Genetica (2009) 135:95–122
DOI 10.1007/s10709-008-9262-x

Microsatellite-based molecular diversity of bread wheat germplasm and association mapping of wheat resistance to the Russian wheat aphid

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Received: 3 September 2007 / Accepted: 14 March 2008 / Published online: 5 April 2008
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Abstract Genetic diversity of a set of 71 wheat accessions, including 53 biotype 2 Russian wheat aphid (RWA2)-resistant landraces and 18 RWA2 susceptible accessions, was assessed by examining molecular variation at multiple microsatellite (SSR) loci. Fifty-one wheat SSR primer pairs were used, 81 SSR loci were determined, and 545 SSR alleles were detected. These SSR loci covered all the three genomes, 21 chromosomes, and at least 41 of the 42 chromosome arms. Diversity values averaged over SSR loci were high with mean number of SSR alleles/locus = 6.7, mean Shannon's index (H) = 1.291, and mean Nei's gene diversity (He) = 0.609. The three wheat genomes ranked as A > D > B and the homoeologous groups ranked as 7 > 3 > 1 > 2 > 6 > 5 > 4 based on the number of alleles per locus. *Xgwm136* on chromosome arm 1AS is the most polymorphic SSR locus with the largest number of observed and effective alleles and the highest H and He. Among all 2485 pairs of wheat accessions, genetic distance (GD) ranged from 0.054 to 1.933 and averaged 0.9832. A dendrogram based on GD matrix showed that all the wheat accessions could be grouped into distinct clusters. Most of the susceptible cultivars (13/18) were clustered into groups that contains all or mostly susceptible accessions. Most of the U.S. cultivars belong to a group that is distinguishable from all the different RWA2 resistant groups. Diversity analysis was also conducted separately for subgroups containing 53 RWA2-resistant accessions and 18 RWA2-susceptible accessions. Association mapping revealed 28 SSR loci significantly associated with leaf chlorosis, and 8 with leaf rolling. New chromosome regions associated with RWA2 resistance were detected, and indicated existence of new RWA resistance genes located on chromosomes of all other homoeologous groups in addition to the groups 1 and 7 in bread wheat. This information is helpful for development of mapping populations for RWA2 resistance genes from different phylogenetic groups, and for wise utilization of the RWA-resistant germplasm in wheat breeding programs.

Keywords Wheat germplasm · Russian wheat aphid resistance · Microsatellite · Molecular diversity · Genetic variation · Association mapping

Abbreviations

AM	Association mapping
LC	Leaf chlorosis
LD	Linkage disequilibrium
LR	Leaf rolling
GD	Genetic distance
PCR	Polymerase chain reaction
PIC	Polymorphic information content
RWA	Russian wheat aphid
SSR	Simple sequence repeat
UPGMA	The un-weighted pair-group method with arithmetic average

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Introduction

The Russian Wheat Aphid (RWA), *Diuraphis noxia* (Kurdjumov), is an important insect pest of bread wheat, *Triticum aestivum* L., in several production areas of the world. The RWA was first discovered in the United States in 1986 (Webster et al. 1987) and has caused extensive damage to wheat production. Yield losses and increased production costs associated with RWA infestations were estimated to be >$800 million in the first 10 years after its introduction (Morrison and Peairs 1998) and additional losses have occurred since then (Peairs FB personal communication, Dep. Bioagricul. Sci. & Pest Mgmt., Colorado State Univ.). Control of RWA in the U.S. has critically relied on insecticides and, since 1996, on host-plant resistance genes effective against biotype 1 RWA (Smith et al. 2004). Most RWA-resistant wheat cultivars planted commercially in the United States contain the *Dn4* gene from PI 372129 (Collins et al. 2005a). However, the emergence of a new biotype (designated as biotype 2) of RWA in 2003 poses a threat to existing wheat cultivars containing resistance genes against biotype 1, particularly *Dn4* and *Dny* (Haley et al. 2004; Collins et al. 2005a; Jyoti and Michaud 2005; Qureshi et al. 2006).

Although the identification of biotype 2 represents the first detectable change in RWA populations in the United States, it is not necessarily the last (Qureshi et al. 2006). RWA populations from other parts of the world show considerable biotypic variation (Shufran et al. 1997; Basky 2003). Puterka et al. (1992) recognized at least eight RWA biotypes worldwide. In the United States, at least eight RWA biotypes were also recently identified, and biotype 2 is currently the predominant biotype (Peairs FB personal communication). Therefore, biotypic variation in RWA, whether introduced from exotic sources or evolved in situ, will likely continue to pose a serious threat to wheat production in the United States, mostly the west central Great Plains (Qureshi et al. 2006). Host-plant resistance is the most cost-effective and environmentally safe means for controlling RWA. Continuous efforts are necessary to identify and introduce additional resistance genes into commercially acceptable cultivars.

Among the 12 RWA resistance genes identified in wheat, rye (*Secale cereale* L.), and *Aegilops tauschii* (Smith et al. 2004; Peng et al. 2007), only the rye-derived *Dn7* and its allelic *Dn2414* are resistant to all the U.S. biotypes including biotype 2 (Anderson et al. 2003; Haley et al. 2004; Lapitan et al. 2007; Peng et al. 2007). The *Dn7* gene has also been shown to provide effective protection from yield losses in field experiments (Collins et al. 2005b). However, *Dn7* and its allelic *Dn2414* are located on a 1RS.1BL wheat-rye translocation (Marais et al. 1994, 1998; Anderson et al. 2003; Lapitan et al. 2007; Peng et al. 2007) which is associated with poor bread-baking quality (Graybosch et al. 1990). In an effort to screen wheat germplasm accessions, Collins et al. (2005a) reported 58 (8.2%) of 709 accessions mainly from central Asia that showed resistance to RWA2. Some of these lines also confer resistance to RWA biotype 1 (http://www.ars-grin.gov/npgs/acc/acc_queries.html). These lines are mainly unimproved landraces of common wheat and thus do not likely contain the *Secalin* genes responsible for the poor bread-baking quality of wheat. To support breeders in selecting parental materials for breeding RWA resistance, knowledge of molecular genetic diversity, phylogenetic relationship of these resistant germplasm resources, and correlation between resistance traits and molecular markers would be helpful.

Simple-Sequence Repeats (SSRs), also known as microsatellites, are a class of molecular markers based on repeats of short (2–6 bp) DNA sequences (Litt and Luty 1989). The high level of polymorphism, combined with a high interspersion rate, makes them an abundant source of genetic markers. The usefulness of SSRs as genetic markers in plants has been demonstrated for several species, including soybean (Akkaya et al. 1995), rice (Wu and Tanksley 1993), maize (Senior and Heun 1993), *Arabidopsis* (Bell and Ecker 1994), and wheat (Bryan et al. 1997; Röder et al. 1998; Song et al. 2002; Peng and Lapitan 2005). These studies indicated that SSRs in plants can be up to ten-fold more variable than other marker systems such as Restriction Fragment Length Polymorphisms (RFLPs). Furthermore, the efficiency of SSR markers was also demonstrated for hexaploid wheat, a self-pollinating species with a relatively low level of intraspecific polymorphism (Plaschke et al. 1995; Röder et al. 1995). In recent years, a large number of SSR markers have been developed and extensively utilized in genomic mapping and marker-assisted breeding (Bryan et al. 1997; Röder et al. 1998; Peng et al. 1999, 2000a, b, c, 2003, 2007; Liu et al. 2001; Song et al. 2002; Arzani et al. 2004; Somers et al. 2004; http://maswheat.ucdavis.edu/), population genetic analyses (Li et al. 2000a, b) and diversity/polymorphism evaluation of germplasm (Fahima et al. 1998, 2002; Huang et al. 2002; Alamerew et al. 2004; Bertin et al. 2004; Khlestkina et al. 2004a, b; You et al. 2004; Roussel et al. 2004, 2005; Teklu et al. 2006; Zhang et al. 2006; Liu et al. 2007) in wheat.

Association mapping (AM) is to detect correlations between genotypes and phenotypes in a sample of individuals on the basis of linkage disequilibrium (LD) (Zondervan and Cardon 2004). As compared to other experimental designs that require sampling within families, AM offers the important advantage of sampling unrelated individuals in the population in the study of genetics of complex traits (Risch 2000). Sampling unrelated genotypes presents several advantages for marker-assisted plant

breeding (Jannink et al. 2001). First, the experimental population can be a representative sample of the population to which inference is desired. Second, AM is more efficient in the use of resources, e.g., several traits can be studied in the same population using the same genotypic data, a higher proportion of molecular markers are likely to be polymorphic, providing better genome coverage than any bi-parental map. Furthermore, multi-year and multi-location phenotypic data may be available at no additional cost in study of elite lines (Rafalski 2002). In wheat, Breseghello and Sorrells (2006) demonstrated that association mapping in elite germplasm can enhance the information from QTL studies toward the implementation of marker-assisted selection.

The main objectives of this study were to determine genetic diversity and phylogenetic relationship among a group of 71 wheat accessions including 53 RWA2–resistant and 18 RWA2-susceptible genotypes for development of mapping populations for RWA2-resistance genes from different phylogenetic groups and for wise utilization of the RWA-resistant germplasm in wheat breeding programs. In the present paper, we report the differentiation and estimation of genetic diversity revealed by SSR markers among different wheat accessions originating primarily from central Asia selected for their high resistance to RWA2, and also potential SSR markers associated with RWA2 resistance.

Materials and methods

Plant materials

Previous studies showed that most of the bread wheat accessions resistant to RWA2 were from central Asia (Collins et al. 2005a; http://www.ars-grin.gov/npgs/acc/acc_queries.html). In this study, a set of 71 wheat genotypes was used. This bread wheat germplasm collection represents mainly landraces from central Asia and cultivars from the USA originating from geographically different locations (Table 1). Among these are 53 RWA2-resistant wheat accessions including 51 central Asian genotypes (38 from Iran, 10 from Afghanistan, 2 from Kazakhstan and 1 from Tajikistan), one Egyptian cultivar, and one Bulgarian cultivar, and 18 RWA2-susceptible wheat accessions including 10 U.S. genotypes developed in Colorado, Kansas, Oklahoma and Texas, six central Asian landraces, one Chinese cultivar/genetic material and one landrace from Sweden. The seeds were obtained from the National Small Grains Collection (NSGC) of the National Plant Germplasm System, USDA-ARS in Aberdeen, Idaho. Table 1 lists the geographic origin and reaction to RWA2 of these materials.

DNA extraction

Fifteen seeds were germinated in 100 × 15 mm Petri dishes (Becton Dickinson and Company, Franklin Lakes, NJ 07417–1886, USA), and equal amount of tissues were collected from 10 seedlings for each of the selected wheat genotypes. The tissue was placed in a 2 ml Eppendorf tube, immediately frozen in liquid nitrogen, and stored in a −80°C freezer. Total genomic DNA was extracted according to a procedure modified from Edwards et al. (1991).

SSR genotyping

In order to have complete coverage of the wheat genome, 51 primer pairs, at least one for each chromosome arm, which amplify the expected wheat SSR fragments were chosen for the analysis. These SSR primers included 48 GWMs (Röder et al. 1998), one BARC (Song et al. 2002), one CFD (Guyomarc'h et al. 2002), and one CWEM (Peng and Lapitan 2005). Primer designation, the amplified loci, the chromosome arm location, the number of alleles, and the range of allele size are presented in Table 2. Primer sequences are available at the GrainGenes web site: http://wheat.pw.usda.gov/cgi-bin/graingenes/browse.cgi?class=marker.

The polymerase chain reactions (PCR) were performed in PTC-200 MJ Thermocyclers (MJ Research, Inc., Watertown, MA). The PCR procedure was the same as in Peng et al. (1999). The PCR-amplified fragments were separated by electrophoresis on a 5% denaturing polyacrylamide gel. The gels were visualized with silver staining (Bio-Rad Kit Protocol #LIT-34 89–0559 689; Morrissey 1981). As an example, Fig. 1 shows the polymorphism pattern of SSR marker *Xgwm161*.

