African Journal of Biotechnology Vol. 11(44), pp. 10170-10175, 31 May, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.2599
ISSN 1684–5315 ©2012 Academic Journals

Full Length Research Paper

Genetic diversity in Indian sub-continental landrace cultivars of the genus *Triticum* L.

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Accepted 18 April, 2012

Narrowing genetic diversity is a limiting factor in wheat breeding. Popularity of semi dwarf cultivars, developed after green revolution, has resulted in genetic erosion as they replaced indigenous cultivars derived from landraces. These old cultivars have a wealth of useful genes that can be incorporated in the modern cultivars to improve their tolerance level against biotic and abiotic stresses. Genetic analysis of these indigenous and advanced cultivars by the SSR markers has shown greater diversity in this valuable Indian sub-continental germplasm and grouped them into seven units. The *Triticum durum* lines T2 and T3 were placed in group A, whereas their counterpart T1 was quite distinct. Of the selections, T4 to T9 that was clustered in Group B, T4 to T7 were of *Triticum sphaerococcum*, whereas the other two were of *Triticum aestivum* type. *T. aestivum* cultivar C-248 also appeared to be distinct and could not be grouped with any other cultivar. Based on genetic divergence, therefore, T1 can be used for enhancing diversity in *T. durum* and C-248 in *T. aestivum*.

Key words: *Triticum*, germplasm, simple sequence repeats, genetic diversity.

INTRODUCTION

Wheat is one of the most important food crops in the world. Two species of wheat viz. *Triticum aestivum* and *Triticum turgidum* are globally cultivated. Durum wheat originated about 10,000 years ago by the fusion of two grasses while bread wheat originated as a result of hybridization between durum and a third grass species about 8,000 years ago (Ginkel and Ogbonnaya, 2007). Wheat landraces / landrace cultivars had been grown in different parts of the world before green revolution since man started wheat cultivation. These landraces have a wealth of genes for tolerance to abiotic and biotic stresses acquired by passing through evolutionary process for a long period of time. Similarly in the sub-continent, landraces were grown which were a mixture of genotypes and species. From the landraces being grown in the region, 25 different types belonging to *Triticum durum*, *Triticum sphaerococum*, and *T. aestivium* were isolated in 1909 (Table 1).

Great variations among these types were observed for their agronomic features such as plant type, grain characters and others. From theses isolations, the first improved variety T9 was released in 1911, later on, it was replaced by T11 (Rehman et al., 2009).

Through a field survey, type 8A was found superior to T11 and was released for general cultivation in 1914. Another type, 9D, which was found to be the best wheat suited for cultivation in Rain fed (Barani) areas was released in 1932.

After a few years, it was felt that no further improvement was possible through pure line selection and hybridization was resorted to, where many desirable characters present in more than one type may be combined in one variety for the creation of still better varieties. So, a number of crosses were attempted by using local germplasm. The varieties developed from these crosses were C-591, C-518, C-217, C-250, C-271 and C-273 (Table 1).

These genotypes are valuable for breeding varieties

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Table 1. *Triticum* landraces derived lines / approved varieties used in genetic diversity analysis.

***Triticum* species**	**Origin**	**Germplasm (lines / approved varieties)**
T. durum (4x)	Selection	T1, T2, T3
T. sphaerococcum (6x)	Selection	T4, T5, T6, T7
	Landrace	C-228, C-245, C-247, C-248, C-258, C-288, Local Tall, Local White
T. aestivum (6x)	Selection	T8, T9, T10, T11, T12, T13, T14, T15, T16, T17, T18, T19, T20, T21, T22, T23, T24, T25, 8A
	Hybridization	C-217 (C-516 x C-591), C-250 (Hard Federation x 9D), C-271 (C-230 x IP165), C-273 (C-209 x C591), C-518 (T9 x 8A), C-591 (T9 x 8B)

