Published online February 23, 2005

Genetic Diversity among and within CIMMYT Wheat Landrace Accessions Investigated with SSRs and Implications for Plant Genetic Resources Management

S. Dreisigacker, P. Zhang, M. L. Warburton, B. Skovmand, D. Hoisington, and A. E. Melchinger*

ABSTRACT

Many wheat (*Triticum aestivum* L.) landrace cultivars (LCs) conserved in seed banks are not sufficiently characterized to inspire breeders' interest for their efficient exploitation. Patterns of genetic variation within and among wheat LCs are usually unknown. Two sets of wheat LCs stored in CIMMYT's plant genetic resources center were assessed for genetic diversity by means of 76 (Set 1) and 44 simple sequence repeat (SSR) markers (Set 2). Set 1 included 36 LC accessions originating from different countries, either collected as bulks, composed of a single LC subline, or an unknown collection method. Set 2 consisted of three to 25 sublines of five Mexican and four Turkish LCs already included in Set 1. In a principal coordinate analysis based on modified Rogers' distance (MRD), only three Turkish LC accessions formed a distinct cluster in Set 1. The Mexican accessions clustered together with a Spanish accession and a close relationship between a Chilean and Nigerian accession was observed. In Set 2, gene diversity (H_e) among the Turkish LCs (0.43) was higher than among the Mexican LCs (0.35). Analyses of molecular variance (AMOVA) revealed considerable genetic diversity within Mexican (52.7%) and within Turkish (67.6%) LCs. Pairwise fixation indices (F_{ST}) were significant, except between two Turkish LCs. Results were discussed in relation to the most suitable collection method of wheat LCs (bulk or individual sublines) as well as to the use of SSRs as a tool for seed bank management.

Wheat landraces are genetically diverse and dynamic populations but are still morphologically recognizable because of a certain integrity (Harlan, 1975). Thousands of landrace cultivars (LCs) in wheat are stored in seed banks worldwide but the majority is inadequately described for an efficient exploitation in plant breeding. High costs and time-lags associated with the extensive search for useful characteristics lead to the fact that breeders rarely resort to these genetic resources (Gollin et al., 2000). Subsequently, intensive prebreeding approaches are required to transfer desired genes from an unimproved LC material into advanced breeding lines (Skovmand and Rajaram, 1990).

Landrace cultivars undoubtedly represent an important source of genetic variation in wheat. One of the prime examples is the use of *Rht* dwarfing genes that became available through the Japanese wheat 'Norin 10', derived from the LC Shiro Daruma (Kihara, 1982). Two important genes, *Rht1* and *Rht2*, were observed to directly effect yield because of reduced lodging. Moreover, a considerable LC diversity was found for resistance to pests such as stem rust (caused by *Puccinia graminis* Pers.: Pers. f. sp. *tritici* Eriks. & E. Henn.), leaf rust [*P. recondita* Roberge ex Desmaz. f. sp. *tritici* (Eriks. & E. Henn.) D.M. Henderson], or Russian wheat aphid (*Diuraphis noxia* Mordv.) (Skovmand and Rajaram, 1990; Skovmand et al., 1994), and for tolerance to abiotic stresses like heat (Hede et al., 1999; Skovmand et al., 2001).

With a few exceptions, all evaluations for desired traits in wheat LCs were done in ex situ collections. Examinations included either a random bulk of LC genotypes or the collections of LC sublines. Preliminary evaluation data were usually recorded during the first seed multiplication and consisted of observations that were highly heritable, easily detectable, and expressed in different environments (DeLacy et al., 2000). However, little information about the genetic variation within LCs and associations among LC accessions is available. It is also still questionable which strategy is the best to ensure an appropriate maintenance of this variation for future generations.

Molecular markers can support a more detailed characterization of genetic resources. A vast potential lies in their ability to identify the structure of genetic diversity within and among accessions, which can be of great importance for the optimization of collections, the planning of seed regeneration, and the successful implementation of prebreeding approaches. Molecular markers provide a direct measure of genetic diversity and go beyond indirect diversity measures based on agronomic traits or geographic origin. Simple sequence repeats are highly polymorphic in wheat and, therefore, suitable for the discrimination of genotypes. They are generally genome specific, abundant, codominant, and cover all 21 wheat chromosomes. They have been successfully employed to characterize genetic diversity in seed bank collections of improved wheat germplasm (Börner et al., 2000; Huang et al., 2002) and wild relatives (Li et al., 2000; Hammer, 2000).

The objectives of our study were to (i) determine SSR-based genetic diversity among and within two sets of hexaploid wheat LCs stored in the plant genetic resources center of CIMMYT, (ii) compare the form of conservation in bulks and individual plant collections, which were applied to maintain these LCs, and (iii) evaluate the use of SSRs as a tool to improve the management of wheat genetic resources.

S. Dreisigacker and A.E. Melchinger, Inst. of Plant Breeding, Seed Science, and Population Genetics, Univ. of Hohenheim, 70593 Stuttgart, Germany; P. Zhang, M.L. Warburton, and D. Hoisington, CIMMYT, Mexico D.F., Mexico; B. Skovmand present address: Nordic Gene Bank, P.O. Box 41, SE-230 53 Alnarp, Sweden. Received 24 Nov. 2003. Crop Breeding, Genetics & Cytology. *Corresponding author (melchinger@uni-hohenheim.de).

Published in Crop Sci. 45:653–661 (2005).

677 S. Segoe Rd., Madison, WI 53711 USA

Abbreviations: AMOVA, analysis of molecular variance; F_{ST}, pairwise fixation indices; H_e, gene diversity; LC, landrace cultivar; MB, multiple bands; MRD, modified Rogers' distance; PC, principal coordinate; PCoA, principal coordinate analysis; SSR, simple sequence repeat.

Table 1. Name, CIMMYT accession number, country of origin, form and year of collection, and storage of 36 landrace cultivar (LC) accessions of wheat.