Genetic data analysis

The SSR markers were treated as co-dominant markers. The visualized polyacrylamide gels were scored using capital alphabets following the user guide of Popgene (Yeh and Yang 2000). The collection of wheat germplasm was classified into three groups, R+S group including all the 71 accessions, R group consisting of 53 RWA2-resistant accessions, and S group consisting of 18 RWA2-susceptible accessions. These three groups were subjected to the following analyses. The actual number of alleles was counted for each amplified locus. The effective number of alleles was estimated as $n = 1 + 4N_e u$ for each locus, where N_e is the effective population size and u is the average mutation rate (Kimura and Crow 1964). Shannon's (1949) information index (H) was estimated for each locus using the formula $H = -\sum p_i \ln p_i (i = 1 \text{ to } S)$, where S is the total number of alleles in the locus, and p_i is the

Table 1 Wheat accessions, their geographical origin and reaction type to infestation of Russian wheat aphid biotype 2 (RWA2)

Line ID	Accession/Name	Country of origin	Reaction to RWA[a]	Improvement status
1	Chinese spring	China	S	Cultivar/genetic material
2	PI 262660 *(Dn2)*	Azerbaijan	S	Cultivar
3	PI 372129 *(Dn4)*	Turkmenistan	S	Cultivar
4	Ankor	Colorado, USA	S	Cultivar
5	Avalanche	Colorado, USA	S	Cultivar
6	CO970547-7	Colorado, USA	S	Breeding line
7	Halt	Colorado, USA	S	Cultivar
8	Jagalene	Texas, USA	S	Cultivar
9	NuFrontier	Kansas, USA	S	Cultivar
10	Stanton	Kansas, USA	S	Cultivar
11	TAM 111	Texas, USA	S	Cultivar
12	Yuma	Colorado, USA	S	Cultivar
13	CItr 2401	Tajikistan	R	Landrace
14	CItr 9355	Sweden	S	Landrace
15	CItr 11349	Bulgaria	R	Cultivar
16	PI 134134	Afghanistan	R	Landrace
17	PI 135064	Afghanistan	R	Landrace
18	PI 140213	Iran	R	Landrace
19	PI 220131	Afghanistan	R	Landrace
20	PI 221482	Afghanistan	R	Landrace
21	PI 243648	Iran	S	Landrace
22	PI 243659	Iran	R	Landrace
23	PI 243679	Iran	S	Landrace
24	PI 245583	Afghanistan	R	Landrace
25	PI 283886	Afghanistan	R	Cultivar
26	PI 347006	Afghanistan	R	Landrace
27	PI 352008	Kazakhstan	R	Cultivar
28	PI 361836	Afghanistan	S	Landrace
29	PI 366103	Egypt	R	Cultivar
30	PI 366518	Afghanistan	R	Landrace
31	PI 366572	Afghanistan	R	Landrace
32	PI 366589	Afghanistan	R	Landrace
33	PI 367053	Afghanistan	S	Landrace
34	PI 572289	Oklahoma, USA	S	Breeding line
35	PI 572652	Kazakhstan	R	Cultivar
36	PI 620913	Iran	R	Landrace
37	PI 620951	Iran	R	Landrace
38	PI 620991	Iran	R	Landrace
39	PI 621019	Iran	R	Landrace
40	PI 621097	Iran	R	Landrace
41	PI 621117	Iran	R	Landrace
42	PI 621123	Iran	R	Landrace
43	PI 621154	Iran	R	Landrace
44	PI 621256	Iran	R	Landrace
45	PI 621362	Iran	R	Landrace
46	PI 621392	Iran	R	Landrace
47	PI 621394	Iran	R	Landrace
48	PI 621421	Iran	R	Landrace
49	PI 621458	Iran	R	Landrace
50	PI 621460	Iran	R	Landrace
51	PI 621462	Iran	R	Landrace
52	PI 621721	Iran	R	Landrace
53	PI 621736	Iran	R	Landrace
54	PI 621837	Iran	R	Landrace

Table 1 continued

Line ID	Accession/Name	Country of origin	Reaction to RWA[a]	Improvement status
55	PI 622843	Iran	R	Landrace
56	PI 622009	Iran	R	Landrace
57	PI 622083	Iran	R	Landrace
58	PI 622205	Iran	R	Landrace
59	PI 622219	Iran	R	Landrace
60	PI 622278	Iran	R	Landrace
61	PI 622769	Iran	R	Landrace
62	PI 622825	Iran	R	Landrace
63	PI 622826	Iran	R	Landrace
64	PI 622831	Iran	R	Landrace
65	PI 622855	Iran	R	Landrace
66	PI 623081	Iran	R	Landrace
67	PI 623611	Iran	R	Landrace
68	PI 623825	Iran	R	Landrace
69	PI 623836	Iran	R	Landrace
70	PI 624151	Iran	R	Landrace
71	PI 624152	Iran	R	Landrace

[a] Based on the leaf shape and chlorosis ratings after infestation of RWA2, i.e., rolling + chlorosis rating of ≥5 means susceptible (S), and flat + chlorosis ratting of <5 means resistant (R)

proportion of *S* made up of the *i*th allele. Gene diversity was estimated according to the formula of Nei (1973) for each locus $He = 1 - \sum P_{ij}^2$, where P_{ij} is the frequency of the *j*th allele for *i*th locus summed across all alleles of the locus. Anderson et al. (1993) referred to gene diversity as the Polymorphic Information Content (PIC). Nei's (1972) genetic identity (*I*) and genetic distance (*D*) were calculated for each pair of tested entries according to the equations: $I = J_{XY}/\sqrt{J_X J_Y}$, and $D = -\ln I$, where J_X, J_Y and J_{XY} are the arithmetic means of $j_X(=\sum x_i^2)$, j_Y $(=\sum y_i^2)$ and j_{xy} $(=\sum x_i y_i)$, respectively, over all loci; x_i and y_i are the frequencies of the *i*th allele in X and Y entry/population, respectively. The un-weighted pair-group method with arithmetic average (UPGMA) was chosen as a clustering method. Based on the Nei's GD, the dendrogram was drawn using UPGMA. All these analyses were conducted using the Popgene 1.32 Window-based computer package (Yeh and Yang 2000).

Association mapping

Leaf chlorosis (LC) and leaf rolling (LR) were scaled to measure reaction of the bread wheat accessions to RWA2 as described by Collins et al. (2005a). Associations between these two traits and the available SSR data were analyzed based on the whole set of 71 bread wheat accessions used in the present study. The AM was conducted using the general linear model by the aid of the computer software TASSEL2.0.1 (http://www.maizegenetics.net/tassel) accounting for population structure estimates from STRUCTURE2.1 software (Prichard et al. 2000, http://pritch.bsd.uchicago.edu/structure.html). The number of permutation run was set as 10,000 to obtain the permutation-based test of marker significance and the experiment-wise *P*-value for marker significance. Markers were declared to be associated with a RWA2 resistance trait only when the markers are significant ($P < 0.05$) in all the three tests, F-test, permutation-based test, and experiment-wise test.

Results and discussion

Genome coverage of the SSR markers

In the present study, the 51 selected primer pairs amplified 81 SSR loci in the set of wheat germplasm collections used (Tables 1 and 2). Each of these SSR primer pairs amplified 1–5 loci. The chromosome arm locations for 67 of these SSR loci were known, and unknown for other 14 (Table 2). The 67 SSR loci were distributed on all but the 7DL chromosome arm of hexaploid wheat (Tables 2 and 3). Therefore, the 81 SSR loci covered all the three genomes, seven homoeologous groups, 21 chromosomes, and at least 41 of the 42 chromosome arms of wheat. In previous studies of genetic diversity in wheat germplasm, scientists used from 20 to 30 SSRs (Fahima et al. 1998, 2002; Huang et al. 2002; Alamerew et al. 2004; Khlestkina et al. 2004a, b; Bertin et al. 2004; Teklu et al. 2006; Zhang et al. 2006; Liu et al. 2007), and a few studies used around 40 SSRs (Roussel et al. 2004, 2005). Thus, the genetic information revealed in this study represents the wheat genome with higher coverage.

SSR polymorphism and genetic diversity

High levels of polymorphism were observed for the SSR markers. A total of 545 alleles were detected on 81 SSR loci in a set (R+S) of 71 wheat collections including both

Table 2 Wheat microsatellite locus, chromosomal location, number of alleles, and genetic variation statistics

Microsatellite locus	CL[a]	Range of allele size(bp)	*Ns*[b]	*Na*[c]			*Ne*[d]			*H*[e]			*He-a*[f]			*He-e*[g]		
				R+S	R	S	R+S[h]	R	S	R+S	R	S	R+S	R	S	R+S	R	S
Xbarc98	4DS	162–186	0	4	4	3	2.765	2.575	2.752	1.129	1.079	1.055	0.000	0.000	0.000	0.638	0.612	0.637
Xcfd45a	–	117–126	0	3	3	3	2.073	1.790	2.624	0.882	0.726	1.021	0.394	0.415	0.333	0.518	0.441	0.619
Xcfd45b	–	150, 156	0	3	3	2	2.093	2.089	1.976	0.797	0.813	0.687	0.000	0.000	0.000	0.522	0.521	0.494
Xcfd45c	6DL	228–238	0	3	3	3	2.161	2.082	2.374	0.889	0.864	0.949	0.549	0.604	0.389	0.537	0.520	0.579
Xcwem6a	1DS	98–104	1	3	2	3	1.220	1.078	1.742	0.362	0.161	0.730	0.000	0.000	0.000	0.180	0.073	0.426
Xcwem6b	1AS	182–190	1	4	4	3	2.336	2.390	2.110	0.959	0.986	0.846	0.000	0.000	0.000	0.572	0.582	0.526
Xcwem6c	1BS	345–370	1	4	4	2	2.270	2.398	1.841	0.927	0.990	0.649	0.000	0.000	0.000	0.559	0.583	0.457
Xgwm11	1BS	180–205	4	10	6	9	3.929	3.030	7.044	1.676	1.324	2.062	0.000	0.000	0.000	0.746	0.670	0.858
Xgwm18	1BS	178–190	0	6	6	5	3.985	3.551	3.951	1.539	1.439	1.461	0.000	0.000	0.000	0.749	0.718	0.747
Xgwm44	7DS	109–137	3	14	14	8	8.278	8.049	6.231	2.334	2.306	1.952	0.000	0.000	0.000	0.879	0.876	0.840
Xgwm88a	6BS	120–160	6	20	18	10	11.227	10.289	8.100	2.695	2.608	2.187	0.000	0.000	0.000	0.911	0.903	0.877
Xgwm88b	6BS	176–186	3	10	9	7	5.268	4.818	4.909	1.850	1.765	1.744	0.000	0.000	0.000	0.810	0.793	0.796
Xgwm106	1DS	115–143	4	14	12	7	5.357	5.840	3.177	2.123	2.086	1.484	0.000	0.000	0.000	0.813	0.829	0.685
Xgwm111a	6DS	93–101	0	4	4	3	1.630	1.625	1.615	0.783	0.784	0.678	0.000	0.000	0.000	0.387	0.385	0.381
Xgwm111b	7DS	102–114	0	6	6	5	2.906	2.627	3.461	1.356	1.291	1.372	0.029	0.019	0.059	0.656	0.619	0.711
Xgwm111c	7BS	120–154	4	17	16	10	10.208	9.522	8.257	2.537	2.470	2.201	0.000	0.000	0.000	0.902	0.895	0.879
Xgwm111d	–	310–340	0	4	4	3	2.301	2.275	2.240	1.062	1.059	0.924	0.000	0.000	0.000	0.565	0.560	0.554
Xgwm113	4BS	98–106	0	4	4	2	2.674	3.118	1.528	1.135	1.234	0.530	0.000	0.000	0.000	0.626	0.679	0.346
Xgwm124	1BL	178–196	3	7	6	3	2.683	2.818	2.051	1.260	1.277	0.828	0.000	0.000	0.000	0.627	0.645	0.512
Xgwm136	1AS	225–400	9	24	21	8	13.588	12.374	5.400	2.866	2.778	1.879	0.000	0.000	0.000	0.926	0.919	0.815
Xgwm146	7BL	148–184	4	18	17	11	11.861	11.656	8.526	2.650	2.614	2.274	0.000	0.000	0.000	0.916	0.914	0.883
Xgwm157	2DL	99–107	1	4	4	3	2.331	2.399	1.742	0.957	0.987	0.730	0.000	0.000	0.000	0.571	0.583	0.426
Xgwm159a	–	116, 118	0	2	2	2	1.953	1.917	2.000	0.681	0.672	0.693	0.000	0.000	0.000	0.488	0.479	0.500
Xgwm159b	5DS	120–128	1	4	4	3	2.101	2.092	1.976	0.872	0.839	0.849	0.000	0.000	0.000	0.524	0.522	0.494
Xgwm159c	–	128, 130	0	2	2	1	1.573	1.772	1.000	0.550	0.627	0.000	0.000	0.000	0.000	0.364	0.436	0.000
Xgwm159d	–	133–139	0	3	3	2	1.222	1.166	1.385	0.381	0.320	0.451	0.000	0.000	0.000	0.182	0.142	0.278
Xgwm159e	5BS	224–228	0	4	4	3	2.034	2.117	1.588	0.884	0.880	0.684	0.000	0.000	0.000	0.508	0.528	0.370
Xgwm161	3DS	133–151	1	9	9	4	5.046	5.939	2.348	1.826	1.926	1.014	0.000	0.000	0.000	0.802	0.832	0.574
Xgwm164	1AL	132–140	1	4	4	3	1.639	1.416	2.160	0.733	0.610	0.854	0.015	0.020	0.000	0.390	0.294	0.537
Xgwm192a	–	96, 98	0	2	2	2	1.355	1.441	1.117	0.431	0.484	0.215	0.000	0.000	0.000	0.262	0.306	0.105
Xgwm192b	4BL	150–162	2	7	7	4	3.217	3.309	2.656	1.383	1.427	1.118	0.000	0.000	0.000	0.689	0.698	0.624
Xgwm192c	5DS	235–260	1	6	5	4	2.477	2.207	2.746	1.206	1.087	1.132	0.000	0.000	0.000	0.596	0.547	0.636
Xgwm194a	4DL	130–144	0	7	7	6	5.149	5.154	4.629	1.790	1.785	1.648	0.000	0.000	0.000	0.806	0.806	0.784
Xgwm194b	4DS	150, 152	0	2	2	2	2.000	1.982	1.800	0.693	0.689	0.637	0.000	0.000	0.000	0.500	0.496	0.444