having tolerance to different abiotic stresses (Aziz, 1960). Characterization of this material, using DNA fingerprinting techniques, will provide quantitative estimates of genetic diversity and the information required for a rational utilization of germplasm in breeding programs. It has been claimed that plant breeding tends to reduce genetic diversity in elite germplasm which seriously jeopardizes any genetic improvement efforts. Genetic diversity in common wheat have been studied using different molecular markers such as random amplified polymorphic DNA (RAPDs) (Joshi and Naguyen, 1993), restriction fragment length polymorphism (RFLPs) (Siedler et al., 1994; Kim and Ward, 2000), amplified fragment length polymorphisms AFLPs (Barrett and Kidwell, 1998; Burkhamer et al., 1998), simple sequence repeats (SSR) (Masmoudi et al., 2006), sequence-tagged site (STS) (Chen et al., 1994), and intersimple sequence repeats (ISSRs) (Nagaoka and Ogihara, 1997).

Plant genome contains large number of simple sequence repeats, also termed as microsatellites (Tauz and Renz, 1984), that provide the basis for development and deployment of PCR based, multiallelic, co-dominant genetic marker system (Saghai Maroof et al., 1994). The utility of SSR markers is due to their abundant distribution, hyper-variability in the whole genome and power to distinguish between closely related genotypes. Furthermore, SSRs are highly reproducible among different laboratories to produce consensus data. A microsatellite linkage map of wheat is available (Roder et al., 1998; Liu et al., 2005) and SSR markers have been used to monitor and maintain germplasm biodiversity (Plaschke et al., 1995; Borner et al., 2000; Cordeiro et al., 2001; Iqbal et al., 2007), genome mapping (Wu and Tanksley, 1993, Roder et al., 1995) gene tagging and QTL analysis (Iqbal et al., 2007).

An analysis of the genetic diversity among different landrace cultivars can be a useful tool to get information about the genetic base of this naturally evolved material. This can contribute to a purposeful and focused utilization of the germplasm in different breeding programs.

MATERIALS AND METHODS

40 germplasm lines of *Triticum* (Table 1), acquired from Wheat Research Institute (Faisalabad, Pakistan) were used in this study. These germplasm lines were planted in pots during the normal growing season. Two or three leaves were collected from the plants after 3 weeks of growth and immediately stored at -7°C for molecular studies. DNA was extracted following the modified cetyltrimethylammonium bromide (CTAB) method (Khan et al., 2004). The genomic DNA concentration of wheat germplasm was quantified through spectrophotometer (CEC, CE 2021). The optical density (OD) of each sample was calculated. The conversion of OD to ug/ml was carried out by using simple formula, that is, OD x DX50 DNA concentration in ug/ml.

For best amplification, three different dilutions (20, 25 and 30 ng/ml) from the stock DNA samples were prepared to optimize the DNA concentration. 50 primers were used in the study for microsatellite analysis and 34 polymorphic primers (Roder et al. 1998) (gives more on sequence information) were selected to amplify the genomic DNA of wheat germplasm. The amplification reactions were performed in a total volume of 25 ul following the SSR (PCR) reaction protocol of Roder et al. (1998). Specifically, PCR reactions were performed in a volume of 25 µl, wherein the reaction mixture contained 250 nM of each primer, 0.2 mM of each deoxynucleotide, 1.5 mM $MgCl_2$, 1 unit *Taq* polymerase, and 30 ng of template DNA. Following an initial denaturation for 3 min at 94°C, 45 cycles were performed with 1 min at 94°C, 1 min at either 55, or 60°C (depending on the individual microsatellite), 2 min at 72°C, and a final extension step of 10 min at 72°C. Amplification products were fractionated on 2% agarose gel. The fingerprints were examined under ultra violet trans-illuminator and photographed using SyneGene Gel Documentation System. The data generated from the detection of polymorphic fragments were analyzed using Popgene 1.44 software (Yeh et al., 2000). All amplification products were scored as present (1) or absent (0) for each of the 40 landraces with all primers. Ambiguous bands that could not be clearly distinguished were not scored. The bands were counted from top to bottom of the lanes. Genetic similarity between all the 40 landraces was estimated by simply matching co-efficient (Sokal and Michener, 1958).

RESULTS AND DISCUSSION

In the present study, 40 *Triticum* germplasm lines were analyzed by SSR using 34 primer pairs (Table 2). Each

Table 2. Polymorphism of pimers in PCRs of 40 Triticum germplasm.