Number	LC	CIMMYT accession	Country	Collection form	Collection year	Year of storage†
			Africa			
1	Tchere	CWI32617	Chad	unknown	unknown	1990
2	Alkana	CWI32616	Chad	unknown	unknown	1990
3	Aethiopicum 1B.18.16	CWI21968	Ethiopia	unknown	unknown	1987
4	Aethiopicum 400	CWI21966	Ethiopia	unknown	unknown	1987
5	Abyssinia 1	CWI9819	Ethiopia	unknown	1962	1989
6	Dikwa 1	CWI74748	Nigeria	bulk	1970s	1999
			Asia			
7	Pissi Khawri	CWI65257	India	LC subline	unknown	1990
8	LPG 1	CWI28879	Nepal	bulk	1950s	1990
9	Kharkovskaya 2	CWI51805	Russia	unknown	1989	1993
10	Shorewaki	BW20313	Pakistan	unknown	unknown	1995
11	86PK1317	CWI28659	Pakistan	LC subline	1986	1990
12	86PK1271	CWI28683	Pakistan	LC subline	1986	1990
			West Europe			
13	Gentil Bianco	CWI42611	Italy	unknown	unknown	1992
14	Barbela	CWI10618	Portugal	unknown	1967	1989
15	Barbela 0248	CWI7874	Spain	unknown	unknown	1990
16	Cologgne Abastrado 11660	CWI17849	Spain	unknown	unknown	1990
17	Barbilla	CWI17538	Spain	unknown	unknown	1990
18	Blanquillo-de-Badajoz	CWI17542	Spain	unknown	unknown	1990
			Turkey			
19	AK Bugday	CWI11215	Turkey	unknown	1969	1989
20	Yayla 305	CWI41983	Turkey	bulk	1985	1992
21	Yilmaz 1	CWI32653	Turkey	LC subline	1985	1991
22	Yilmaz 11	CWI32659	Turkey	LC subline	1985	1991
23	AK 702	CWI11164	Turkey	bulk	unknown	1989
24	84TK520.001.01	CWI28416	Turkey	LC subline	1984	1984
25	84TK523.006.02	CWI28421	Turkey	LC subline	1984	1984
26	84TK538.002.02	CWI28427	Turkey	LC subline	1984	1984
27	84TK567.001	CWI28013	Turkey	LC subline	1984	1984
			Central America			
28	Pillon	CWI31398	Mexico	LC subline	1990	1990
29	Barbon	CWI31424	Mexico	LC subline	1990	1990
30	Quartito	CWI31470	Mexico	LC subline	1990	1990
31	Caña Morado	CWI31499	Mexico	LC subline	1990	1990
32	Tzumutaro	CWI31604	Mexico	LC subline	1990	1990
33	Crillo GTM National V	CWI74755	Guatemala	unknown	unknown	
			South America			
34	Trigo Blanco	CWI59547	Chile	unknown	unknown	1995
35	Trigo Africano	CWI12244	Chile	unknown	unknown	1989
36	Trigo Azul	CWI27062	Chile	unknown	unknown	1990

† Year since the accessions were placed in CIMMYT's plant genetic resources center for storage.

MATERIALS AND METHODS

Plant Materials

The collection and maintenance of wheat LCs in seed banks is conducted either in bulks or as individual plant collections. Bulks are usually created as a random sample of spikes per LC, harvested and threshed together in one bag. Individual plant collections are composed of a number of LC sublines, whose seeds are kept separately.

Two sets of germplasm were used to analyze the genetic variation of hexaploid wheat LCs stored in CIMMYT's plant genetic resource center. Set 1 included 36 LCs accessions, either collected as a bulk, composed of a single LC subline, or of an unknown collection method (Table 1). Set 2 consisted of supplementary individual plant collections of five Mexican and four Turkish LCs already included in Set 1 (refer to Table 2 for the names and available number of sublines per LC).

The LC accessions in Set 1 were chosen because they expressed several characteristics of particular interest to breeders (e.g., salt tolerance, zinc, or flooding tolerance). The individual plant collections of the Mexican LCs in Set 2 were collected by B. Skovmand, in Michoacan, Mexico in 1989 in cooperation with the Mexican Organization for the Study of Biodiversity (Skovmand et al., 1992). The collections were performed within the framework of a larger collection mission at 219 Mexican sites. It was assumed that the LCs, still commercially grown at the time of collection, were introduced from Spain in about 1550. The individual plant collections of the Turkish LCs in Set 2 were collected in 1984 by R. Metzger, together with researchers from the Turkish Ministry of Agriculture. Collection sites were located in the mountain regions of Hakkari, in southeast Turkey (Skovmand et al., 1994). Since the beginning of their storage at CIMMYT, all wheat LC accessions have been regenerated once, by sowing 100 seeds per accession.

SSR Analyses

Genomic DNA of each LC accession in Set 1 was extracted from fresh leaves of 10 to 12 randomly selected seedlings by a modified CTAB (cetyltrimethylammonium bromide) method (Hoisington et al., 1994). Quality and quantity of the isolated DNA was determined on 1% (w/v) agarose gels by comparing bands to known concentrations of lambda DNA. Equal quantities of eight DNA samples per LC accession were bulked together. For Set 2, genomic DNA was extracted from each LC subline, applying the same method.

Table 2. Number of sublines per landrace cultivar (LC), average number of alleles per locus, percentage of heterozygosity, number of unique alleles, monomorphic loci, and gene diversity (H_e) in each of five Mexican and four Turkish LCs.

LC	Number of sublines	Average number of alleles per locus†	Heterozygosity	Unique alleles†	Monomorphic loci	H_e
			Mexican LCs			
Pillon	20	1.9	2.6	27.3	15	0.31
Barbon	24	2.1	4.9	24.5	10	0.37
Quartito	17	1.9	1.6	37.0	12	0.27
Caña Morado	25	2.3	1.9	41.3	8	0.41
Tzumutaro	13	2.3	1.7	45.9	14	0.41
Total/mean	99	4.6	2.5	–	5	0.35
			Turkish LCs			
84TK523.006.02	4	1.4	2.5	46.9	20	0.49
84TK538.002.02	7	1.4	5.0	33.5	10	0.55
84TK567.001	6	1.4	3.1	19.8	14	0.44
84TK567.002	3	1.5	0.0	7.2	32	0.20
Total/mean	20	3.7	2.7	–	6	0.43

† Standardized values calculated by resampling ten sublines per Mexican and two per Turkish LC without replacement. The mean was then calculated from 5000 repetitions.

A total of 76 SSRs was applied to fingerprint the LC accessions in Set 1. On the basis of these results the 44 most polymorphic SSRs, equally distributed over the entire genome, were selected for the analyses of Set 2. Simple sequence repeat information was provided by the Institute of Plant Genetics and Crop Plant Research, Gatersleben, Germany (Röder et al., 1998; Röder, unpublished data, 2000) and DuPont (Wilmington, DE) (Eujayl et al., 2002; DuPont, unpublished data, 2001). In addition, the marker *WMC56* developed by the Wheat Microsatellite Consortium (Agrogene, France) was used. Information on map location, repeat type, annealing temperature, fragment sizes, number of alleles, as well as polymorphic information content for each SSR is available at http://www.cimmyt.org/english/webp/support/publications/support_materials/ssr_mw1.htm (verified 14 Nov. 2004). PCR amplification and allele detection were performed with an ABI-Prism Sequencer 377 in combination with the computer software GeneScan 3.1 and Genotyper 2.1 (PerkinElmer Biotechnologies, Foster City, CA), as described in detail by Dreisigacker et al. (2004).