Table 2 continued

Microsatellite locus	CL[a]	Range of allele size(bp)	*Ns*[b]	*Na*[c]			*Ne*[d]			*H*[e]			*He-a*[f]			*He-e*[g]		
				R+S	R	S	R+S[h]	R	S	R+S	R	S	R+S	R	S	R+S	R	S
Xgwm194c	–	233, 235	0	2	2	2	1.953	1.999	1.528	0.681	0.693	0.530	0.000	0.000	0.000	0.488	0.500	0.346
Xgwm257	2BS	169–175	0	3	3	3	2.926	2.818	2.946	1.086	1.068	1.089	0.000	0.000	0.000	0.658	0.645	0.661
Xgwm293a	7BS	106–112	0	3	3	2	1.813	1.790	1.800	0.770	0.776	0.637	0.000	0.000	0.000	0.448	0.441	0.444
Xgwm293b	5AS	106–124	2	9	9	6	6.247	6.736	4.378	1.943	1.998	1.615	0.000	0.000	0.000	0.840	0.852	0.772
Xgwm294	2AL	73–102	4	12	10	8	4.521	3.455	3.951	1.882	1.628	1.706	0.000	0.000	0.000	0.779	0.711	0.747
Xgwm295	2DS	155–171	2	8	6	7	4.880	4.237	3.682	1.723	1.593	1.581	0.000	0.000	0.000	0.795	0.764	0.728
Xgwm299	3BL	210–228	3	9	9	6	4.899	4.369	4.263	1.763	1.667	1.613	0.000	0.000	0.000	0.796	0.771	0.765
Xgwm314	3DL	162–215	3	16	14	9	10.568	8.918	7.044	2.522	2.381	2.062	0.000	0.000	0.000	0.905	0.888	0.858
Xgwm332	7AL	215–230	1	4	4	2	1.423	1.362	1.528	0.610	0.535	0.530	0.000	0.000	0.000	0.297	0.266	0.346
Xgwm337	1DS	109–137	3	14	14	9	7.150	7.296	5.586	2.279	2.277	1.956	0.000	0.000	0.000	0.860	0.863	0.821
Xgwm357	1AL	170–188	2	7	6	3	2.277	2.261	2.160	1.180	1.151	0.901	0.000	0.000	0.000	0.561	0.558	0.537
Xgwm369	3AS	169–193	3	12	12	5	5.269	4.552	2.382	1.972	1.912	1.165	0.000	0.000	0.000	0.810	0.780	0.580
Xgwm372	2AS	136–166	1	15	15	9	12.206	10.930	7.364	2.589	2.526	2.091	0.000	0.000	0.000	0.918	0.909	0.864
Xgwm391	3AL	104–110	0	3	3	3	2.235	2.027	2.323	0.874	0.764	0.960	0.333	0.392	0.167	0.553	0.507	0.569
Xgwm427a	6AL	196–209	1	12	10	7	5.506	4.306	4.909	2.025	1.790	1.744	0.000	0.000	0.000	0.818	0.768	0.796
Xgwm427b	–	218–242	0	6	6	3	3.090	2.793	2.656	1.406	1.298	1.026	0.000	0.000	0.000	0.676	0.642	0.624
Xgwm427c	–	248–264	2	9	6	7	6.347	4.744	5.586	1.951	1.657	1.817	0.000	0.000	0.000	0.842	0.789	0.821
Xgwm427d	–	275–287	1	6	6	3	2.601	2.693	2.314	1.167	1.211	0.937	0.000	0.000	0.000	0.616	0.629	0.568
Xgwm427e	–	290–320	3	10	8	6	3.840	3.290	3.767	1.700	1.478	1.504	0.000	0.000	0.000	0.740	0.696	0.735
Xgwm455a	6BL	126, 128	0	2	2	2	1.990	1.999	1.800	0.691	0.693	0.637	0.000	0.000	0.000	0.498	0.500	0.444
Xgwm455b	2DS	138–178	0	4	4	3	3.614	3.643	2.219	1.332	1.340	0.934	0.000	0.000	0.000	0.723	0.726	0.549
Xgwm455c	6BL	225, 230	0	2	2	2	1.994	1.997	1.800	0.692	0.692	0.637	0.000	0.000	0.000	0.498	0.499	0.444
Xgwm469	6DS	160–205	3	13	10	9	6.840	5.392	7.044	2.177	1.950	2.062	0.000	0.000	0.000	0.854	0.815	0.858
Xgwm471	7AS	90–116	2	13	11	10	6.590	4.972	7.044	2.157	1.905	2.135	0.000	0.000	0.000	0.848	0.799	0.858
Xgwm493	3BS	96–132	2	7	6	5	2.303	2.453	1.841	1.222	1.230	0.961	0.000	0.000	0.000	0.566	0.592	0.457
Xgwm494a	6AS	105, 114	0	2	2	2	1.391	1.251	1.800	0.454	0.353	0.637	0.000	0.000	0.000	0.281	0.201	0.444
Xgwm494b	6AL	175, 203	0	2	2	2	1.391	1.251	1.800	0.454	0.353	0.637	0.000	0.000	0.000	0.281	0.201	0.444
Xgwm494c	1BS	205–211	0	3	3	2	2.226	2.235	1.800	0.870	0.893	0.637	0.000	0.000	0.000	0.551	0.553	0.444
Xgwm494d	4AL	214–220	1	3	3	2	1.121	1.121	1.117	0.249	0.254	0.215	0.000	0.000	0.000	0.108	0.108	0.105
Xgwm501	2BL	101–121	2	10	8	5	2.037	1.727	3.177	1.250	1.019	1.378	0.000	0.000	0.000	0.509	0.421	0.685
Xgwm513	4BL	245–255	0	5	4	4	2.906	2.289	3.177	1.318	1.071	1.259	0.000	0.000	0.000	0.656	0.563	0.685
Xgwm526a	2AL	115–125	1	4	4	2	2.146	2.238	1.670	0.859	0.908	0.591	0.000	0.000	0.000	0.534	0.553	0.401
Xgwm526b	2BL	130–140	1	5	3	5	3.271	2.709	3.951	1.303	1.041	1.461	0.000	0.000	0.000	0.694	0.631	0.747
Xgwm537a	5BL	152–160	1	4	4	3	1.845	1.800	1.976	0.836	0.823	0.849	0.000	0.000	0.000	0.458	0.444	0.494

Table 2 continued

Microsatellite locus	CL[a]	Range of allele size(bp)	*Ns*[b]	*Na*[c]			*Ne*[d]			*H*[e]			*He-a*[f]			*He-e*[g]		
				R+S	R	S	R+S[h]	R	S	R+S	R	S	R+S	R	S	R+S	R	S
Xgwm537b	7BS	195–270	3	14	11	9	7.081	5.864	6.149	2.236	2.040	2.003	0.000	0.000	0.000	0.859	0.830	0.837
Xgwm537c	–	300–310	1	4	4	3	1.467	1.486	1.409	0.643	0.662	0.557	0.000	0.000	0.000	0.318	0.327	0.290
Xgwm538	4BL	150, 0	0	2	2	2	1.183	1.206	1.117	0.290	0.313	0.215	0.000	0.000	0.000	0.155	0.171	0.105
Xgwm544	5BS	185–200	0	4	4	3	2.603	3.090	1.409	1.127	1.232	0.557	0.000	0.000	0.000	0.616	0.676	0.290
Xgwm550	1BS	241, 243	0	2	2	2	1.372	1.262	1.710	0.443	0.362	0.606	0.000	0.000	0.000	0.271	0.208	0.415
Xgwm554	5BL	260–300	0	5	5	3	2.805	3.240	1.780	1.255	1.352	0.778	0.000	0.000	0.000	0.644	0.691	0.438
Xgwm565	5DL	115–121	0	3	3	3	2.286	2.228	2.314	0.899	0.871	0.937	0.000	0.000	0.000	0.563	0.551	0.568
Xgwm595a	–	108–116	0	4	4	4	2.740	2.875	2.141	1.171	1.184	1.006	0.000	0.000	0.000	0.635	0.652	0.533
Xgwm595b	5AL	140–156	1	8	6	7	3.924	3.163	5.667	1.662	1.437	1.824	0.000	0.000	0.000	0.745	0.684	0.824
Xgwm610	4AS	196–210	2	7	4	7	3.836	2.986	6.000	1.529	1.228	1.850	0.000	0.000	0.000	0.739	0.665	0.833
Xgwm613	6BS	198–204	0	3	3	3	2.105	2.082	2.160	0.893	0.889	0.901	0.000	0.000	0.000	0.525	0.520	0.537
Xgwm637	4AL	185–203	3	8	7	5	2.446	2.110	2.909	1.265	1.141	1.249	0.000	0.000	0.000	0.591	0.526	0.656
Xgwm642	1DL	120–128	0	4	4	4	2.039	1.601	3.657	0.965	0.722	1.334	0.000	0.000	0.000	0.510	0.375	0.727
Sum			104	545	494	362	300.2	279.7	259.9									
Mean			1.3	6.7	6.1	4.5	3.71	3.45	3.21	1.291	1.226	1.134	0.016	0.018	0.012	0.609	0.591	0.581

[a] CL = Chromosomal location

[b] *Ns* = Accession-specific alleles, i.e., those detected in only one accession

[c] *Na* = Observed number of polymorphic bands/alleles

[d] *Ne* = Effective number of alleles (Kimura and Crow 1964)

[e] *H* = Shannon's information index (Shannon and Weaver 1949)

[f] *He-a* = Observed heterozygosity, proportion of observed heterozygotes at a given locus

[g] *He-e* = Nei's (1973) expected heterozygosity or gene diversity

[h] R refers to the subgroup only including RWA2-resistant genotypes, S refers to the subgroup only including RWA2-susceptible genotypes, and R+S refers to the whole group containing both the RWA2-resistant and -susceptible genotypes

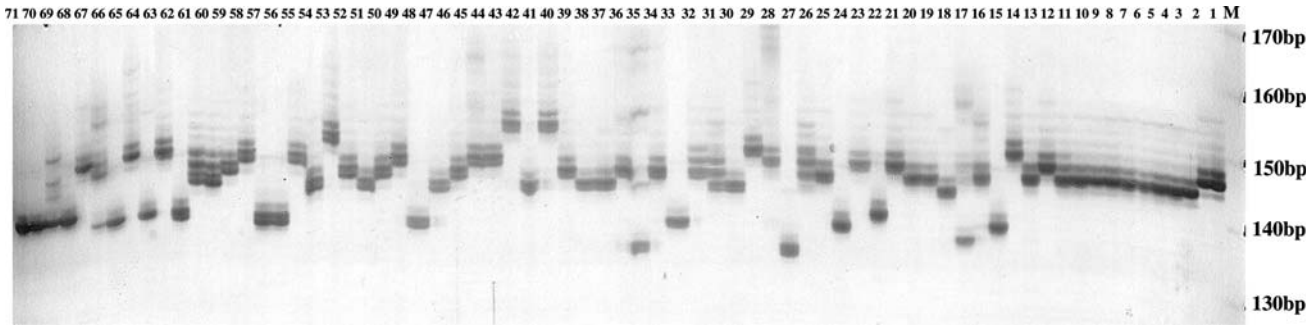


Fig. 1 Amplification profiles of SSR marker *Xgwm161* in the 71 wheat accessions/cultivars. Lanes 1–71 corresponds to line ID in Table 1. M = 10 bp DNA ladder