S/N	Primer name	Number of band	Polymorphic band	Polymorphism (%)
1	CWM -231	6	6	100
2	CWM -155	7	6	85.71
3	CWM-216	7	7	100
4	CWM-29	8	8	100
5	CWM-183	6	6	100
6	CWM-334	8	8	100
7	CWM-311	5	4	80
8	CWM-178	9	9	100
9	CWM-302	5	4	80
10	GWM-437	6	5	83
11	GWM-3	6	6	100
12	GWM-213	6	6	100
13	GWM-46	8	8	100
14	GWM-48	8	8	100
15	GWM-455	9	9	100
16	GWM-261	8	8	100
17	GWM-636	6	6	100
18	GWM484	5	5	100
19	GWM-102	8	8	100
20	GWM-314	5	5	100
21	GWM-190	6	6	100
22	GWM-164	4	4	100
23	GWM-448	5	4	80
24	GWM-247	6	6	100
25	GWM-57	4	3	75
26	GWM-304	3	3	100
27	GWM-210	4	4	100
28	GWM135	5	5	100
29	GWM-88	-	-	-
30	GWM-497	6	6	100
31	GWM276	6	6	100
32	GWM-296	7	7	100
33	GWM-544	4	4	100
34	GWM-194	6	6	100

primer-template yielded distinct, easily detectable bands of variable intensities. Indistinct bands produced by non-specific amplification were ignored. The bands used for fingerprinting were those that were reproducible over repeated runs, and were of sufficient intensity to detect presence or absence unambiguously.

All the tested primers amplified a total of 202 bands (amplicons ranging in size from 250 to 50 bp) for 40 *Triticum* lines, of which 194 were polymorphic, showing 96.03% overall polymorphism. Although the level of polymorphism observed was quite adequate for our research objective, use of more sensitive techniques for DNA fragment size analysis, for example, polyacrylamide gel electrophoresis or capillary electrophoresis, silver staining or using fluorescence sequencer, is also expected to reveal a higher number of amplicons and/or rate of polymorphism. The polymorphism percentage was higher than that obtained by Roder et al. (1998) from wheat genomic DNA (30%) and that by Stack et al. (2000) from wheat ESTs (50%). Our findings were in contrast to Kantety et al. (2002), but in agreement with Varshney et al. (2002). The most frequent occurrence (71%) of the motif was in the coding regions, as reported by other researchers for sugarcane (Cordeiro et al., 2001), barley (Thiel et al., 2003), rice, maize, and sorghum (Kantety et al., 2002; Varshney et al., 2002). In rice, among the more than 7000 SSRs found in predicted genes, 92% were trinucleotides and such motifs were most common (Temnykh et al., 2001; Goff et al., 2002; Yu et al., 2002).

In this study, the number of amplicons produced per line ranged from 46 to 132, with an average of 105.45