Statistical Analyses

The proportion of SSRs showing multiple bands (MB) was determined to estimate the genetic variation of each accession in Set 1. The presence of MB indicates that for a given SSR more than one allele was observed, which may reflect residual heterozygosity and/or segregation at the respective SSR marker. Ordinary *t* tests were calculated to compare the observed genetic variation of LC accessions composed of bulks or single LC sublines (SAS Institute, 1990).

For the comparison of the LCs in Set 2, which was based on different numbers of LC sublines, standardized average numbers of observed alleles per locus and standardized numbers of unique alleles were calculated. Standardized values were computed by resampling 10 sublines per Mexican and two per Turkish LC and taking means over 5000 repetitions. Gene diversity of each Mexican and Turkish LC was calculated according to Nei (1973).

Analyses of molecular variance (AMOVA) were conducted on the basis of SSR data to divide the genetic variation in Set 2 into components attributable to variance components among and within LCs. Pairwise fixation indices were determined to estimate the extent of LC isolation by distance within the two countries, Mexico and Turkey. Significance levels were computed by permuting sublines between LCs.

Modified Rogers' distance was calculated for each pairwise combination in Set 1 and Set 2 according to the following equation (Wright, 1978):

$$MRD = \sqrt{\frac{1}{2m}\sum_{i=1}^{m}\sum_{k=1}^{a_i}\left(p_{ij} - q_{ij}\right)^2},$$

where p_{ij} and q_{ij} are the allele frequencies of the jth allele at the ith marker; a_i refers to the number of alleles at the ith marker; and m is the number of SSRs. The allele frequencies of the accessions in Set 1 were estimated on the basis of the peak area and height of each band in the electrophoresis detected by GeneScan 3.1. Standard errors of the MRD estimates were obtained by a bootstrap procedure with resampling 1000 times over markers (Weir, 1996). Principal coordinate analyses (PCoA) were performed on the basis of the MRDs to visualize the dispersion of genotypes in Set 1 and Set 2 (Gower, 1966).

The AMOVA and pairwise F_{ST} values were calculated by the software package Arlequin (Schneider et al., 2000). All other analyses were performed by applying the Plabsim software (Frisch et al., 2000), which is implemented as an extension of the statistical software R (Ihaka and Gentleman, 1996).

RESULTS

Genetic Diversity among 36 Wheat Landrace Cultivar Accessions

The 76 SSRs assayed in Set 1 resulted in a total of 419 alleles, with 11 SSRs detecting monomorphic bands. The average number of alleles per locus accounted for 6.0 alleles with a minor variation among the three genomes (Table 3). Most of the SSR loci of the LC accessions were homozygous. On average 10.0% of the SSRs showed MB.

In Set 1, SSRs amplifying more than two distinct al-

Table 3. Number of SSRs and alleles per locus, as well as percentage of SSRs with multiple bands (MB) per accession determined for the three genomes in 36 landrace cultivar (LC) accessions of wheat.

Genome	No. of SSRs	Alleles per locus		SSRs with MB per accession†	
		Average	Range	Average	Range
A	20	5.9	1–13	11.5	0.0–47.1
B	27	6.8	2–16	9.0	0.0–48.0
D	24	5.8	1–17	9.5	0.0–45.8
Total	76‡	6.0	1–17	10.0	0.0–44.9

† MB for a given SSR in one accession reflect to residual heterozygosity and/or segregation.
‡ Genome location of 5 SSRs was unknown.