Table 3 Distribution of microsatellite loci and alleles among homoeologous groups, genomes and chomosomes in wheat

Group	Genome												Sum			Accession-specific alleles
	A				B				D							
	Long arm		Short arm		Long arm		Short arm		Long arm		Short arm					
	Locus	Allele	Locus	Allele	Locus	Allele	Locus	Allele	Locus	Allele	Locus	Allele	Locus	Allele	A/L[a]	
1	2	11	2	28	1	7	5	25	1	4	3	31	14	106	7.57	29
2	2	16	1	15	2	15	1	3	1	4	2	12	9	65	7.22	12
3	1	3	1	12	1	9	1	7	1	17	1	9	6	57	9.50	12
4	2	11	1	7	3	14	1	4	1	7	2	6	10	49	4.90	8
5	1	8	1	9	2	9	2	8	1	3	2	10	9	47	5.22	6
6	2	14	1	2	2	7	3	33	1	3	2	17	11	76	6.91	13
7	1	4	1	13	1	18	3	24	0	0	2	31	8	90	11.25	17
Total-arm	11	67	8	86	12	79	16	104	6	38	14	116				
A/L-arm	6.09		10.75		6.58		6.5		6.33		8.29					
Total-genome	19	153			28	183			20	154			67	490		
A/L-genome	8.05				6.54				7.70						7.31	
Accession-specific alleles	37				33				27							97

[a] A/L, alleles per locus

the RWA2-resistant and -susceptible lines, with a range of 2–24 and average of 6.7 alleles per SSR locus. The most polymorphic SSR locus was *Xgwm136* on chromosome arm 1AS which had the largest number of observed (24) and effective (13.6) alleles, and the highest Shannon's index and Nei's gene diversity (Table 2). Among the three genomes of hexaploid wheat, the A genome had the highest number of SSR alleles per locus (8.05), followed by D (7.70) and B (6.54) genomes. Homoeologous group 7 had the highest number of alleles per locus (11.25), followed by group 3 (9.50), group 1 (7.57), group 2 (7.22), and other groups (< 7.0) (Table 3).

For the R and S subgroups of the wheat accessions, the total number of detected SSR alleles was 494 and 362, respectively, and the average number of SSR alleles was 6.1 and 4.5, respectively (Table 2). In comparison with the R+S group, the R subgroup had slightly lower total and average numbers of alleles, but the S subgroup had a obviously lower total and average numbers of detected alleles (Table 2). Thus, high levels of polymorphism still occur in the RWA2-resistant germplasm, but relatively lower polymorphism exists in the RWA2-susceptible accessions. The origin and geographic distribution of the wheat germplasm showed in Table 1 may explain this result.

The number of alleles per SSR locus is one of the most important parameters describing polymorphism and varies from 4.6 to 18.2 in previous studies of wheat genetic diversity (Fahima et al. 1998, 2002; Huang et al. 2002; Bertin et al. 2004; Khlestkina et al. 2004b; Roussel et al. 2004, 2005; You et al. 2004; Teklu et al. 2006; Liu et al. 2007). Many of these wheat diversity studies treated a SSR

marker as a single locus. However, studies have shown that many wheat SSR markers could actually determine multiple loci (Röder et al. 1998; Peng et al. 2000c; Sourdille et al. 2004). We strictly differentiated the SSR loci from markers in this study. Therefore, the previous studies usually reported higher number of alleles per locus than we did in the present study (Table 2).

An allele found in only one accession is termed an accession-specific allele. A total of 104 SSR alleles were found to be accession-specific, and amounted to 19.08% of the total number of alleles (Table 2). In spelt wheat, Bertin et al. (2004) found that 17% of the SSR alleles were accession specific. This indicates that some aspect of SSR polymorphism in the analyzed bread wheat germplasm is slightly higher than that in spelt wheat (19% vs. 17%). Chromosome locations are known for 97 of the 104 accession-specific alleles (Table 2). These SSR alleles were randomly distributed among the three genomes, i.e., 37, 33, and 27 on A, B, and D genomes, respectively ($\chi^2 = 1.57$, $P = 0.4566$). However, distribution of accession-specific alleles was not random among homoeologous groups ($\chi^2 = 24.70$, $P = 0.0004$), i.e., 29 on group 1, 12 on each of groups 2 and 3, 8 on group 4, 6 on group 5, 13 on group 6 and 17 on 7. Thus, homoeologous groups 1 and 7 showed higher numbers of accession-specific SSR alleles (Table 3). Out of the 71 wheat accessions, 14 showed three or more accession-specific alleles. Chinese Spring originally from China, PI 135064 from Afghan, and PI 243659 from Iran, showed 7, 4, and 4 accession-specific alleles, respectively (Table 2).

If all the alleles in an SSR locus were equally frequent, the proportion ($F \approx 1/(1 + 4N_e u)$) of homozygotes would be the reciprocal of the number of alleles at the locus maintained in the population. Therefore, $n = 1/F$ may be used as a measure of the effective number of alleles maintained in the population, which in general will be less than the actual number (Kimura and Crow 1964). The effective number of alleles ranged from 1.121 to 13.585 with an average of 3.71 for the 81 SSR loci detected in the R+S group (Table 2). This effective number of alleles is positively correlated with the observed number of alleles ($r = 0.93$, $P < 0.001$). This means that the proportion of homozygotes would decrease, or heterozygosity/polymorphism would increase, with increase of the actual number of alleles at a locus in the analyzed bread wheat germplasm.

In comparison with the R+S group, both the R and S subgroups had lower total and average numbers of effective alleles estimated. For the R+S group, and R and S subgroups, the total number of effective alleles was 300.2, 279.7, and 259.9, respectively, and the average number of effective alleles was 3.71, 3.45, and 3.21, respectively (Table 2). However, this difference of number of effective alleles among the three groups of wheat accessions seems not as significant as that for the observed number of alleles.

Diversity indices provide important information about rarity and commonness of alleles at a locus. The Shannon diversity index (*H*) is one common diversity index often used to characterize allele diversity in a locus. Shannon's index accounts for both abundance and evenness of the alleles present (Shannon and Weaver 1949), and is useful for understanding allele structure at an SSR locus. Among the 81 SSR loci in the R+S group of wheat accessions, *H* averaged 1.291 and ranged from 0.249 to 2.866. For those loci with $H > 2$, the number of alleles must be $\geq$12, and for those loci with $H < 1$, the number of alleles would be $\leq$4 (Table 2). Thus, *H* is positively correlated with the allele number ($r = 0.94$, $P < 0.001$), and can be used to quantify the diversity or polymorphism of SSR markers. In 15 wild emmer wheat (*T. dicoccoides*) populations representing a wide range of ecological conditions of soil, temperature, and water availability in Israel and Turkey, this parameter (*H*) averaged 0.84 and ranged 0.166–1.307 (Fahima et al. 2002), and was lower than those (mean = 1.291, 1.226 and 1.134 for R+S, R, and S group or subgroup, respectively) reported in the present study (Table 2). Therefore, there is high genetic diversity as revealed by SSR markers in the bread wheat germplasm analyzed, and this diversity is even higher than that in the wild emmer wheat, the progenitor of cultivated tetraploid durum and hexaploid bread wheats. The genetic diversity of wheat thus have not decayed but increased during the long process of domestication of wild progenitor and cultivation/spread from Middle East to other parts of the world. This may be because of the narrowing of the wheat germplasm base can be averted and the genetic diversity can be subsequently increased through the introgression of novel materials, as Reif et al. (2005) reported.

From the R+S to R, and S group or subgroup, *H* decreased from 1.291 to 1.226, and to 1.134 (Table 2). There was little difference (0.065) between R+S group and R subgroup for *H*. This difference of *H* between R+S group and S subgroup was much larger (0.157), and also large (0.092) between R and S subgroups. This indicates again that there exists high genetic diversity in the RWA2-resistant wheat germplasm.

Nei's (1973) gene diversity or expected heterozygosity (*He*) is another common diversity index in population genetics and is equivalent to PIC. In the present study, gene diversity varied from 0.108 to 0.926 and averaged 0.609. *He* was >0.9 for SSR loci *Xgwm136*, *Xgwm88a*, *Xgwm111c*, *Xgwm146*, *Xgwm314*, and *Xgwm372*. Each of these loci had an allele number >15 (Table 2). The correlation between gene diversity and allele number is positive and highly significant ($r = 0.79$, $P < 0.001$). Bertin et al. (2004) found a value of *He* equal to 0.78 (0.323–0.936), analyzing spelt wheat accessions. Fahima et al. (2002) reported a *He* values of 0.5 (0.094–0.736) in analyzing wild emmer wheat accessions. Huang et al. (2002) showed a *He* value of 0.77

(0.43–0.94) in analysis of a set of common wheat germplasm from across all wheat producing regions. Roussel et al. (2004) revealed 0.662 (0.214–0.868) of *He* value in evaluating French bread wheat accessions. Roussel et al. (2005) also found a *He* value of 0.650 (0.211–0.899) in testing Eurasian bread wheat varieties. Khlestkina et al. (2004b) obtained a value of *He* equal to 0.70 (0.46–0.82) in evaluating Siberian common spring wheat. Teklu et al. (2006) analyzed Ethiopian tetraploid wheat landraces and found a *He* value equal to 0.684–0.688. Liu et al. (2007) showed a *He* value of 0.56 (0.18–0.80) in analyzing a Chinese wheat gene pool from recurrent selection. In the present study, *He* was estimated as 0.609, 0.591, and 0.581 for R+S, R, and S group or subgroup, respectively (Table 2). There was minor *He* difference (0.010–0.018) among these three group/subgroups of wheat accessions. Therefore, gene diversity of RWA2-resistant subgroup of wheat accessions is similar to that of the whole group involving resistant and susceptible accessions. Gene diversity as reflected by *He* index in the present study (Table 2) is comparable with previously published results of different wheat species or populations, but the range (0.818 = 0.926–0.108 for R+S, 0.846 = 0.919–0.073 for R subgroup, and 0.883 = 0.883–0.000 for S subgroup) is the greatest. This indicates that gene diversity is highly variable among different SSR loci in the bread wheat germplasm used in the present study.

The observed heterozygosity (*He-a*), proportion of observed heterozygotes at a given locus, was estimated for each of the 81 loci. The result showed that heterozygosity occurred only on five loci, accounting for 6.17% of the total SSR loci investigated. Furthermore, these five loci showed heterozygosity only distributed in 1AL, 3AL, 6DL, 7BS and an unknown chromosome regions (Table 2). It seems that heterozygote is a rare event detected only by a few SSR loci on a few chromosome regions in the bread wheat germplasm resistant or susceptible to RWA2. The average heterozygosity for the R+S, R, and S group or subgroups was 0.016, 0.018, and 0.012, respectively. This low rate of observed heterozygosity is expected because wheat is a typical self-pollinated crop species with outcrossing rates <1% and more than 1% for wheat plants grown in close proximity (Waines and Hegde 2003), and may explain why the observed heterozygosity has been simply ignored in the previous studies in wheat germplasm (Fahima et al. 1998, 2002; Huang et al. 2002; Alamerew at al 2004; Khlestkina et al. 2004a, b; Bertin et al. 2004; Roussel et al. 2004, 2005; Teklu et al. 2006; Zhang et al. 2006; Liu et al. 2007).