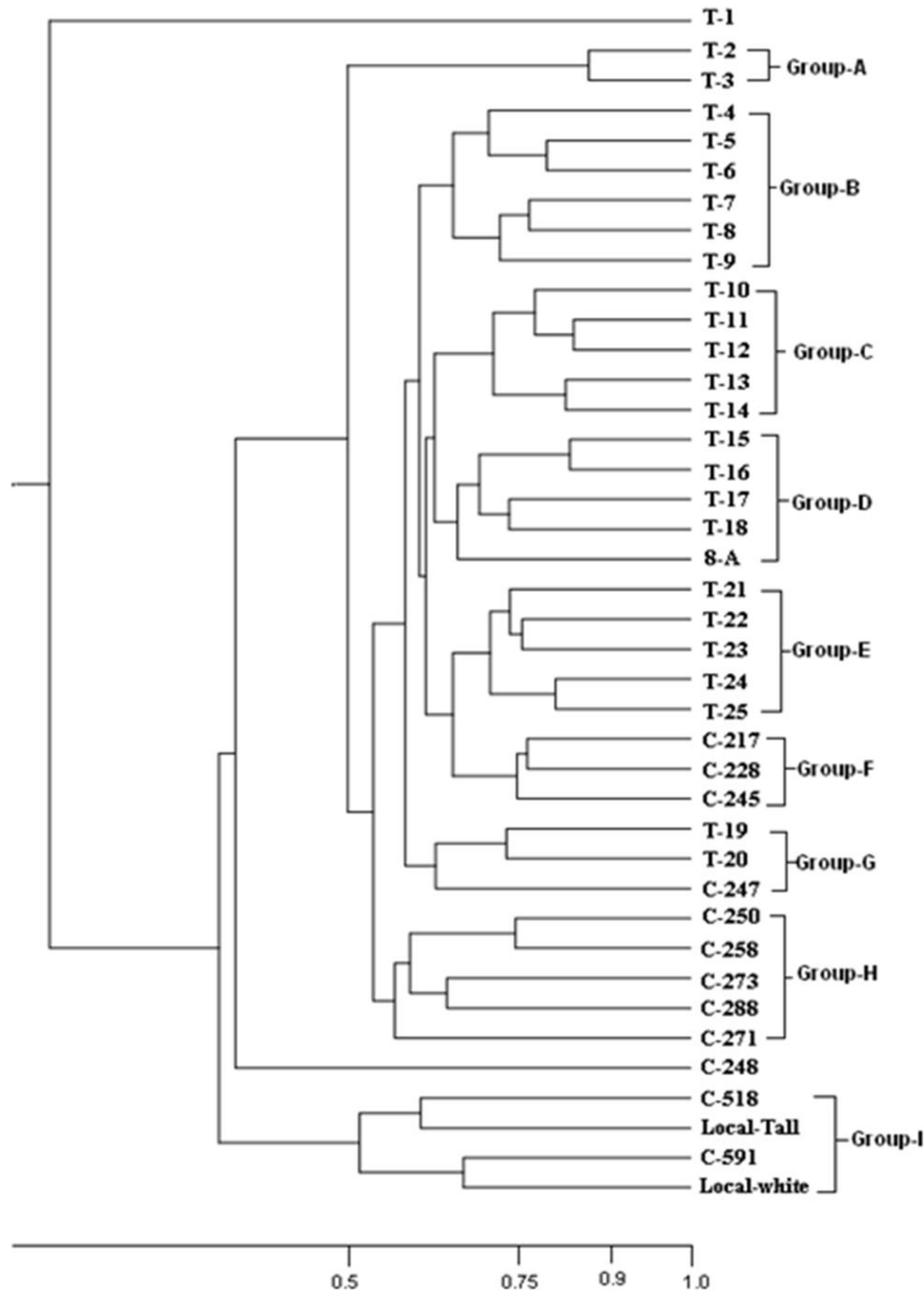

Figure 1. Dendrogram of the 40 *Triticum* germplasm lines obtained from SSR derived genetic similarity matrix.

amplicons. The maximum number of amplicons (132) was produced by the variety T11, while the minimum number (46) was produced by T1. The low degree of similarity (monomorphic bands) indicated high divergence between the germplasm lines. The number of amplification products produced per primer varied from 3 to 9 with an average of 6.02 amplicons (Table 2). The maximum number of amplicons bands was produced by the primer GWM-29, while primer GWM-577, GWM-314 and GWM-135 produced the minimum number of bands (Table 2). Multivariate analysis, to estimate genetic distance and relatedness of wheat landraces, was conducted to generate a similarity matrix using Popgene32 software, version 1.44 (Yeh et al., 2000) based on Nei's Unweighted Paired Group of Arithmetic Means Averages (UPGMA). Dendrogram drawn for the genetic distances is shown in Figure 1. Maximum similarity (87.38%) was observed between genotypes T2

and T3, while the maximum dissimilarity (36.40%) was observed between genotypes T1 and C-228.