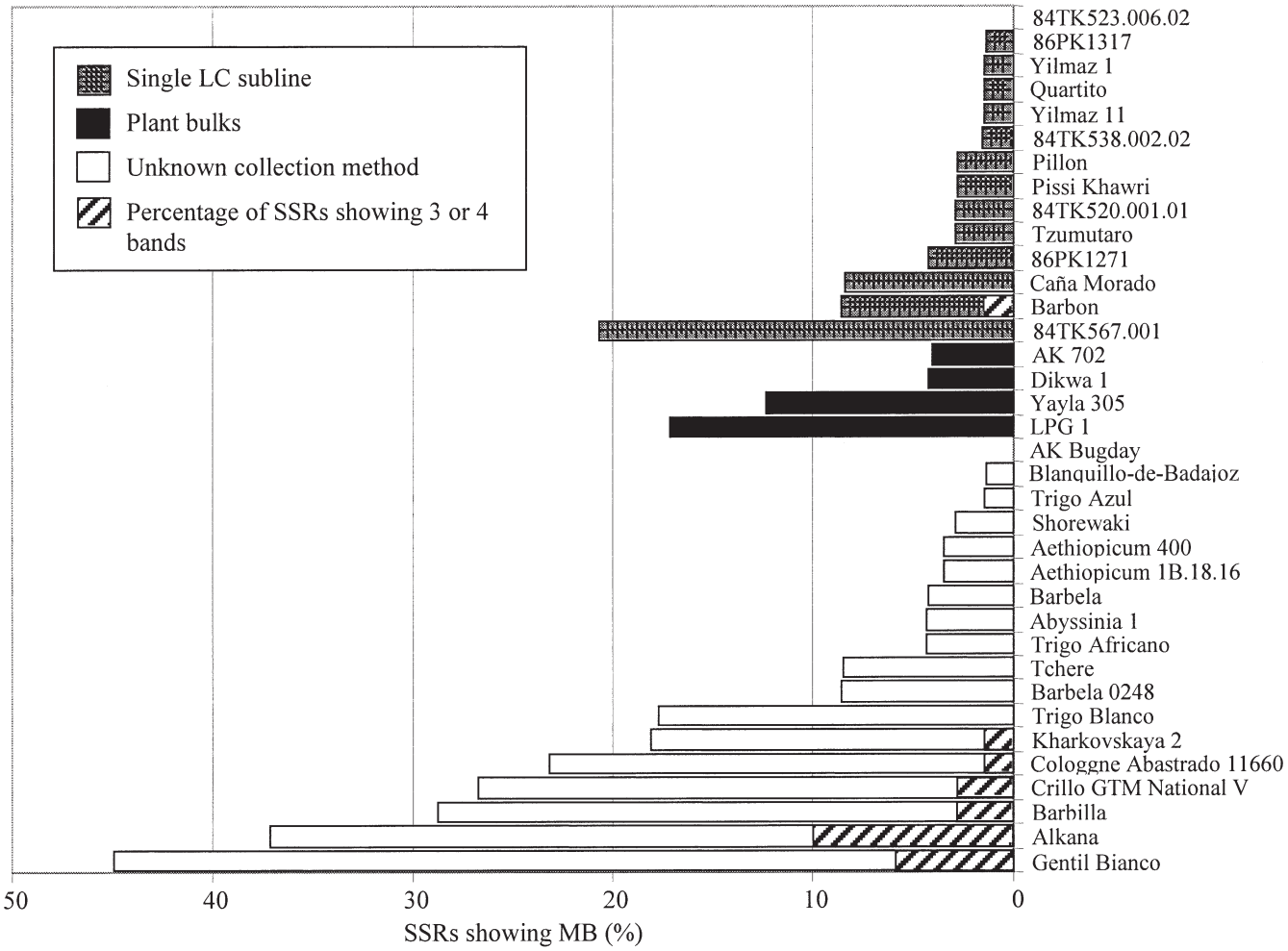

Fig. 1. Proportion of SSR loci with multiple bands (MB) determined in each of 36 landrace cultivar (LC) accessions of wheat.

leles per SSR were found in LC accessions with generally high allelic variation. Accession 27 (84TK567.001) showed a fairly high proportion of SSRs with MB (20.6%), although it was based on a single LC subline (Fig. 1). The mean proportion of SSRs with MB did not significantly differ ($P < 0.05$) between accessions composed of bulks and single sublines.

The MRD between LC accessions of Set 1 averaged 0.69. The lowest MRD value (0.16) was observed between the LC accessions Barbela and Barbela 0248 and the highest value (0.82) between the LC accessions Aethiopicum 400 and Yilmaz 1. Standard errors of MRD estimates ranged from 0.02 to 0.06. In the PCoA based on MRD estimates, the first three principal coordinates (PC) explained 8.7, 7.8, and 6.9% of the total variation, respectively (Fig. 2). The accessions did not group according to their continent or country of origin for the most part. Three Turkish accessions (84TK520.001.01, 84TK523.006.02, and 84TK567.001) formed a distinct cluster. The accessions from Mexico and Guatemala, were separated together with accession 18 (Blanquillo-de-Badajoz) from Spain and accession 5 (Abyssinia 1) from Ethiopia on the basis of PC3. A close relationship was revealed between accession 35 (Trigo Africano) originating from Chile and accession 6 (Dikwa 1) originating from Nigeria (MRD = 0.18).

Genetic Diversity within and between Mexican and Turkish Landrace Cultivars

Tzumutaro and Caña Morado were the most diverse (H_e = 0.41) Mexican LCs in Set 2 on the basis of the high average number of alleles per locus and the number of unique alleles (Table 2). The lowest number of unique alleles was observed in Barbon, which was still highly diverse (H_e = 0.37) becaue of heterozygosity. Among the Turkish LCs in Set 2, gene diversity was highest (H_e = 0.55) in 84TK538.002.02. Only three LC sublines were available from 84TK567.002, which revealed 32 monomorphic and no segregating loci.

In the AMOVA, 18.4% of the total variance was found between the combined populations of Mexican vs. Turkish LCs in Set 2. Considering exclusively Mexican LCs, the variance within the populations accounted for 52.3%. All Mexican LCs were significantly ($P < 0.05$) different from each other, whereas corresponding pairwise F_{ST} values ranged from 0.37 to 0.68 (Table 4). Variance within was twice as large (67.6%) than between the Turkish LCs. The highest F_{ST} value (0.62) was found between