Contribution of A, B, D genomes to the genetic variation of wheat

Among the 67 SSR loci of known chromosome locations, 19 were located in A, 28 in B, and 20 in D genome, and the detected allele numbers were 153, 183, and 154 for A, B, and D genome, respectively (Table 3). The average number of alleles per locus for A, B, and D genome was 8.05, 6.54, and 7.70, respectively. This means that the alleles/locus of A genome is 34.6% and 4.5% higher than B and D genome, respectively; and D genome is 17.7% higher than B genome. Therefore, for the set of wheat accessions including both the RWA2-resistant and -susceptible used in this study, the contribution of A, B, and D genome to the genetic variation revealed by SSR markers can be ranked as A > D > B. But the three genomes can be ranked as D (1.470) > A (1.280) > B (1.264) based on Shannon index (*H*), and D (0.667) > B (0.613) > A (0.610) based on Nei's gene diversity (*He*) (Table 3). Roussel et al. (2004) also reported the inconsistent ranking based on allele number/locus and gene diversity. In a group of stripe rust-resistant *T. dicoccoides* accessions, the A genome possessed 20% higher number of SSR alleles than the B genome (Fahima et al. 1998). In an analysis of quantitative trait locus (QTL) in *T. dicoccoides*, the number of domestication-related QTL effects and domestication syndrome factors in the A genome was found to be higher than in the B genome due to the higher polymorphism for expressed traits in the A genome (Peng et al. 2003). In a set of 998 bread wheat accessions from 68 countries worldwide, the three genomes were ranked as B > A > D for both the SSR allele number and gene diversity (Huang et al. 2002). In a set of 96 random accessions of Chinese bread wheat, the three genomes were also ranked as B > A > D for SSR allele number (You et al. 2004). In French bread wheat accessions, the three genomes were ranked as A > D > B based on SSR alleles/locus and B > D > A based on gene diversity or PIC value (Roussel et al. 2004). In Ethiopian hexaploid wheat, the three genomes were ranked as B > A > D based on SSR alleles per locus (Alamerew et al. 2004). In Siberian common spring wheat, more SSR alleles were also detected in B genome than in A and D genomes (Khlestkina et al. 2004b). The A genome was more polymorphic than the B genome in the three Ethiopian tetraploid wheat species, *T. durum*, *T. dicoccon*, and *T. turgidum* (Teklu et al. 2006). In general, the A genome is more polymorphic than B or D genome in tetraploid wheat and some hexaploid wheat materials/ populations, and B genome is more polymorphic than A or D genome in many of the hexaploid wheat accessions.

Genetic distance

With the aid of Popgene computer program (Yeh and Yang 2000), Nei's (1972) GD and Nei's (1978) unbiased GD were estimated for the 71 × (71 − 1)/2 = 2485 possible pairs/combinations of wheat accessions. The results indicated that the GD is quite similar to the unbiased genetic

distance in the present study. The GD ranged from 0.054 to 1.933 with an average of 0.9832 (Appendix 1). The wheat collections used in this study have a wide geographical distribution and are mainly landraces. Large GD is thus expected in this set of wheat collections. The two Iranian landraces PI 621458 and PI 621462, both collected from East Azerbaijan, have the closest GD (0.054). The largest GD (1.933) occurred between Colorado breeding line CO970547-7, susceptible to RWA2, and Egyptian cultivar Bouhi 12 (PI 366103), resistant to RWA2. Thus a cross between CO970547-7 and Bouhi 12 could be used to develop a mapping population for the RWA2 resistance gene carried by Bouhi 12 because high SSR mapping efficiency would be expected in this cross.

The SSR-based GD is 0.69 (0.018–0.964) among a set of stripe rust-resistant *T. dicoccoides* accessions (Fahima et al. 1998), 1.862 (0.876–3.320) among 15 *T. dicoccoides* populations (Fahima et al. 2002), 0.30 (0.08–0.71) in set of Siberian common spring wheat varieties (Khlestkina et al. 2004b), 0.26 between *T. durum* and *T. turgidum*, 0.38 between *T. turgidum* and *T. dicoccon*, 0.34 between *T. durum* and *T. dicoccon* (Teklu et al. 2006). The GD among the bread wheat accessions (R+S group) in the present study is smaller than that among *T. dicoccoides* populations, but larger than that between *T. dicoccoides* accessions, Siberian common spring wheat cultivars, and even tetraploid wheat species. This again indicates a high genetic polymorphism existing in the bread wheat accessions used in this study.

We also estimated separately the pair-wise GD for R and S group of wheat accessions (data not shown) using the same set of 81 SSR loci. The results showed that the GD estimated in R or S subgroup was the same as that obtained in the R+S group for a specific pair of accessions. This is because of the large GD (Appendix 1) and the low observed heterozygosity (Table 2) in the wheat accessions used in the present study. The number of individuals to be used for estimating GD can be very small if the genetic distance is large and the average heterozygosity of the two species compared is low (Nei 1978).

Phylogenetic analysis

Based on Nei's original GD (Appendix 1), cluster analysis was carried out using the UPGMA method and resulted in a phylogenetic dendrogram shown in Fig. 2. The 71 wheat accessions could be divided into two mega-groups. Mega-group I included 20 wheat accessions that can be further clustered into four subgroups and mega-group II contained 51 accessions that could be clustered into nine subgroups. The pattern of clustering for most of the wheat accessions corresponded with the geographic distribution of wheat collections. This result is in agreement with several previous studies (Fahima et al. 1998, 2002; Huang et al. 2002; Bertin et al. 2004; You et al. 2004; Roussel et al. 2005).

Subgroup Ia contained only two accessions with relatively large GD (0.722), the genetically well characterized cultivar Chinese Spring, and a Colorado modern cultivar Yuma, both of which are susceptible to RWA2. Subgroup Ib contained five RWA2-resistant Iranian landraces. Subgroup Ic contained eight RWA2-susceptible modern cultivars developed and/or deployed in the western Great Plains of USA. Subgroup Id contained five central Asian accessions (one from Tajikistan, two from Kazakhstan, and one from each of Afghanistan and Iran), one of which was RWA2-susceptible (Fig. 2).

Subgroup IIa consisted of 18 accessions of which the majority (16) were RWA2-resistant Iranian landraces, one was a RWA2-resistant Egyptian cultivar, and one was a RWA2-susceptible Azerbaijan landrace. Subgroup IIb consisted of four accessions including one RWA2-resistant Bulgarian cultivar CItr 11349 (Varna 20), one RWA2-resistant Iranian landrace, one RWA2-resistant Afghanistan landrace, and one RWA2-susceptible Iranian landrace. Subgroup IIc contained one RWA2-susceptible Turkmenistan cultivar and two RWA2-resistant Iranian landraces. Subgroup IId contained four accessions including only one RWA2-resistant Iranian landrace and three RWA2-susceptible accessions (one Sweden landrace, one Afghanistan landrace and one US breeding line). Subgroup IIe contained seven RWA2-resistant Afghanistan landraces or cultivated materials and one RWA2-susceptible Iranian landrace. Subgroup IIf consisted of only two RWA2-resistant landraces, one from each of Iran and Afghan. Subgroup IIg consisted of eight RWA2-resistant landraces of which seven from Iran and one from Afghan. Subgroup IIh contained two RWA2-resistant Iranian landraces with GD of 0.587. Subgroup IIi consisted of two genetically distant RWA2-resistant Iranian landraces with GD of 0.919 (Fig. 2, Appendix 1).

As indicated in Fig. 2, among the 18 RWA2-susceptible accessions, 11 (61%) belonged to the mega-group I and the other seven (39%) belonged to mega-group II. Further analysis indicated that 13 (72%) of the 18 susceptible accessions were assigned to subgroups Ia, Ic and IId; 100% of the accessions in subgroups Ia and Ic, and 75% of the accessions in subgroup IId were susceptible to RWA2. Among the 53 RWA2-resistant accessions, 9 (17%) belonged to the mega-group I, and the other 44 (83%) belonged to the mega-group II. These resistant accessions distributed among >10 subgroups, indicating the existence of a rich genetic diversity among these bread wheat germplasm.

Phylogenetic analyses were also conducted for the R and S subgroup of the wheat accessions. The resulting dendrograms were presented in Figs. 3 and 4. It was indicated that the 53 RWA2-resistant wheat accessions

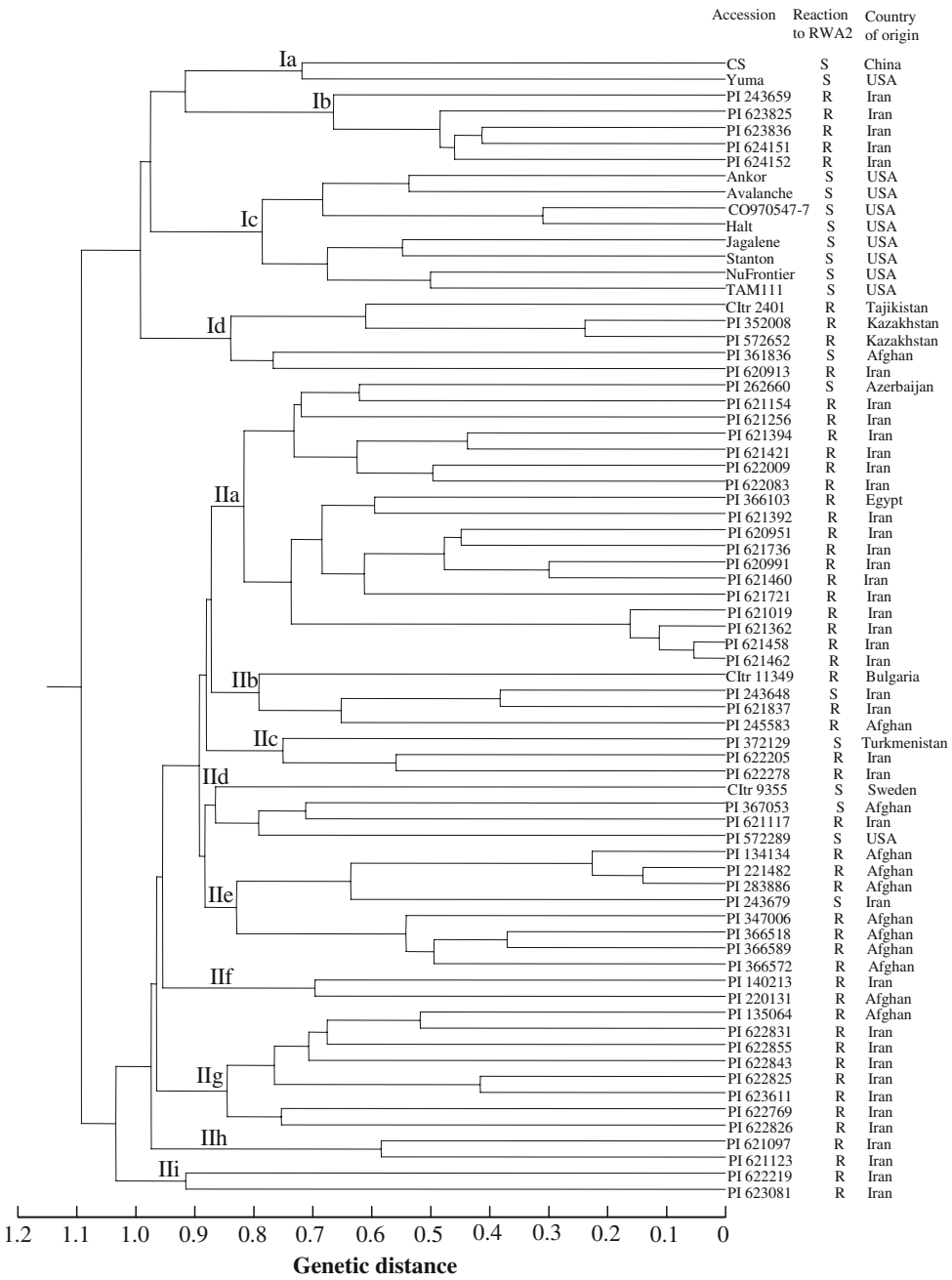


Fig. 2 Dendrogram of 71 wheat genotypes based on the Nei's (1972) original genetic distance calculated from data of 81 SSR loci, using the UPGMA as the clustering method. Marked groups are described in the section of result and discussion

could still be divided into two mega groups and further into many distinguishable subgroups (Fig. 3), and the classification was similar to that of whole set of accessions shown in Fig. 2. For example, all the resistant accessions in mega-group I (Fig. 2) were still in the same mega-group as showed in Fig. 3 with one exception of PI 621256 that belonged to mega-group II in analysis of the R+S group. In this analysis of R subgroup, majority (43) of the 53 RWA2-resistant accessions were still classified into the mega-group II, but each of the subgroups IIa and IIe in Fig. 2 were further divided into two subgroups in Fig. 3. In the analysis of S subgroup including 18 RWA2-susceptible accessions, the 11 accessions belonging to mega-group I and the 7 accessions belonging to mega-group II in Fig. 4 maintained basically the same classification as shown in Fig. 2. The 7 RWA2-susceptible accessions classified into five subgroups in Fig. 2 could be re-classified into two phylogenetic subgroups (Fig. 4).

