI-QUETTA	Quetta
19.	011517	*Triticum*	*aestivum*		ARI-QUETTA	Quetta
20.	011518	*Triticum*	*aestivum*		ARI-QUETTA	Quetta
21.	011519	*Triticum*	*aestivum*		ARI-QUETTA	Quetta
22.	011520	*Triticum*	*aestivum*		ARI-QUETTA	Quetta
23.	011556	*Triticum*	*aestivum*	LISHI	PARC/ICARDA	Mastung
24.	011750	*Triticum*	*aestivum*	GHALLAM	PARC/NIAR	Kalat
25.	011751	*Triticum*	*aestivum*	GHALA	PARC/NIAR	Kalat
26.	011752	*Triticum*	*aestivum*	GHALLAM	PARC/NIAR	Khuzdar
27.	011753	*Triticum*	*aestivum*	KHOLAM	PARC/NIAR	Khuzdar
28.	011754	*Triticum*	*aestivum*	GHALLAM	PARC/NIAR	Kharan
29.	011755	*Triticum*	*aestivum*	GHALLAM	PARC/NIAR	Kharan
30.	011756	*Triticum*	*aestivum*	GHALLAM	PARC/NIAR	Kharan
31.	011757	*Triticum*	*aestivum*	GHALLAM	PARC/NIAR	Panjgur
32.	011758	*Triticum*	*aestivum*	GHALLAM	PARC/NIAR	Panjgur
33.	011759	*Triticum*	*aestivum*	GHALLAM	PARC/NIAR	Turbat
34.	011760	*Triticum*	*aestivum*	GHALLAM	PARC/NIAR	Turbat
35.	011761	*Triticum*	*aestivum*	GHALLAM	PARC/NIAR	Turbat
36.	011762	*Triticum*	*aestivum*	GHALLAM	PARC/NIAR	Khuzdar
37.	011773	*Triticum*	*aestivum*		PARC/NIAR	Khuzdar
38.	011779	*Triticum*	*aestivum*	MULKI	PARC/NIAR	Noshki

Table 1. (Cont'd.).

Sr. no.	Acc. no.	Genus	Species	L. name	O. name	District name
39.	012113	*Triticum*	*aestivum*	DAN	PARC/ICARDA	Mastung
40.	012114	*Triticum*	*aestivum*	DHAK	PARC/ICARDA	Kharan
41.	012115	*Triticum*	*aestivum*	DAYAK	PARC/ICARDA	Kharan
42.	012116	*Triticum*	*aestivum*	DAN	PARC/ICARDA	Kharan
43.	012117	*Triticum*	*aestivum*	DAYAK	PARC/ICARDA	Kharan
44.	012118	*Triticum*	*aestivum*	DAYAK	PARC/ICARDA	Kharan
45.	012119	*Triticum*	*aestivum*	SHAG	PARC/ICARDA	Panjgur
46.	012120	*Triticum*	*aestivum*	KHOLUM	PARC/ICARDA	Kharan
47.	012121	*Triticum*	*aestivum*	KHOLUM	PARC/ICARDA	Kalat
48.	012122	*Triticum*	*aestivum*	GHANAM	PARC/ICARDA	Mastung
49.	012150	*Triticum*	*aestivum*		PARC/SVP	Quetta
50.	012151	*Triticum*	*aestivum*		PARC/SVP	Loralai
51.	012153	*Triticum*	*aestivum*	KHOLUM	PARC/JICA	Loralai
52.	012154	*Triticum*	*aestivum*	KHOLUM	PARC/JICA	Quetta
53.	012156	*Triticum*	*aestivum*	KHOLUM	PARC/JICA	Quetta
54.	012158	*Triticum*	*aestivum*	KHOLUM	PARC/JICA	Quetta
55.	012159	*Triticum*	*aestivum*	KHOLUM	PARC/JICA	Mastung
56.	012160	*Triticum*	*aestivum*	KHOLUM	PARC/JICA	Mastung
57.	012161	*Triticum*	*aestivum*	KHOLUM	PARC/JICA	Mastung
58.	012162	*Triticum*	*aestivum*	KHOLUM	PARC/JICA	Mastung
59.	012163	*Triticum*	*aestivum*	KHOLUM	PARC/JICA	Mastung
60.	012170	*Triticum*	*aestivum*	KHOLUM	PARC/JICA	Mastung
61.	012171	*Triticum*	*aestivum*	KHOLUM	PARC/JICA	Mastung
62.	012172	*Triticum*	*aestivum*	KHOLUM	PARC/JICA	Mastung
63.	012173	*Triticum*	*aestivum*	KHOLUM	PARC/JICA	Mastung
64.	012174	*Triticum*	*aestivum*	KHOLUM	PARC/JICA	Mastung
65.	012175	*Triticum*	*aestivum*	GHALLA	PARC/JICA	Mastung
66.	012176	*Triticum*	*aestivum*	GHALLA	PARC/JICA	Mastung
67.	012177	*Triticum*	*aestivum*	GHALLA	PARC/JICA	Mastung
68.	012179	*Triticum*	*aestivum*	KHOLUM	PARC/JICA	Noshki
69.	012180	*Triticum*	*aestivum*	GHALLA	PARC/JICA	Noshki
70.	012181	*Triticum*	*aestivum*	GHALLA	PARC/JICA	Noshki
71.	012183	*Triticum*	*aestivum*	GHALLA	PARC/JICA	Kalat
72.	012184	*Triticum*	*aestivum*	KHOLAM	PARC/JICA	Kalat
73.	012185	*Triticum*	*aestivum*	KHOLAM	PARC/JICA	Kalat
74.	012186	*Triticum*	*aestivum*	KHOLAM	PARC/JICA	Kalat
75.	012187	*Triticum*	*aestivum*	KHOLAM	PARC/JICA	Kalat