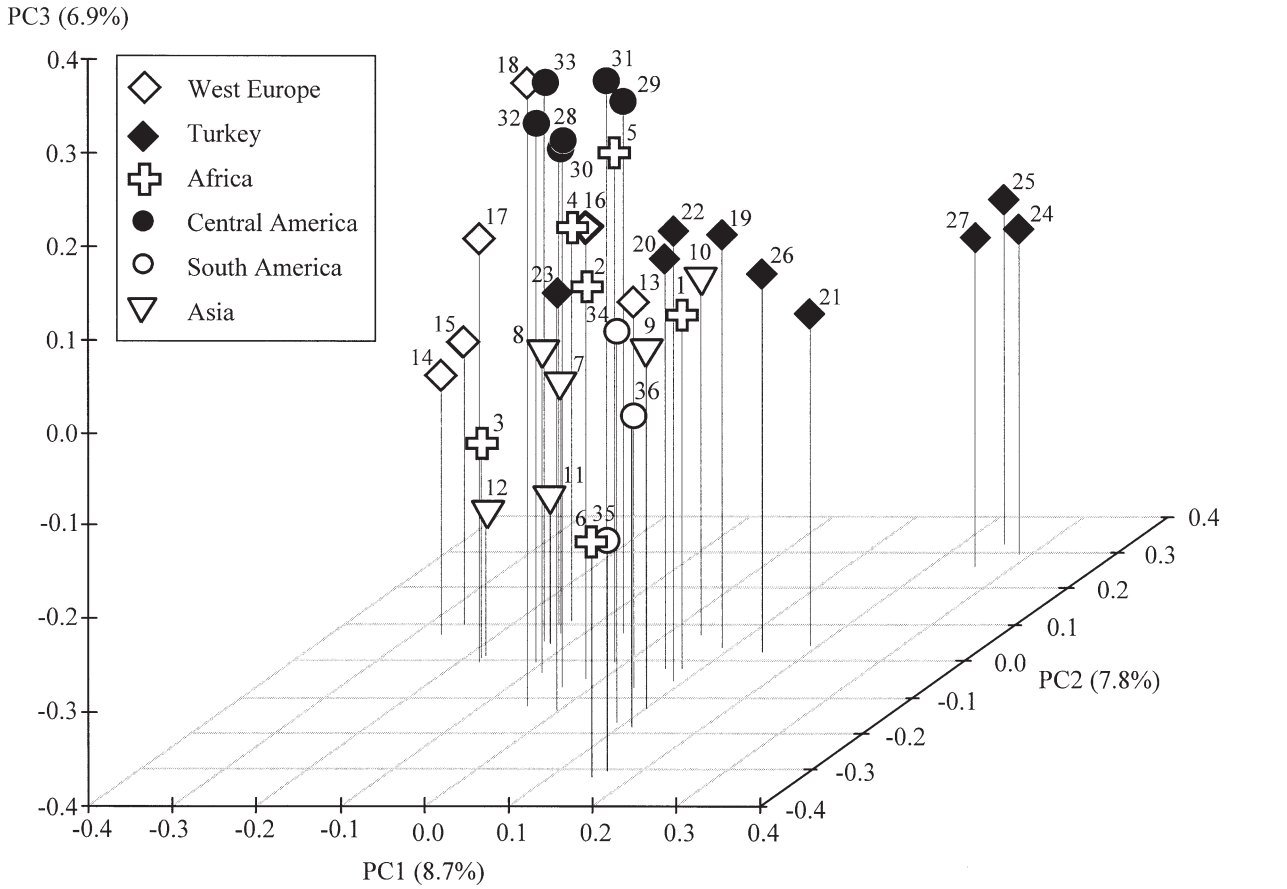

Fig. 2. Associations among 36 landrace cultivar (LC) accessions of wheat revealed by principal coordinate analysis (PCoA) performed with modified Rogers' distances (MRD) calculated from 76 SSRs. Numbers refer to the list in Table 1. Geographic origin is designated by symbols (see legend).

84TK523.001.01 and 84TK567.002 by far the smallest value (0.07) between 84TK.567.001 and 84TK.567.002. A considerably higher range of F_{ST} values was observed for the Mexican LCs.

Principal coordinate analysis revealed a clear grouping among the Mexican LCs with the exception of two sublines of Quartito that were located outside its main cluster and closer to sublines from other LCs (Fig. 3A). However, mean genetic distances of these two sublines to the LC main cluster were smaller (MRD = 0.72 and 0.82) than the maximum genetic distance within the main cluster (MRD = 0.83). Groupings of the Turkish LCs were less clear (Fig. 3B) as reflected by the larger variation within the Turkish LCs in the AMOVA. Sublines of 84TK538.002.02 and 84TK567.001 were widely dispersed and did not form a single main cluster.

DISCUSSION

Wheat Landrace Cultivar Diversity

The genetic variability of LCs has been affected by various factors throughout their evolutionary history. In autogamous crops, outcrossing and fitness-relevant mutations generate an intrapopulation diversity, whereas directed natural or human selection and bottleneck effects lead to an increase in interpopulation diversity (Ennos, 1983).

In our study, a considerable genetic diversity was revealed within rather than between Mexican and Turkish LCs. A surprisingly high intrapopulation diversity seemed to be in contrast to the high selfing rate of wheat but was consistent with previous results found in Pakistani wheat LCs analyzed with protein markers (Tahir et al., 1996) and Italian LCs of emmer [*Triticum turgidum* L. subsp. *dicoccum* (Schrank ex Schübl.) Thell.] analyzed with RAPDs (Barcaccia et al., 2001). The higher diversity observed within the Turkish than within the Mexican LCs can be explained by a much longer evolutionary history of wheat in Turkey. Furthermore, wheat LCs

Table 4. Pairwise fixation index (F_{ST}) for five Mexican and four Turkish landrace cultivars (LCs).

	LC			
LC	**1**	**2**	**3**	**4**
	Mexican LCs			
1: Pillon				
2: Barbon	**0.50***			
3: Quartito	**0.68***	**0.42***		
4: Caña Morado	**0.50***	**0.37***	**0.50***	
5: Tzumutaro	**0.47***	**0.37***	**0.54***	**0.39***
	Turkish LCs			
1: 84TK523.001.01				
2: 84TK538.002.02	**0.31***			
3: 84TK567.001	**0.41***	**0.17***		
4: 84TK567.002	**0.62***	**0.38***	**0.07**	

*** Significant at the 0.05 probability level.**

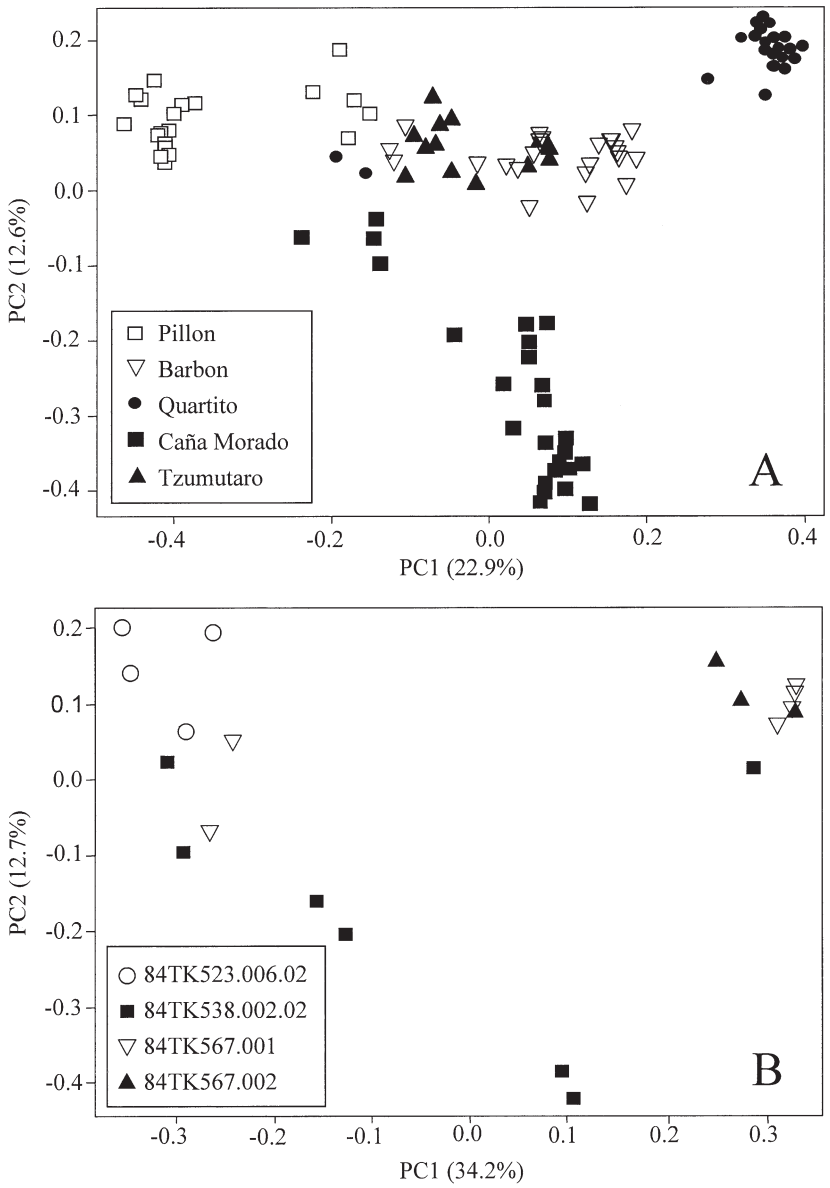

Fig. 3. Associations among five Mexican (A) and four Turkish (B) landrace cultivars (LC) of wheat revealed by principal coordinate analyses (PCoA) performed with modified Rogers' distances (MRD) calculated with 44 SSRs. The LCs consisted of 3 to 25 sublines.

or varieties mainly transferred from Spain to the New World were presumably limited in population size, thus resulting in a founder effect.