This phylogenetic information of the relatedness of RWA2-resistant wheat accessions is of value for wheat breeding programs worldwide. The RWA2-susceptible USA cultivars belonged to a separate phylogenetic mega-group or subgroups from that of RWA2-resistant accessions originating from central Asia. Thus, the crosses

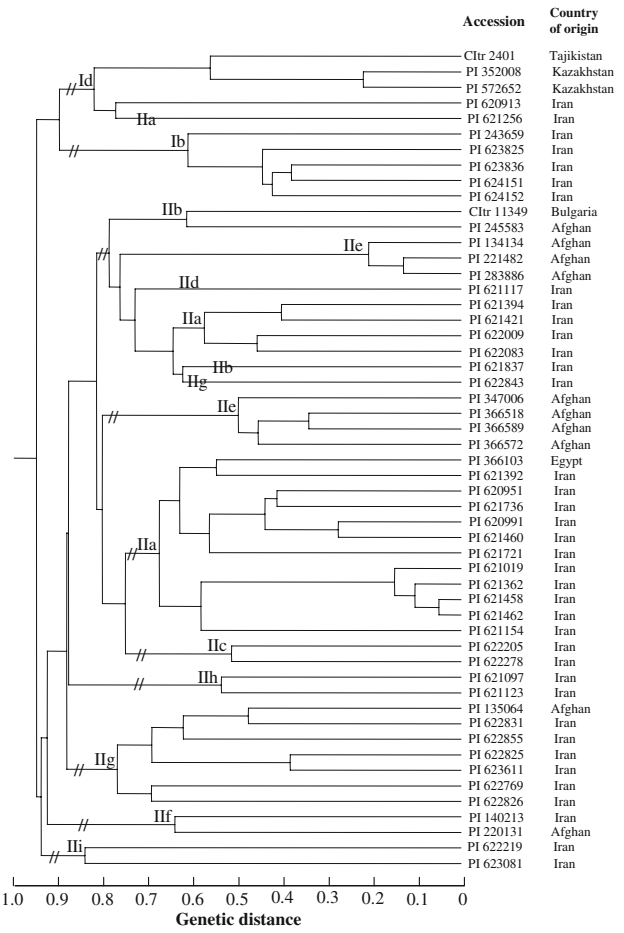


Fig. 3 Dendrogram of 53 RWA2-resistant wheat accessions based on the Nei's (1972) original genetic distance calculated from data of 81 SSR loci, using the UPGMA as the clustering method. The subgroup codes are correspondent to those shown in Fig. 2

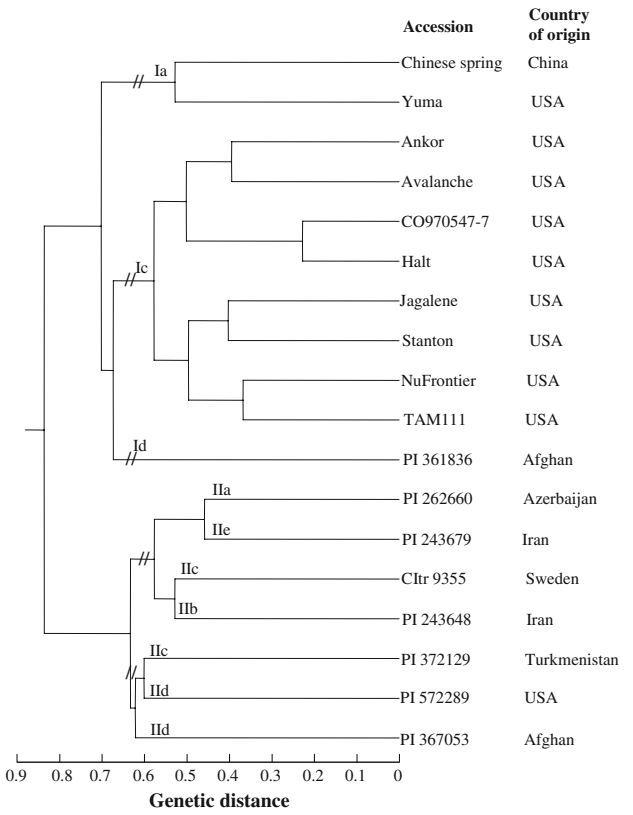


Fig. 4 Dendrogram of 18 RWA2-susceptible wheat accessions based on the Nei's (1972) original genetic distance calculated from data of 81 SSR loci, using the UPGMA as the clustering method. The subgroup codes are correspondent to those shown in Fig. 2

between the susceptible U.S./Colorado cultivars and the RWA2-resistant central Asian landraces or cultivars could introduce RWA resistance genes into Colorado wheat cultivars and meanwhile enhance the genetic diversity that is essential for sustainability of wheat cultivars. The phylogenetic grouping shown in Figs. 2 and 3 can be utilized to diversify the source of resistance genes against RWA2, and perhaps increase the chance of diversity in response to new biotypes of RWA. It is likely that individuals in one cluster carry genes that are different from individuals in another cluster. However, this hypothesis needs to be tested using gene mapping approaches and further allelism tests for those located in the same chromosome regions.

In an attempt to estimate the different contributions of each genome to the genetic variation of wheat, GDs were calculated based on each genome set of SSR loci separately. The resulting dendrograms (Appendix 2–4) showed that the clustering patterns are different from each other among A, B, and D genome, and from the whole wheat genome shown in Fig. 2. However, the grouping of RWA2-susceptible U.S. cultivars was similar for the genome subset of SSR loci and the whole genome, and particularly the A genome that had grouping pattern most similar to that based on the whole genome.

Association between RWA resistance and SSR markers

LC and LR are the two important traits used to determine reaction of wheat plants to infestation of the Russian wheat aphid (Anderson et al. 2003; Collins et al. 2005a, 2005b; Peng et al. 2007). AM could detect association between phenotype and genotype based on LD (Zondervan and Cardon 2004). In the present study, we used the AM approach to find SSR markers and chromosome regions potentially associated or linked with RWA2 resistance traits. The results showed that there were as many as 28 SSR marker loci showed significant correlation with LC (p-M < 0.01, p-P < 0.01, p-adj < 0.05). *Xgwm427b* showed marginally significant association with LC (p-M < 0.01, p-P < 0.01, p-adj $= 0.0596$). These marker loci could explain 10.49–40.80% of the total variation after fitting the other model effects (Table 4). However, there

Table 4 Chromosomes and SSR markers associated with RWA-resistance traits

Chromosome arm	Marker locus	Leaf chlorosis				Leaf rolling			
		R^{2a}	p-M[b]	p-P[c]	p-adj[d]	R^2	p-M	p-P	p-adj
1BL	*Xgwm124*	0.2190	0.0001	0.0016	0.0034	0.2409	0.0008	0.0012	0.0203
1DS	*Xcwem006a*	0.1718	<0.0001	0.0001	0.0001				
1DS	*Xgwm106*	0.3625	<0.0001	0.0002	0.0001	0.3686	0.0006	0.0006	0.0151
1DL	*Xgwm642*	0.1792	0.0003	0.0008	0.0053				
2AS	*Xgwm372*	0.2919	0.0009	0.0020	0.0310				
2AL	*Xgwm294*	0.4080	<0.0001	0.0001	0.0001				
2BL	Xgwm501	0.2346	0.0007	0.0047	0.0366				
2BL	*Xgwm526b*	0.2496	<0.0001	0.0002	0.0001				
3AS	*Xgwm369*	0.2625	0.0008	0.0060	0.0407	0.3136	0.0015	0.0021	0.0549
3BL	*Xgwm299*	0.2297	0.0005	0.0016	0.0136				
3DS	*Xgwm161*	0.2882	<0.0001	0.0003	0.0001	0.2723	0.0011	0.0017	0.0369
3DL	*Xgwm314*	0.3196	0.0006	0.0025	0.0269				
4BS	*Xgwm113*	0.1491	0.0008	0.0013	0.0204				
4BL	*Xgwm513*	0.2621	<0.0001	0.0001	0.0001	0.2359	0.0001	0.0003	0.0018
4DL	*Xgwm194a*	0.3045	<0.0001	0.0001	0.0001				
5BS	*Xgwm544*	0.1403	0.0013	0.0014	0.0385				
5BL	*Xgwm554*	0.1853	0.0003	0.0012	0.0057				
5DS	*Xgwm192c*	0.2352	<0.0001	0.0007	0.0001				
6AL	*Xgwm427a*	0.2684	0.0005	0.0023	0.0215				
6DL	Xcfd45c	0.1049	<0.0001	0.0036	0.0001	0.1077	0.0044	0.0058	0.0130
7AS	*Xgwm471*	0.3591	0.0005	0.0046	0.0215				
7BS	*Xgwm537b*	0.3655	<0.0001	0.0003	0.0001				
7DS	Xgwm111b	0.2029	0.0004	0.0013	0.0091	0.2604	0.0002	0.0004	0.0043
Unknown	*Xcfd45a*	0.2260	<0.0001	0.0009	0.0001				
Unknown	Xgwm111d	0.1468	0.0012	0.0017	0.0425				
Unknown	*Xgwm194c*	0.1153	0.0003	0.0001	0.0023				
Unknown	*Xgwm427b*	0.1709	0.0017	0.0024	0.0596	0.2409	0.0004	0.0003	0.0058
Unknown	*Xgwm427c*	0.2481	0.0002	0.0007	0.0026	0.2948	0.0004	0.0004	0.0067
Unknown	*Xgwm427e*	0.3302	<0.0001	0.0001	0.0001				

Number of permutations run = 10,000

[a] R^2 = The fraction of the total variation explained by the marker after fiting the other model effects

[b] p-M = Probability based on the F-distribution for specific markers

[c] p-P = The permutation-based test for marker significance of individual markers

[d] p-adj = The experiment-wise p-value for marker significance that controls the error-rate for the entire set of hypotheses

were only eight SSR marker loci showed significant association with LR (p-M < 0.01, p-P < 0.01, p-adj < 0.05). *Xgwm369* showed marginally significant association with LR (p-M < 0.01, p-P < 0.01, p-adj = 0.0549). These marker loci could explain 10.77–36.86% of the total variation (Table 4). According to Breseghello and Sorrells (2006), before marker-assisted selection based on the markers identified via AM can be applied to the progeny, a simple and essential step of confirmation is required for individual cultivars involved in crosses. This confirmation is necessary because the marker alleles are correlated with, but not entirely predictive of, the gene alleles. To confirm the association with the marker locus, for example, we can genotype F_2 plants and phenotype F_3 progeny as we did in wheat genome mapping (Peng et al. 1999, 2000a, b, 2003, 2007; Lapitan et al. 2007).

Up to date, 12 resistance genes against RWA have been identified, and most of them are located on group 1 and group 7 chromosomes of Triticeae (1D, 1R, and 7D) (Smith et al. 2004; Peng et al. 2007). In the present study, we found, with the aid of AM method, 29 SSR loci significantly associated with RWA resistance reflected by LC and/or LR in a set of bread wheat germplasm including both the RWA2-resistant and -susceptible accessions.

Theses loci distributed on at least 16 chromosomes and 21 chromosome arms. Except for the groups 1 and 7 chromosomes harboring the currently known RWA resistance genes, other 11 chromosomes belonging to 2, 3, 4, 5 and 6 homoeologous groups were also found to be associated with RWA2 resistance, especially LC (Table 4). Nevertheless, the number of chromosomes and homoeologous groups associated with LR was obviously smaller than those with LC, and additional homoeologous groups were 3, 4, and 6 except for the group 1 and 7. LC and LR are important traits reflecting RWA resistance but are not necessarily controlled by the same genes. Many loci associated with LC may not be correlated with LR. But all the loci for LR showed significant associations with LC (Table 4). Thus many genetic loci control LC, and part (1/3 ~ 9/29) of them control both LR and LC. The loci associated with RWA2 resistance on homoeologous groups 2, 3, 4, 5, and 6 must be new genes that have not been mapped, yet.

Acknowledgements This study was supported in part by the Chinese Academy of Sciences under the Important Directional Program of Knowledge Innovation Engineering Grant No. KSCX2-YW-Z-0722, the Colorado Wheat Research Foundation, the U.S. Department of Agriculture under Cooperative Agreements USDA Contract No. 2001–52100-11293 and USDA Contract No. 2003-34205-13636, the National Research Initiative of USDA's Cooperative State Research, Education and Extension Service, CAP Grant No. 2006-55606-16629, and Hatch Funds Project No. 644.