Results and Discussions

Detection of polymorphism in single seedlings: To determine the reproducibility of the RAPD bands, three replications of the same DNA sample were screened with 23 primers, which yielded consistent amplifications. Additionally, the banding patterns of 10 individual plants within each of the five wheat accessions also revealed consistent results. DNA samples of 10 individual plants from five wheat accessions were bulked and the banding pattern revealed from the bulked samples was also consistent with that of the pattern revealed from individual plants. Ten primers screened as polymorphic in a previous study (Khan *et al*., 2005) were tested on the present material. With these primers, a total of 76 DNA fragments were observed among the 10 individual plants of the five different accessions when tested separately. Three polymorphic loci were detected which showed the genetic differentiation within a cultivar. These types of genetic changes are, however, absent when a variety is developed by tissue culturing (Fu *et al*., 2002). This methodology of detection of polymorphism in single seedling thus increased confidence for the reproducibility and the consistency of the RAPD markers (Fu *et al*., 2002). Therefore, further characterization of the accessions was carried out following the procedure described previously (Khan *et al*., 2005).

Characterisation of wheat germplasm with RAPD markers: For affecting genetic improvement through selection and breedings the presence and estimation of genetic diversity in a crop species is a prerequisite. The results reported here pertain to the estimation of genetic diversity among 75 accessions of wheat using RAPD markers. RAPD technique has been extensively used for genetic characterization of wheat (Wang *et al*., 1995; Rashed *et al*., 2008). A total of 177 DNA fragments were amplified in the 75 accessions of wheat with the 20 RAPD primers with an average of about 8.85 bands per primer (Table 2). The number of bands that a primer yielded ranged from 4 (GLA-17 and GLB-9) to 16 (GLA-20). Of the total, 142 fragments were polymorphic among the wheat accessions, which indicated ~ 80.22% polymorphism. Rest of the bands (35) were monomorphic in the wheat accessions. The maximum polymorphism was revealed by the primers GLB-9 and GLB-16, while minimum polymorphism was produced by the primer GLC-9. The wheat accessions could be distinguished with the RAPD primers. The use of abundant and high polymorphic DNA markers eliminates the limitations associated with morphological and biochemical characterization, especially for closely related varieties (Asif *et al*., 2005: Schulman, 2007).

It was clear from the dendrogram (Fig. 1) that the accession 011226 was more dissimilar genetically from the other accessions and made a separate cluster. The accessions 011226 and 012184 were found genetically most dissimilar than the other accessions and had 60% similarity.

It may be concluded from this part of the studies that a good degree of polymorphism existed in the material examined, yet the genetic similarity among the accessions was medium to high (95.51% to 56.74%). The accession 011242 collected from Sibi district and 011286 collected from Pishin district had the greatest similarity 95.51%. The accessions 011226 and 012122 both collected from Lorali district were least similar. The accession 012173 collected from Mastung district also showed least similarity with accession number 011226. Thus, the polymorphism revealed in the wheat accessions appeared to be distributed with the collection site. However, the level of genetic diversity expected in the landrace material did not appear in the collections examined.

Table 2. Selected primers and their sequence and level of polymorphism.