The LCs in Set 1 were not grouping according to their continent or country of origin in the PCoA (Fig. 2). We speculate that many LCs analyzed in our study were relatively late in history transferred from the Near East or Europe to other parts of the world and/or environmental adaptation changed their genetic composition only little. The Turkish LCs, which formed a distinct cluster, were collected in the primary center of diversity of wheat in proximal locations in Hakkari, Turkey. All three LCs show resistance to Russian wheat aphid. As expected, the two LCs Barbela and Barbela 0248 were closely related, the latter being considered as a subrace of Barbela, a very old Portuguese LC showing impressively wide adaptation to different environments, in particular high acid soil and drought tolerance. The Chilean LC Trigo Africano clustered together with the African LC Dikwa 1, which was collected in a small homonymous region in the northeast of Nigeria (Zeven, 1974). The Spanish name Trigo Africano directly refers to the continent of origin, Africa, but not necessarily to the Nigerian region. In view of the lack of historical records, a larger number of accessions per country should be fingerprinted before drawing any firm conclusions about the evolutionary relationships of accessions.

Diversity within wheat LCs rests more on the allelic variation between individual plants than on heterozygous individuals. In our study, this was reflected by a low mean of heterozygosity (2.6%) observed in the sublines of Mexican and Turkish LCs, which was similar to the mean (2.5%) reported for improved lines from CIMMYT's wheat breeding program (Dreisigacker et

al., 2004). An exception was the Turkish LC accession 27 (84TK567.001), which was supposedly composed of a single subline, but showed an extremely high percentage of SSRs (20.6%) with MB (Fig. 2). This high variation might be due to outcrossing, seed contamination, or experimental errors. Employing the formula of Crow and Kimura (1970, p. 93), our estimate of mean heterozygosity corresponds to 1.3% outcrossing rate and is thus slightly higher than reported in the literature (Martin, 1990; Hucl, 1996). This outcrossing rate is sufficient to generate off-types by contamination with foreign pollen. Outcrossing might also explain why some sublines of the Mexican and Turkish LCs were positioned separately from their main clusters in the PCoA (Fig. 3). Furthermore, the Turkish LCs could be intercrossed with wild species, such as goatgrass [*Triticum tauschii* (Coss.) Schmal.], which are still widely grown in the mountain regions of Hakkari (Braun et al., 2001).

Bulk versus Individual Plant Conservation

In early expeditions of genetic resources acquisition, collections of bulks were preferred since the prime focus was to collect as much material as possible in a short time and to cover widely diverse geographic regions. Collecting individual plants separately was first advocated by Bennett (1970) and later reinforced by Ford-Lloyd and Jackson (1986). On one hand, the conservation in bulks offers the advantage of including seed of many different plants, which prevents a dramatic reduction in the original population size and simplifies the procedure of sampling and conservation (Frankel, 1977; Marshall, 1990). On the other hand, the presence of different genotypes makes a precise characterization of bulks difficult. Bulk accessions must therefore be "de-bulked" or evaluated on a larger scale before the best individuals are identified and used in prebreeding programs.

We observed a low molecular variation in LC accessions conserved as bulks. In general, the variance of genetic diversity measures increases with reduced numbers of examined genotypes (Weir, 1996). The variance of gene diversity in the Turkish LCs of Set 2 was higher than in Mexican LCs, the former being composed of only three to seven LC sublines (Table 2). The regeneration procedure at CIMMYT, where only 100 seeds are sown per accession, could be an additional reason for the low molecular variation observed in the bulk accessions. Small effective population sizes lead to the risk of losing molecular variation during seed regeneration. Major threats are genetic drift and selection as shown in previous studies on barley (*Hordeum vulgare* L.) (Parzies et al., 2000), rice (*Oryza sativa* L.) (Gao et al., 2000), and rye (*Secale cereale* L.) (Chwedorzewska et al., 2002). Therefore, larger samples for seed regeneration are recommended in the literature. Assuming a population with 20 000 polymorphic loci and two alleles per locus, Lawrence et al. (1995) concluded that about 172 plants are sufficient to conserve nearly all alleles with frequencies not lower than 0.05. According to Crossa and Vencovsky (1999), for 5 to 100 loci and 2 to 20 alleles per locus, between 105 and 335 plants per population are required to maintain alleles at a 5% frequency. Some of the bulk accessions in our study were probably subsamples received from or shared with cooperators. These samples usually contain only 100 to 200 seeds, which could be another reason for a loss of variation.

In individual plant collections, alleles are usually fixed in each accession. Because of their uniformity, the accessions can be more precisely characterized and, hence, exploitation by breeders may proceed more rapidly (De-Lacy et al., 2000). Its disadvantages are extensive space and labor costs essential for conservation and seed regeneration. The genetic variation within individual plant collections directly depends on the number of collected sublines. The Mexican and Turkish LCs in Set 2 might therefore represent only a part of the variation present in the original LCs. Indigenous knowledge of LCs would be extremely useful for the optimization of the sampling of sublines of each particular LC (for a review see Zeven, 2002).

Both ex situ conservation methods maintain only a part of the original LCs genetic variation and disregard their integrity. For instance, low input agriculture relies on the buffering effect of LCs, which is responsible for their broad adaptation but requires the intact original level of diversity. Thus, a combination of both conservation forms could be a reasonable solution: the storage of (i) a large bulk to preserve the natural state of the LC variation in a simple manner, and (ii) separate LC sublines representing potentially useful variants for breeding programs.

Implications of SSR-Based Genetic Diversity for Seed Bank Management

Currently some of the limiting factors in the use of LC ex situ collections are (i) missing or incomplete passport data, and (ii) the precise characterization of the collections. Passport data were not available for half of the 36 CIMMYT wheat LC accessions used in our study. Most collecting expeditions were of such a short duration that it was difficult to locate and interview all relevant landowners at the collection sites. Additionally, personnel, management, and political changes in seed banks may have contributed to the incompleteness of the records. Molecular markers may provide new and reliable information for the description and optimization of LC collections in seed banks.