Appendix 1

Genetic distance and genetic identity matrix of 71 wheat accessions/cultivars[a]

Pop ID	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	–	0.367	0.404	0.293	0.409	0.211	0.262	0.379	0.415	0.357	0.471	0.486	0.299	0.349	0.299	0.329
2	1.004	–	0.429	0.368	0.372	0.260	0.312	0.280	0.390	0.331	0.322	0.399	0.461	0.470	0.362	0.360
3	0.907	0.847	–	0.293	0.297	0.347	0.399	0.379	0.427	0.331	0.322	0.361	0.374	0.432	0.375	0.447
4	1.229	1.000	1.229	–	0.583	0.520	0.574	0.440	0.414	0.548	0.470	0.439	0.375	0.277	0.322	0.258
5	0.895	0.990	1.213	0.539	–	0.444	0.485	0.440	0.568	0.527	0.593	0.453	0.304	0.342	0.367	0.279
6	1.558	1.347	1.059	0.653	0.811	–	0.733	0.440	0.333	0.489	0.395	0.391	0.329	0.266	0.367	0.279
7	1.341	1.166	0.919	0.555	0.725	0.311	–	0.430	0.373	0.479	0.410	0.431	0.331	0.217	0.331	0.343
8	0.971	1.275	0.971	0.820	0.822	0.822	0.844	–	0.489	0.577	0.539	0.480	0.417	0.353	0.311	0.286
9	0.880	0.941	0.850	0.882	0.566	1.099	0.987	0.715	–	0.451	0.605	0.422	0.360	0.380	0.380	0.266
10	1.031	1.105	1.105	0.601	0.640	0.715	0.735	0.551	0.796	–	0.559	0.489	0.387	0.339	0.319	0.280
11	0.754	1.133	1.133	0.754	0.523	0.929	0.892	0.619	0.503	0.582	–	0.404	0.391	0.323	0.342	0.279
12	0.722	0.919	1.018	0.824	0.791	0.938	0.841	0.735	0.862	0.716	0.907	–	0.325	0.392	0.357	0.386
13	1.207	0.774	0.984	0.980	1.190	1.111	1.105	0.874	1.021	0.950	0.938	1.124	–	0.379	0.370	0.374
14	1.052	0.755	0.840	1.285	1.073	1.325	1.530	1.043	0.968	1.083	1.130	0.937	0.970	–	0.357	0.432
15	1.209	1.016	0.982	1.135	1.002	1.002	1.105	1.167	0.968	1.142	1.073	1.031	0.996	1.030	–	0.406
16	1.111	1.021	0.805	1.355	1.278	1.278	1.071	1.253	1.323	1.272	1.278	0.951	0.984	0.840	0.900	–
17	1.031	0.851	1.013	1.162	0.965	1.230	1.182	0.996	0.916	0.979	0.932	0.804	0.894	0.965	1.008	0.978
18	1.272	0.889	1.127	1.402	1.210	1.344	1.247	1.353	1.169	1.224	1.231	1.173	1.173	1.031	1.013	1.089
19	1.089	0.874	0.935	1.134	1.297	1.344	1.163	1.089	1.056	1.192	1.092	1.204	1.050	1.115	0.822	0.951
20	1.127	0.904	0.788	1.237	1.169	1.169	0.948	1.228	1.190	1.161	1.210	0.998	1.068	0.913	0.851	0.242
21	1.105	0.827	0.948	1.399	1.146	1.467	1.461	1.050	1.035	1.221	1.035	1.083	0.882	0.722	0.749	0.813
22	1.065	0.995	1.222	0.901	0.885	1.163	1.331	1.029	0.798	0.903	0.798	0.942	0.942	0.989	0.923	1.140
23	1.320	0.627	0.833	1.308	1.371	1.371	1.228	1.555	1.153	1.472	1.153	1.294	0.919	0.840	0.900	0.663
24	1.050	0.932	0.871	1.313	1.207	1.127	1.121	1.163	1.186	1.373	1.207	1.160	1.083	1.092	0.671	0.731
25	1.228	1.018	0.919	1.259	1.169	1.297	1.124	1.137	1.253	1.141	1.320	1.059	1.059	0.897	1.049	0.211
26	0.886	0.839	0.969	1.210	1.043	1.536	1.415	1.004	1.025	1.072	0.905	0.948	0.983	0.964	1.037	0.902
27	1.209	0.932	1.188	1.105	1.130	1.212	1.124	0.783	0.935	0.923	1.055	1.067	0.579	1.086	1.145	1.231
28	1.204	0.932	1.105	0.752	0.967	1.053	0.795	0.916	0.935	0.947	1.001	0.782	0.755	1.214	0.879	1.105
29	1.114	0.876	0.735	1.484	1.177	**1.933**	1.452	1.095	1.157	1.291	1.137	1.037	0.904	0.760	1.215	0.803
30	1.038	0.971	0.938	1.380	1.234	1.529	1.467	1.159	1.234	1.086	1.133	1.137	1.099	0.783	1.089	0.862
31	0.971	0.819	0.971	1.285	1.114	1.588	1.272	1.083	1.234	1.124	1.096	0.959	0.992	1.108	1.016	0.805
32	0.971	0.907	0.847	1.285	1.153	1.717	1.365	1.012	1.077	1.086	0.957	1.099	1.027	0.783	1.052	0.987

Appendix 1 continued

Pop ID	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
33	1.074	0.971	0.877	1.431	1.114	1.421	1.317	1.242	0.974	1.342	1.133	1.099	1.176	0.825	0.885	0.877
34	1.086	0.822	0.822	1.054	0.982	1.252	1.161	1.134	0.854	0.953	0.869	1.036	1.073	0.939	0.891	0.897
35	1.074	0.938	1.190	1.108	1.173	1.300	1.018	0.938	1.007	0.962	1.024	1.053	0.648	1.070	1.209	1.444
36	0.938	0.971	0.877	1.000	1.024	1.133	0.919	0.862	0.822	0.913	0.926	0.761	0.761	0.948	1.016	1.092
37	1.294	0.802	0.802	1.205	1.231	1.368	1.225	1.272	1.056	1.512	1.150	1.247	0.856	0.921	0.837	0.935
38	1.341	0.672	0.802	1.340	1.190	1.471	1.412	1.147	1.056	1.303	1.150	1.015	0.799	0.829	0.837	0.935
39	1.323	0.850	0.880	1.047	0.993	1.176	1.130	1.193	1.062	1.167	1.136	1.092	0.777	0.903	1.111	0.941
40	1.383	1.121	1.047	0.973	1.143	1.579	1.405	1.358	1.247	1.449	1.225	1.240	1.080	1.052	1.221	1.408
41	1.007	0.880	0.741	1.012	0.961	1.303	1.130	1.234	1.099	1.127	1.099	0.987	1.130	0.843	0.968	0.822
42	1.256	1.024	1.024	0.867	0.868	1.176	1.130	1.371	0.961	1.127	0.961	1.150	1.150	0.968	0.968	1.041
43	1.018	0.624	0.830	1.248	1.004	1.863	1.412	1.272	1.021	1.303	1.190	1.050	0.827	0.773	0.962	0.935
44	0.984	0.802	1.294	1.205	1.004	1.275	1.068	1.147	0.923	1.171	0.907	0.981	0.981	1.058	1.105	1.071
45	1.278	0.822	0.941	1.083	1.099	1.259	1.169	1.234	1.099	1.167	1.062	1.210	0.805	0.903	1.150	0.880
46	1.256	0.858	0.800	1.361	1.259	1.569	1.253	1.181	1.095	1.230	1.194	1.043	1.157	0.881	0.866	0.814
47	1.177	0.835	0.926	1.272	1.029	1.180	1.058	1.273	0.961	1.237	1.201	1.049	1.014	0.827	0.756	0.738
48	1.191	0.719	0.919	1.089	0.971	1.377	1.108	1.191	1.058	1.021	1.114	0.965	0.965	0.938	0.913	0.858
49	1.190	0.777	0.833	1.026	1.153	1.234	1.187	1.092	1.007	1.067	0.974	1.250	0.774	0.900	1.070	0.877
50	1.194	0.696	0.923	1.292	1.285	1.485	1.325	1.281	1.043	1.315	1.118	1.037	0.873	0.957	0.869	0.971
51	1.323	0.822	0.910	1.083	1.099	1.216	1.169	1.193	1.099	1.127	1.062	1.297	0.833	0.903	1.111	0.794
52	1.374	0.828	1.212	0.981	1.194	1.328	1.422	1.073	1.023	1.219	1.154	1.070	0.755	0.905	0.946	1.212
53	1.053	0.748	0.748	1.124	1.111	1.190	1.105	0.874	0.777	0.984	0.847	0.916	0.669	0.859	0.996	0.935
54	1.150	0.954	1.092	1.458	1.193	1.474	1.582	1.092	1.077	1.272	1.114	1.207	0.830	0.811	0.965	0.819
55	1.247	0.932	0.813	0.994	1.166	1.294	1.288	1.050	1.108	1.480	1.341	1.047	0.824	0.769	0.959	0.841
56	1.147	0.855	0.948	1.039	1.150	1.373	1.227	1.188	1.002	1.173	1.111	1.031	0.929	0.886	1.030	0.811
57	0.981	0.813	0.901	1.313	1.018	1.441	1.160	1.291	1.001	1.373	1.127	1.047	1.047	1.019	0.959	0.932
58	1.086	0.964	0.706	1.141	1.207	1.495	1.121	1.015	1.035	1.070	0.984	1.012	0.978	1.131	0.993	0.841
59	0.975	0.991	1.262	1.058	1.047	1.334	1.428	1.080	1.102	1.141	1.121	0.939	1.077	1.048	1.095	1.177
60	1.166	0.951	0.802	1.124	1.190	1.320	1.183	1.001	1.210	1.213	1.074	1.086	0.856	1.022	0.929	0.874
61	1.153	1.193	1.114	1.243	1.027	1.350	1.393	1.193	0.993	1.252	1.062	0.923	0.987	1.073	0.903	0.794
62	0.910	0.941	1.077	1.383	1.062	1.259	1.210	1.007	0.993	1.209	1.027	0.987	1.210	1.037	1.234	1.077
63	1.114	1.041	1.041	1.160	1.136	1.303	1.130	1.193	0.993	1.209	1.062	1.056	1.092	1.278	0.935	1.007
64	0.919	0.984	0.984	1.205	0.938	1.231	1.032	0.874	0.923	1.055	0.938	0.744	0.981	0.921	0.996	0.844
65	1.225	1.338	1.086	1.256	1.146	1.035	1.065	1.247	1.146	1.221	1.146	1.047	1.047	1.224	1.028	0.901
66	1.247	1.143	1.032	1.181	1.341	1.341	1.012	1.204	1.365	1.425	1.341	1.121	1.201	1.131	1.315	0.901
67	0.879	1.008	0.942	1.164	1.102	1.358	1.259	0.958	0.994	1.248	1.029	0.971	1.005	1.105	1.240	1.008
68	0.836	0.926	0.990	1.029	0.868	1.350	1.393	0.822	0.757	0.840	0.731	0.971	1.074	0.985	1.111	1.153
69	0.923	1.056	0.862	0.926	0.910	1.278	1.108	0.938	0.822	0.921	0.880	0.830	0.859	1.080	1.016	1.056
70	0.849	1.157	0.849	0.945	0.994	1.180	1.134	0.926	0.898	0.877	0.929	0.971	0.907	1.145	1.039	1.157
71	1.029	1.065	1.140	1.108	1.015	1.163	1.118	0.995	0.827	1.115	0.981	0.792	1.008	1.312	1.099	1.311
Mean	1.103	0.934	0.962	1.114	1.053	1.268	1.154	1.068	1.003	1.100	1.027	1.006	0.966	0.990	1.010	0.974

Pop ID	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
1	0.357	0.280	0.337	0.324	0.331	0.345	0.267	0.350	0.293	0.412	0.299	0.300	0.328	0.354	0.379	0.379
2	0.427	0.411	0.417	0.405	0.438	0.370	0.534	0.394	0.361	0.432	0.394	0.394	0.417	0.379	0.441	0.404
3	0.363	0.324	0.393	0.455	0.388	0.295	0.435	0.419	0.399	0.380	0.305	0.331	0.480	0.391	0.379	0.429
4	0.313	0.246	0.322	0.290	0.247	0.406	0.271	0.269	0.284	0.298	0.331	0.472	0.227	0.252	0.277	0.277
5	0.381	0.298	0.273	0.311	0.318	0.413	0.254	0.299	0.311	0.352	0.323	0.380	0.308	0.291	0.328	0.316
6	0.292	0.261	0.261	0.311	0.231	0.313	0.254	0.324	0.273	0.215	0.298	0.349	**0.145**	0.217	0.204	0.180
7	0.307	0.288	0.313	0.388	0.232	0.264	0.293	0.326	0.325	0.243	0.325	0.451	0.234	0.231	0.280	0.256