Cluster analysis defined 9 distinct groups (A to I); Group A consisted of 2 wheat lines (T2 and T3) showing close similarity among them, GroupB consisted of 6 wheat landraces (T4, T5, T6, T7, T-8 and T-9) showing close similarity among them, GroupC consisted of 5 wheat landraces (T10, T11, T12, T13 and T-14) showing close similarity among them, Group D consisted of 5 wheat landraces (T15, T16, T17, T18 and 8-A) showing close similarity among them. In Group-D, T15, T16, T17, and T18 were clustered together while 8-A remained uncluttered showing a distinct behavior from other varieties of the Group D. Group E consisted of 5 landraces (T21, T22, T23, T24 and T25) showing close similarity among them. In Group E, T22, T23 and T24, T25 was clustered together while T21 remained uncluttered showing a distinct behavior from other wheat landraces varieties of the Group-E. Group F consisted of 3 landraces (C-217, C-245 and C-228), with C-217 and C-228 showing close similarity among them and were clustered together while C-245 remained uncluttered showing a distinct behavior from other landraces of the Group F. Group G consisted of 3 landraces (T19, T21 and C-247) with T19 and T21 showing close similarity among them while C-247 remained uncluttered showing a distinct behavior from other landraces of the Group-G. Group-H consisted of 6 landraces (C-250, C-258, C-288, C-271and C-273) with C-250, C-258, C-288 and C-273 showing close similarity among them. In Group-H, C-271 remained uncluttered showing a distinct behavior from other varieties of the Group H. Group I consisted of 4 landraces (C-518, Local tall, C-591 and Local white), with C-518, Local tall, C-591 and Local white showing close similarity among them. Interestingly, lines C-248 and T1 did not cluster with any group, showing a distinct behavior from other wheat lines.

ACKNOWLEDGEMENT

The help of Dr Yong-Bi Fu (Plant Gene Resources, Canada) is greatly appreciated for the primers used in this study.

REFERENCES

Aziz MA (1960). Cereals and Pulses, Resume of fifty years research work at Punjab Agricultural College and Research Institute, Lyallpur. Department of Agriculture, West Pakistan.

Barret BA, Kidwell KK (1998). AFLP-based genetic diversity assessment among wheat cultivars from the Pacific Northwest. Crop Sci., 38: 126-127.

Borner A, Chebotar S, Korzun V (2000). Molecular characterization of the genetic integrity of wheat (*Triticum aestivum* L.) germplasm after long term maintenance. Theor. Appl. Genet., 100: 494-497.

Burkhamer RL, Lanning SP, Martens RJ, Martin JM, Talbert LE (1998). Predicting progeny variance from parental divergence in hard red spring wheat. Crop Sci., 38: 243-248.

Chen H, Martin JM, Lavin M, Talbert LE (1994). Genetic diversity in hard red spring wheat based on sequence-tagged-site PCR markers. Crop Sci., 34: 1628-1632.

Cordeiro GM, Casu R, McIntyre CL, Manners JM, Henry RJ (2001). Microsatellite markers from sugarcane (*Saccharum* spp.) ESTs cross transferable to erianthus and sorghum. Plant Sci., 160: 115-123.

Ginkel MV, Ogbonnaya F (2007). Novel genetics diversity from synthetic wheats in breeding cultivars for changing production conditions. Field Crops Res., 104: 86-94.

Goff SA, Ricke D, Lan TH, Presting G, Wang R (2002). A draft sequence of the rice genome (*Oryza sativa*). Plant Sci., 143: 1001-1012.

Iqbal N, Firdissa E, Khlestkina K, Weidner A, Roder MS, Borner A (2007). The use of simple sequence repeat (SSR) markers to identify and map alien segments carrying genes for effective resistance to leaf rust in bread wheat. Plant Genet. Resour., 5: 100-103.

Joshi CP, Nguyen HT (1993). RAPD (random amplified polymorphic DNA) analysis based intervarietal genetic relationships among hexaploid wheats. Plant Sci., 93: 95-103.

Khan IA, Awan FS, Ahmad A, Khan AA (2004). A modified mini-prep method for economical and rapid extraction of genomic DNA in plants. Plant Mol. Biol. Repor., 22: 89a-89e.

Kim HS, Ward RW (2000). Patterns of RFLP-based genetic diversity in germplasm pools of common wheat with different geographical or breeding program origins. Euphytica, 115: 197-208.

Kantety RV, Rota ML, Mathews DE, Sorrells MS (2002). Data mining for simple seqence repeats in expressed sequence tags from barley, maize, rice sorghum and wheat. Plant Mol. Biol., 48: 501-510.