Sr. no.	Primer	Sequence	Total No. of bands	No. of polymorphic bands	Percentage of polymorphic bands
1	GL DecamerA-01	CAGGCCCTTC	6	5	83.33
2	GL DecamerA-05	AGGGGTCTTG	10	8	80.00
3	GL DecamerA-07	GAAACGGGTG	5	4	80.00
4	GL DecamerA-08	GTGACGTAGG	12	10	83.33
5	GL DecamerA-09	GGGTAACGCC	7	5	71.42
6	GL DecamerA-15	TTCCGAACCC	7	6	85.71
7	GL DecamerA-17	GACCGCTTGT	4	3	75.00
8	GL DecamerA-19	CAAACGTCGG	7	5	71.42
9	GL DecamerA-20	GTTGCGATCC	16	15	93.75
10	GL DecamerB-09	TGGGGGACTC	4	4	100.00
11	GL DecamerB-12	CCTTGACGCA	6	4	66.67
12	GL DecamerB-16	TTTGCCCGGA	5	5	100.00
13	GL DecamerB-19	ACCCCCGAAG	7	6	85.71
14	GL DecamerC-02	GTGAGGCGTC	13	12	92.30
15	GL DecamerC-05	GATGACCGCC	9	7	77.77
16	GL DecamerC-07	GTCCCGACGA	13	10	76.92
17	GL DecamerC-09	CTCACCGTCC	12	6	50.00
18	GL DecamerC-10	TGTCTGGGTG	12	11	91.66
19	GL DecamerC-15	GACGGATCAG	9	8	88.88
20	GL DecamerC-20	ACTTCGCCAC	13	8	61.53
Total	20	---	177	142	---
Average	---	---	8.85	7.1	80.22

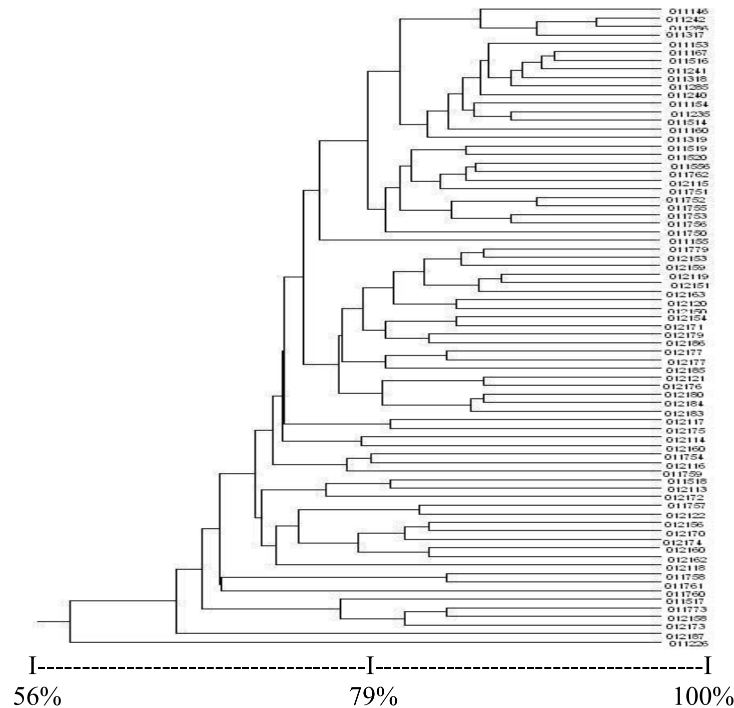

Fig. 1. Dendrogram of 75 wheat accessions collected from various districts of the Balochistan Province of Pakistan based on genetic similarities calculated from the data using 20 RAPD primers.

Conclusions

Food security through sustainable wheat production in the country is absolutely crucial for the sustenance of the people of Pakistan. However, in spite of concerted efforts by the wheat breeders, wheat yield and production is facing stagnancy in Pakistan for the last many years. The present assessment of the 'presumably' landrace material does not show the expected level of genetic diversity in the wheat germplasm even from those areas of Balochistan, which are still away from the access of the modern agriculture. It appears that the indigenous landrace material being gown in those centers of genetic diversity has been replaced by the short stature, fertilizer responsive high yielding varieties of wheat. This also appears that the genetic diversity has been drastically narrowed down after Green Revolution and same parental source has been used consistently for breeding the fertilizer responsive wheat varieties and eroded the natural variability existed in the form of landraces even in the centers of genetic diversity of wheat. Therefore, there is a need to develop wheat varieties with a diverse genetic background and augment variability into the existing wheat gene pool (Sud *et al*, 2005). Furthermore, the situation demands finding new solutions, which could substantiate the breeding programmes to increase wheat productivity. Enrichment of wheat germplasm resources through the creation of synthetic hexaploid wheat, development of high efficiency TILLING (Targeted Induced Local Lesions IN Genomes) populations and development of transgenics seems to offer hope to revamp the eroded genetic variability and generate sources of genetic variation for the development of commercial wheat cultivars.

Acknowledgement

The study was supported by PARC under Agriculture Linkage Programme and the seed supplied by PGRI, PARC, Islamabad is gratefully acknowledged.

References

Asif, M., M. Rahman and Y. Zafar. 2005. DNA fingerprinting studies of some wheat (*Triticum aestivum* L.) genotypes using random amplified polymorphic DNA (RAPD) analysis. *Pak. J. Bot*., 37(2): 271-277, 2005.