The increasing costs to efficiently manage large ex situ collections encourage curators to identify redundant germplasm accessions. Verifying duplications is complex, because their definition can vary from "accessions with similar passport data" to "identical genotypes" (Hintum, 2000). Suspected duplicates were identified in collections of sorghum [*Sorghum bicolor* (L.) Moench] and barley by means of 15 and 35 SSRs in combination with passport data and AMOVA as a biometrical tool (Dean et al., 1999; Lund et al., 2003). In our study, 84TK567.001 and 84TK567.002 were assumed to be closely related, because of adjacent collection sites. Applying AMOVA, these LCs showed nonsignificant differences, further strengthening the notion to manage these two individual

collections as one LC. However, in wheat, which is particularly suitable for seed storage, conserving either new or existing accessions in perpetuity (including regeneration in 25-yr intervals, germination tests, etc.) is currently still more cost-effective than DNA fingerprinting even with a relatively small number of SSRs (Dreher et al., 2000; Pardey et al., 2001). Thus, the identification and removal of suspected duplicates should not be considered as the main role of molecular screenings in seed bank collections.

The assessment of LC variability in seed banks demands large-scale screenings of collections. According to Zhang et al. (2002), 300 to 400 alleles are required to reflect stable relationships between wheat accessions and effectively establish core collections. In our study, 256 alleles detected with 44 SSRs (two SSRs per chromosome) were sufficient to differentiate individual genotypes of Mexican and Turkish LCs. Moreover, half of the SSRs applied were developed from expressed sequence tags, which are generally less polymorphic but might reflect functional diversity more accurately. Future opportunities to combine these markers and phenotypic data in association studies may narrow the search for new alleles at loci of interest (Thornsberry et al., 2001).

In conclusion, SSRs provide important information about the genetic variation of wheat LCs and demonstrate a powerful tool for the future tasks of seed bank management. However, the high costs warrant the further optimization of SSR application. The standardization of molecular methods, for instance, would allow to coordinate collections of different seed banks and the incorporation of new technologies like GPS, to relate the molecular diversity with their geographic dispersion.

ACKNOWLEDGMENTS

The authors are indebted to E. Villordo and L. Diaz for their technical assistance, to M. van Ginkel and H. Parzies for critical reading of the manuscript, as well as to the Vater & Sohn Eiselenstiftung, Ulm, and the Federal Ministry for Economic Co-operation and Development (BMZ) for their financial support and collaboration. This paper is dedicated to Prof. Dr. H.H. Geiger on the occasion of his 65th birthday, whose teaching and research were most influential on the careers of the first and corresponding authors.

REFERENCES

Barcaccia, G., L. Molinari, O. Porfiri, and F. Veronesi. 2001. Molecular characterization of emmer (*Triticum diccon* Schrank) Italian landraces. Genet. Resour. Crop Evol. 49:415–426.

Bennett, E. 1970. Tactics of plant exploration. p. 157–179. *In* O.H. Frankel and E. Bennett (ed.) Plant genetic resources in plants: Their exploration and conservation. F.A. Davis Co., Philadelphia.

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

Braun, H.J., N. Zencirci, F. Altay, A. Atli, M. Avci, V. Eser, M. Kambertay, and T.S. Payne. 2001. Turkish wheat pool. p. 851–879. *In* A.P Bonjean and W.J Agnus (ed.) The world wheat book: A history of wheat breeding. Lavosier Publishing, Paris.

Chwedorzewska, K.J., P.T. Bednarek, and J. Puchalski. 2002. Studies on changes in specific rye genome regions due to seed aging and regeneration. Cell. Mol. Biol. Lett. 7:569–576.

Crossa, J., and R. Vencovsky. 1999. Sample size and variance effective population size for genetic resource conservation. FAO/IBPGR Plant Genet. Resour. Newsl. 119:15–25.

Crow, J.F., and M. Kimura. 1970. An introduction to population genetics theory. Harper & Row, New York.

Dean, R.E., J.A. Dahlberg, M.S. Hopkins, S.E. Mitchell, and S. Kresovich. 1999. Genetic diversity and redundancy among 'Orange' accessions in the U.S. national sorghum collection as assessed with simple sequence repeat (SSR) markers. Crop Sci. 39:1215–1221.

DeLacy, I.H., B. Skovmand, and J. Huerta. 2000. Characterization of Mexican wheat landraces using agronomically useful attributes. Genet. Resour. Crop Evol. 47:591–602.

Dreher, K., M.L. Morris, M.M. Khairallah, J.M. Ribaut, S. Pandey, and G. Sirinivasan. 2000. Is marker-assisted selection cost-effective compared to conventional plant breeding methods? p. 203–236. *In* R.E. Evenson and D. Zilberman (ed.) Economic and social issues in agriculture biotechnology. CABI Publishing, CAB International, Wallingford, UK.

Dreisigacker, S., P. Zhang, M. van Ginkel, M. Warburton, D. Hoisington, M. Bohn, and A.E. Melchinger. 2004. SSR and pedigree analyses of genetic diversity among CIMMYT wheat lines targeted to different mega-environments. Crop Sci. 44:381–388.

Ennos, R.A. 1983. Maintenance of genetic variation in plant populations. p. 129–155. *In* M.K. Hecht et al. (ed.) Evolutionary biology. Vol. 16. Plenum Press, New York.

Eujayl, I., M.E. Sorrells, M. Braun, P. Wolters, and W. Powell. 2002. Isolation of EST-derived microsatellite markers for genotyping the A and B genomes of wheat. Theor. Appl. Genet. 104:399–407.

Ford-Lloyd, B., and M. Jackson. 1986. Plant genetic resource: An introduction to their conservation and use. Eduard Arnold, London.

Frankel, O.H. 1977. Genetic resources. *In* P.R. Day (ed.) The genetic basis of epidemics in agriculture. Ann. N.Y. Acad. Sci. 287:332–334.

Frisch, M., M. Bohn, and A.E. Melchinger. 2000. Plabsim: Software for simulation of marker-assisted backcrossing. J. Hered. 91:86–87.

Gao, L.Z., A. Schaal, C.H. Zhang, J.Z. Jia, and Y.S. Dong. 2000. Assessment of population genetic structure in common wild rice *Oryza rufipogon* Griff. using microsatellite and allozyme markers. Theor. Appl. Genet. 106:173–180.

Gollin, D., M. Smale, and B. Skovmand. 2000. Searching an *ex situ* collection of wheat genetic resources. Am. J. Agric. Econ. 82:812–827.