Liu ZH, Anderson JA, Hu J, Friesen TL, Rasmussen JB, Faris JD (2005). A wheat intervarietal genetic linkage map based on microsatellite and target region amplified polymorphism markers and its utility for detecting quantitative trait loci. Theor. Appl. Genet., 111: 782-794.

Masmoudi K, Rebai A, Ellouz R (2006). AFLP and SSR fingerprinting to evaluate genetic diversity among bread wheat cultivars in Tunisia. Cereal Res. Commun., 34: 871-878.

Nagaoka T, Ogihara Y (1997). Applicability of inter-simple sequence repeat polymorphisms in wheat for use as DNA markers in comparison to RFLP and RAPD markers. Theor. Appl. Genet. 94: 597-602.

Plaschke J, Ganal MW, Roder MS (1995). Detection of genetic diversity in closely related bread wheat using microsatellite markers. Theor. Appl. Genet., 91: 1001-1007.

Rehman AU, Khan MA, Muhammad F, Ahmad N, Hussain M (2009). Review of wheat breeding in Punjab-Pakistan. The Cereal Rusts, Volumes 1-2, Bushnell and Roelfs, eds. Retrieve from: http://www.globalrust.org/traction?type=single&proj.

Roder MS, Korzun V, Wendehake K, Plaschke J, Tixier MH, Leroy P, Ganal MW (1998). A microsatellite map of wheat. Genet., 149: 2007-2023.

Roder MS, Plaschke J, Konig SU, Borner A, Sorrels MS (1995). Abundance, variability and chromosomal location of microsatellites in wheat. Mol. Gen. Genet., 246: 327-333.

Roder MS, Korzun V, Gill BS, Ganal MW (1998). The physical mapping of microsatellite markers in wheat. Genome, 41: 278-283.

Siedler H, Messmer MM, Schachermayr GM, Winzeler H, Winzeler M, Keller B (1994). Genetic diversity in euorpean wheat and spelt breeding material based on RFLP data. Theor. Appl. Genet., 88: 994-1003.

Saghai Maroof MA, Biyashev RM, Yang GP, Zhang Q, Allard RW (1994). Extraordinarily polymorphic microsatellite DNA in barley: species diversity, chromosomal locations, and population dynamics. Proc. Natl. Acad. Sci. USA, 91: 5466-5470.

Stack S, Campbell I, Henderson K, Eujayl I, Hanafey M, Powell W, Wolters P (2000). Development of EST derived microsatellite markers for mapping and germplasm analysis. In: Wheat plant and animal genome conference. San Diego, California, USA, p. 102.

Sokal RR, Michener CD (1958). A statistical method for evaluating systematic relationships. Univ. Kansas Sci. Bull., 38: 1409-1438.

Tautz D, Renz M (1984). Simple sequences are ubiquitous repetitive components of eukaryotic genomes. Nucl. Acids. Res., 12: 4127-4138.

Temnykh S, Declerck D, Lukashova A, Lipovich L, Cartinhour S, McCouch S (2001). Computational and experimental analysis of microsatellites in rice: frequency, length variation, transposon association and genetic marker potential. Genome Res., 11: 1441-1452.

Thiel T, Michalek W, Varshney RK, Garner A (2003). Exploiting EST databases for the development and characterization of gene derived SSR markers in barley (*Hordeum vulgare* L.) Theor. Appl. Genet., 106: 411-422.

Varshney RK, Thiel T, Stein N, Langridge P, Garner (2002). In silico analysis of frequency and distribution of microsatellites in ESTs of some cereal species. Cell. Mol. Biol. Lett., 7: 537-546.

Wu KS, Tansksley SD (1993). Abundance, polymorphism and genetic mapping of microsatellites in rice. Mol. Gen. Genet., 241: 225-235.

Yeh FC, Yang R, Boyle TJ, Ye Z, Xiyan JM (2000). Popgene 32, Microsoft Windows Based Freeware for Population Genetic Analysis Molecular Biology and Biotechnology Center, University of Alberta, Edmonton.

Yu J, Hu S, Wong J, Wong GK, Li S (2002). A draft sequence of the rice genome. Sci., 286: 79-92.