Bai, G., P. Guo and F.L. Kolb. 2003. Genetic relationships among head blight Resistant cultivars of wheat assessed on the basis of molecular markers. *Crop Sci*., 43: 498-507.

Chalmers, J.K., A.W. Campbell, J. Krestschmer, A. Karakousis, H.S. Henschke, P. Pierens, N. Harker, M. Pallotta, G.B. Cornish, M.R. Shariflou, L.R. Rampling, A. McLauchlan, G. Daggard, P.J. Sharp, T.A. Holton, M.W. Sutherland, R. Appels and P. Langridge. 2001. Construction of three linkage maps in bread wheat *Triticum aestivium* L. *Aust. J. Agric. Res*., 52: 1089-1119.

Fu, Y.B., G. Peterson, A. Diederichsen and K.W. Richards. 2002. RAPD analysis of genetic relationships of seven flax species in genus *Linum* L. *Genet. Resour. Crop Evo*., 49: 253-259.

Karp, A., P.G. Isaac and G.S. Ingram. 1998. *Molecular Tools for Screening Biodiversity: Plants and Animals*. Chapman & Hall, Thompson Sci., London.

Keller, M., B. Keller, G. Schachermayr, M. Winzeler, J.E. Schmid, P. Stamp and M.M. Messmer. 1999. Quantitative trait loci for resistance against powdery mildew in a segregating wheat × spelt population. *Theor. Appl. Genet*., 98: 903-912.

Khan, I.A., F.S. Awan, A. Ahmad and A.A. Khan. 2004. A modified mini-prep method for economical and rapid extraction of genomic DNA in plants. *Plant Mol. Biol. Repor*., 22: 89a-89e.

Khan, I.A., F.S. Awan, A. Ahmad, Y.B. Fu and A. Iqbal. 2005. Genetic diversity of Pakistan wheat germplasm as revealed by RAPD markers. *Genet. Resour. Crop Evo*., 52: 239-244.

Korzun, V., A. Borner, A.J. Worland, C.N. Law and M.S. Roder. 1997. Application of microsatellite markers to distinguish inter-varietal chromosome substitution lines of wheat (*Triticum aestivum* L.). *Euphytica*, 95: 149-155.

Lawson, W. R., K. C. Goulter, R. J. Henry, G. A. Kong and J. K. Kochman. 2006. RAPD markers for a sunflower rust resistance gene. *Aust. J. Agric. Res*., 46(4): 375-379.

Martin A., A. Cabrera, P. Hernandez, M.C. Ramirez, D. Rubiales and J. Ballesteros. 2000. Prospect for the use of *Hordeum chilense* in durum wheat breeding. Durum wheat improvement in the Mediterranean region: new challenges. In: *Proc. Sem., Zaragoza, Spain*, 12-14 April, Options Mediterranéennes. Série A, No. 40: 111-115.

Nei, N. and W. Li. 1979. Mathematical model for studying genetics variation in term of restriction endonucleases. *Acta. Hortica*., 262: 305-310.

Rahman, M. and Y. Zafar. 2001. Genotyping of a new strain of Lentil (*Lens culinaris* Medik.) by DNA fingerprinting. *Pak. J. Bot*., 33 (4): 423-428.

Rashed, M.A., M.H. Abou-Deif, M.A.A. Sallam and W.A. Ramadan. 2008. Estimation of genetic diversity among thirty bread wheat varieties by RAPD analysis. *J. Appli. Sci. Res.*, 4(12): 1898-1905.

Roder, M.S., J. Plaschke, S.U. Konig, A. Borner, M.E. Sorrels, S.D. Tanksley and M.W. Ganal. 1995. Abundance, variability and chromosomal location of microsatellites in wheat. *Mol. General Genet*., 246: 327-333.

Schulman, A. H. 2007. Molecular markers to assess genetic diversity. *Euphytica*, 158: 313-321.

Seyfarth, R., C. Feuillet, G. Schachermayr, M. Winzeler B. Keller. 1999. Development of a molecular marker for the adult plant leaf rust resistance gene *Lr35* in wheat. *Theor. Appl. Genet*., 99: 554-560.

Sud, S., N.S. Bains and G.S. Nanada. 2005. Genetic relationships among wheat genotypes, as revealed by microsatellite markers and pedigree analysis. *J. Appl. Genet*., 46: 375-379.

Wang, R. R.C., J. Cheng and L.R. Joppa. 1995. Production and identification of chromosome specific RAPD markers for durum wheat 'Langdon' disomic substitution lines. *Crop Sci*., 35: 886-888.

Zenglu, L., L. Qiub, J. A. Thompsonc, M. M. Welshd and R. L. Nelson. 2001. Molecular genetic analysis of U.S. and Chinese soybean ancestral lines. *Crop Sci*., 41:1330-1336.

(Received for publication 25 May 2009)