Gower, J.C. 1966. Some distance properties of latent root and vector methods used in multivariate analysis. Biometrika 53:325–388.

Harlan, J.R. 1975. Our vanishing genetic resources. Science (Washington, D.C.) 188:618–621.

Hammer, K. 2000. Microsatellite markers—A new tool for distinguishing diploid wheat species. Genet. Resour. Crop Evol. 47:497–505.

Hede, A.R., B. Skovmand, M.P. Reynolds, J. Crossa, A.L. Vilhelmsen, and O. Stolen. 1999. Evaluating genetic diversity for heat tolerance traits in Mexican wheat landraces. Genet. Resour. Crop Evol. 46: 37–45.

Hintum, T.J.L. 2000. Duplications within and between germplasm collections. III. A quantitative model. Genet. Resour. Crop Evol. 47:507–513.

Hoisington, D., M. Khairallah, and D. Gonzalez-de-Leon. 1994. Laboratory protocols: CIMMYT Applied Molecular Genetics Laboratory. 2nd ed. (Available online at http://www.cimmyt.org/ABC/Protocols/manualABC.html; verified 14 Nov. 2004). CIMMYT, Mexico, D.F.

Hucl, P. 1996. Out-crossing rates for 10 Canadian spring wheat cultivars. Can. J. Plant Sci. 76:423–427.

Huang, X.Q., A. Börner, M.S. Röder, and M.W. Ganal. 2002. Assessing genetic diversity of wheat (*Triticum aestivum* L.) germplasm using microsatellite markers. Theor. Appl. Genet. 105:699–707.

Ihaka, R., and R. Gentleman. 1996. R: A language for data analysis and graphics. J. Comput. Graph. Stat. 5:299–314.

Kihara, H. 1982. Wheat studies: Retrospect and prospects. Elsevier Science Ltd., St. Louis, MO.

Lawrence, M.J., D.F. Marshall, and P. Davies. 1995. Genetics of genetic conservation. I. Sample size when collecting germplasm. Euphytica 84:89–99.

Li, Y.-C., T. Fahima, J.H. Peng, M.S. Röder, V.M. Kirzhner, A. Beiles, A.B. Korol, and E. Nevo. 2000. Edaphitic microsatellite DNA divergence in wild emmer wheat, *Triticum dicoccoides*, at a microsite: Tabigha, Israel. Theor. Appl. Genet. 101:1029–1038.

Lund, B., R. Ortiz, I.M. Skovgaard, R. Waugh, and S.B. Anderson.

2003. Analysis of potential duplicates in barley seed bank collections using re-sampling of microsatellite data. Theor. Appl. Genet. 106:1129–1138.
Marshall, D.R. 1990. Crop genetic resources: Current and emerging issues. *In* A.H.D. Brown et al. (ed.) Plant population genetics, breeding, and genetic resources. Sinauer Assoc. Inc., Sunderland, MA.
Martin, T.J. 1990. Outcrossing in twelve hard red winter wheat cultivars. Crop Sci. 30:59–62.
Nei, M. 1973. Analyses of gene diversity in subdivided populations. Proc. Natl. Acad. Sci. USA 70:3321–3323.
Pardey, P.G., Bonwoo Koo, B.D. Wright, M.E. van Dusen, B. Skovmand, and S. Taba. 2001. Costing the conservation of genetic resources: CIMMYT's ex-situ maize and wheat collection. Crop Sci. 41:1286–1299.
Parzies, H.K., W. Spoor, and R.A. Ennos. 2000. Genetic diversity of barley landrace accessions (*Hordeum vulgare* ssp. *vulgare*) conserved for different lengths of time in *ex situ* gene banks. Heredity 84:476–486.
Röder, M.S., V. Korzun, K. Wendehake, J. Plaschke, M.H. Tixier, P. Leroy, and M.W. Ganal. 1998. A microsatellite map of wheat. Genetics 149:2007–2023.
SAS Institute. 1990. SAS Institute Inc., Cary, NC.
Schneider, S., and D. Roessli. amd L. Excoffier. 2000. Arlequin version 2.000: A software for population genetics data analysis. Genetics and Biometry Laboratory, Univ. of Geneva, Switzerland. (Available online at http://lgb.unige.ch/arlequin/; verified 14 Nov. 2004).
Skovmand, B., and S. Rajaram. 1990. Utilization of genetic resources in the improvement of hexaploid wheat. *In* J.P. Srivastava and A.B. Damania (ed.) Wheat genetic resources: Meeting diverse needs. J. Wiley & Sons, Chichester, UK.
Skovmand, B., G. Varughese, and G.P. Hettel. 1992. Wheat genetic resources at CIMMYT: Their preservation, enrichment, and distribution. CIMMYT, Mexico, D.F.
Skovmand, B., L. Bertschinger, and J. Robinson. 1994. Quantifying heterogeneity for reactions to the Russian wheat aphid in accessions of Turkish hexaploid wheat landraces previously identified as resistant. p. 139–142. *In* Proc. of the Russian Wheat Aphid Workshop, 6, Fort Collins, CO. 23–25 Jan. 1994.
Skovmand, B., M.P. Reynolds, and I.H. DeLacy. 2001. Mining wheat germplasm collections for yield enhancing traits. Euphytica 119: 25–32.
Tahir, M., T. Turchetta, R. Anwar, and D. Lafiandra. 1996. Assessment of genetic variability in hexaploid wheat landraces of Pakistan based on polymorphisms for HMW glutein subunits. Genet. Resour. Crop Evol. 43: 221–220, 1996.
Thornsberry, J.M., M.M. Goodman, J. Kresovich, S. Nielsen, and E. Buckler. 2001. Dwarf8 polymorphisms associate with variation in flowering time. Nat. Genet. 28:286–289.
Weir, B.S. 1996. Genetic data analysis II. Sinauer Associates, Sunderland, MA.
Wright, S. 1978. Evolution and genetics of populations. University Chicago Press, Chicago.
Zeven, A.C. 1974. Indigenous bread wheat varieties from northern Nigeria. Acta Bot. Neerl. 23:137–144.
Zeven, A.C. 2002. Traditional maintenance breeding of landraces: 2. Practical and theoretical considerations on maintenance of variation of landraces by farmers and gardeners. Euphytica 123:147–158.
Zhang, X.Y., C.W. Li, L.F. Wang, H.M. Wang, G.X. You, and Y.S. Dong. 2002. An estimation of the minimum number of SSR alleles needed to reveal genetic relationships in wheat varieties. I. Information from large scale planted varieties and cornerstone breeding parents in Chinese wheat improvement and production. Theor. Appl. Genet. 106:112–117.