Appendix 1 continued

Pop ID	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
8	0.370	0.259	0.337	0.293	0.350	0.357	0.211	0.313	0.321	0.367	0.457	0.400	0.334	0.314	0.339	0.363
9	0.400	0.311	0.348	0.304	0.355	0.450	0.316	0.305	0.286	0.359	0.393	0.393	0.315	0.291	0.291	0.341
10	0.376	0.294	0.304	0.313	0.295	0.405	0.229	0.253	0.320	0.342	0.397	0.388	0.275	0.338	0.325	0.338
11	0.394	0.292	0.335	0.298	0.355	0.450	0.316	0.299	0.267	0.405	0.348	0.368	0.321	0.322	0.334	0.384
12	0.447	0.309	0.300	0.369	0.339	0.390	0.274	0.314	0.347	0.387	0.344	0.458	0.355	0.321	0.383	0.333
13	0.409	0.309	0.350	0.344	0.414	0.390	0.399	0.339	0.347	0.374	0.561	0.470	0.405	0.333	0.371	0.358
14	0.381	0.357	0.328	0.401	0.486	0.372	0.432	0.336	0.408	0.381	0.338	0.297	0.468	0.457	0.330	0.457
15	0.365	0.363	0.440	0.427	0.473	0.398	0.406	0.511	0.350	0.355	0.318	0.415	0.297	0.337	0.362	0.349
16	0.376	0.337	0.386	0.785	0.444	0.320	0.516	0.481	0.810	0.406	0.292	0.331	0.448	0.422	0.447	0.373
17	–	0.374	0.377	0.396	0.391	0.367	0.325	0.404	0.374	0.410	0.424	0.449	0.434	0.411	0.449	0.386
18	0.984	–	0.497	0.344	0.426	0.352	0.436	0.329	0.288	0.407	0.306	0.361	0.358	0.361	0.399	0.386
19	0.975	0.699	–	0.406	0.477	0.289	0.424	0.370	0.334	0.328	0.376	0.426	0.361	0.371	0.358	0.371
20	0.926	1.068	0.901	–	0.464	0.390	0.573	0.451	0.869	0.381	0.338	0.370	0.481	0.430	0.467	0.430
21	0.939	0.853	0.741	0.768	–	0.398	0.481	0.547	0.414	0.422	0.409	0.359	0.438	0.438	0.400	0.450
22	1.004	1.044	1.240	0.942	0.923	–	0.433	0.322	0.327	0.443	0.385	0.410	0.357	0.357	0.395	0.370
23	1.124	0.830	0.859	0.557	0.731	0.838	–	0.406	0.498	0.491	0.394	0.350	0.423	0.485	0.534	0.497
24	0.907	1.111	0.995	0.795	0.603	1.134	0.901	–	0.455	0.389	0.313	0.377	0.400	0.378	0.378	0.353
25	0.984	1.247	1.096	0.141	0.882	1.118	0.696	0.789	–	0.361	0.319	0.342	0.497	0.424	0.424	0.424
26	0.893	0.899	1.114	0.966	0.864	0.815	0.712	0.945	1.019	–	0.355	0.468	0.465	0.563	0.589	0.589
27	0.859	1.185	0.979	1.086	0.894	0.955	0.932	1.161	1.144	1.037	–	0.441	0.355	0.318	0.406	0.356
28	0.801	1.020	0.853	0.995	1.026	0.891	1.050	0.975	1.074	0.760	0.819	–	0.381	0.366	0.391	0.341
29	0.836	1.028	1.020	0.732	0.825	1.031	0.861	0.916	0.699	0.765	1.036	0.965	–	0.540	0.451	0.527
30	0.889	1.018	0.992	0.844	0.827	1.029	0.725	0.973	0.859	0.575	1.147	1.006	0.617	–	0.603	0.689
31	0.800	0.919	1.027	0.761	0.916	0.929	0.627	0.973	0.859	0.529	0.900	0.940	0.796	0.507	–	0.615
32	0.953	0.951	0.992	0.844	0.799	0.995	0.699	1.041	0.859	0.529	1.034	1.077	0.641	0.372	0.486	–
33	0.970	0.935	0.741	0.774	0.813	0.995	0.847	0.989	0.967	0.935	1.209	1.153	0.884	1.038	0.938	0.847
34	0.864	0.894	0.756	0.878	0.830	1.004	0.866	0.947	0.959	1.015	1.122	1.052	0.828	0.913	0.978	0.913
35	0.913	1.250	1.035	1.272	1.015	1.029	1.092	1.464	1.441	1.137	0.239	0.948	1.114	1.150	0.938	1.038
36	0.836	1.001	0.889	0.967	1.124	1.029	1.092	1.204	1.071	0.969	0.870	0.771	0.955	1.056	0.892	0.777
37	1.121	0.973	0.932	0.916	0.653	0.975	0.735	0.678	0.909	1.019	1.164	0.838	0.732	0.866	0.809	0.927
38	0.942	0.909	0.813	0.948	0.755	1.008	0.761	0.929	0.973	0.883	0.851	0.838	0.585	0.728	0.809	0.809
39	1.052	0.987	1.056	0.892	0.844	0.856	0.822	1.071	0.954	0.938	0.968	0.830	0.792	0.941	0.767	0.974
40	1.115	1.147	1.137	1.177	0.955	1.111	0.995	0.988	1.319	1.128	1.080	1.058	0.867	1.056	1.020	1.020
41	1.089	0.987	0.987	0.777	0.935	0.916	0.910	0.935	0.805	0.858	1.325	0.844	0.820	0.794	0.910	0.741
42	0.999	1.210	1.169	0.971	0.935	0.948	0.794	1.018	1.056	1.062	1.055	1.089	0.991	0.941	1.077	0.941
43	0.975	1.114	0.871	0.948	0.755	1.008	0.788	0.929	1.006	0.867	0.882	0.853	0.632	0.927	0.781	0.754
44	0.942	1.236	1.105	1.086	0.929	0.879	1.108	0.929	1.153	1.056	0.913	0.898	1.037	0.992	1.062	0.992
45	1.052	0.954	0.954	0.805	0.844	0.827	0.767	0.967	0.892	0.938	0.968	0.830	0.738	0.850	0.741	0.880
46	1.076	0.965	1.008	0.716	0.700	0.976	0.758	0.921	0.900	0.985	1.212	1.058	0.598	0.772	0.828	0.772
47	1.009	0.846	0.915	0.775	0.656	0.916	0.765	0.752	0.817	0.858	1.021	0.927	0.783	0.725	0.806	0.650
48	0.863	0.833	0.982	0.870	0.766	0.942	0.745	0.712	0.862	0.867	1.148	0.752	0.729	0.836	0.993	0.865
49	1.049	0.984	0.951	0.802	0.871	0.809	0.738	0.998	0.919	0.902	0.965	0.932	0.832	0.892	0.751	0.892
50	0.929	0.850	0.758	0.858	0.755	0.867	0.684	0.916	1.046	0.919	1.000	0.783	0.631	0.854	0.884	0.980
51	1.052	0.923	0.954	0.777	0.844	0.827	0.767	0.967	0.833	0.938	1.002	0.889	0.820	0.910	0.794	0.974
52	0.959	0.924	1.168	1.231	0.979	0.910	0.952	1.013	1.310	1.040	0.866	0.897	0.904	1.064	1.102	1.140
53	0.790	0.940	0.841	1.086	0.809	0.942	0.844	1.029	1.153	0.821	0.851	0.809	0.758	0.837	0.754	0.866
54	0.978	0.889	0.984	0.802	0.384	1.029	1.038	0.706	0.830	0.870	0.965	1.068	0.861	0.923	1.021	0.923
55	0.845	1.150	1.140	0.824	0.806	1.259	0.998	0.806	0.845	1.173	1.010	1.198	0.769	0.878	0.973	0.940

Appendix 1 continued

Pop ID	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
56	0.859	0.905	0.808	0.851	0.863	1.138	0.900	0.790	0.815	1.018	0.863	0.894	0.933	0.908	1.007	0.877
57	0.907	1.038	0.867	0.945	0.725	1.096	0.964	0.835	0.875	1.091	1.083	0.792	0.916	1.041	1.114	0.973
58	0.907	1.111	0.995	0.755	0.926	1.134	0.841	0.926	0.845	0.980	1.010	0.975	0.825	1.041	0.909	0.848
59	0.900	1.032	1.058	1.095	1.020	1.128	1.099	1.020	1.270	0.939	1.115	1.151	1.046	0.966	0.842	1.147
60	0.790	0.909	0.932	0.771	0.728	1.044	0.967	0.809	0.878	0.948	0.979	0.838	0.706	0.809	0.927	0.959
61	0.982	1.130	1.021	0.892	0.935	0.856	1.041	1.071	0.862	1.226	1.002	1.186	0.942	1.007	1.153	1.234
62	0.796	1.169	1.210	1.130	0.904	1.124	1.278	0.935	1.130	1.025	1.037	1.001	1.008	1.153	1.007	1.077
63	0.825	1.130	1.253	1.021	1.108	1.015	1.114	0.874	0.954	1.062	1.037	0.935	0.974	1.193	0.941	1.007
64	0.520	1.041	1.032	0.856	0.867	1.080	1.147	0.809	0.792	1.056	1.013	0.898	0.814	0.959	0.896	0.992
65	0.680	1.121	1.243	0.929	0.864	1.174	0.998	0.700	0.913	0.945	1.141	0.975	1.016	0.916	0.799	0.948
66	0.939	1.111	1.140	0.945	1.137	1.305	1.032	0.895	1.003	1.239	1.203	0.958	1.128	1.153	0.878	1.236
67	0.933	1.340	1.237	0.971	0.904	1.128	1.044	0.985	0.947	1.232	0.969	1.131	0.993	1.108	1.000	1.108
68	0.840	1.190	1.169	1.092	0.935	0.598	1.234	1.053	1.111	0.858	0.951	1.018	0.991	1.024	0.926	0.957
69	0.913	1.218	1.166	0.967	1.050	0.678	1.169	1.068	1.027	1.004	1.016	0.932	0.939	1.159	1.121	1.121
70	0.933	1.554	1.154	1.058	1.234	0.716	1.240	1.171	1.164	1.046	0.969	0.904	1.102	1.229	1.035	1.187
71	0.969	1.380	1.198	1.198	1.174	0.681	1.311	1.259	1.285	1.088	1.179	0.891	1.031	1.180	1.140	1.222
Mean	0.941	1.073	1.022	0.931	0.912	0.984	0.963	0.980	0.993	0.973	1.016	0.948	0.928	0.976	0.939	0.959

Pop ID	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48
1	0.342	0.338	0.342	0.391	0.274	0.262	0.266	0.251	0.365	0.285	0.361	0.374	0.279	0.285	0.308	0.304
2	0.379	0.440	0.391	0.379	0.449	0.511	0.427	0.326	0.415	0.359	0.536	0.449	0.440	0.424	0.434	0.487
3	0.416	0.440	0.304	0.416	0.449	0.449	0.415	0.351	0.477	0.359	0.436	0.274	0.390	0.449	0.396	0.399
4	0.239	0.348	0.330	0.368	0.300	0.262	0.351	0.378	0.364	0.420	0.287	0.300	0.339	0.256	0.280	0.337
5	0.328	0.375	0.310	0.359	0.292	0.304	0.370	0.319	0.383	0.420	0.367	0.367	0.333	0.284	0.357	0.379
6	0.242	0.286	0.272	0.322	0.255	0.230	0.309	0.206	0.272	0.309	0.155	0.280	0.284	0.208	0.307	0.252
7	0.268	0.313	0.361	0.399	0.294	0.244	0.323	0.245	0.323	0.323	0.244	0.344	0.311	0.286	0.347	0.330
8	0.289	0.322	0.391	0.422	0.280	0.318	0.303	0.257	0.291	0.254	0.280	0.318	0.291	0.307	0.280	0.304
9	0.378	0.426	0.365	0.440	0.348	0.348	0.346	0.288	0.333	0.383	0.360	0.398	0.333	0.335	0.383	0.347
10	0.261	0.386	0.382	0.401	0.221	0.272	0.311	0.235	0.324	0.324	0.272	0.310	0.311	0.292	0.290	0.360
11	0.322	0.419	0.359	0.396	0.317	0.317	0.321	0.294	0.333	0.383	0.304	0.404	0.346	0.303	0.301	0.328
12	0.333	0.355	0.349	0.467	0.288	0.363	0.335	0.289	0.373	0.317	0.350	0.375	0.298	0.352	0.350	0.381
13	0.308	0.342	0.523	0.467	0.425	0.450	0.460	0.340	0.323	0.317	0.438	0.375	0.447	0.314	0.363	0.381
14	0.438	0.391	0.343	0.387	0.398	0.436	0.405	0.349	0.431	0.380	0.462	0.347	0.405	0.414	0.437	0.392
15	0.413	0.410	0.299	0.362	0.433	0.433	0.329	0.295	0.380	0.380	0.382	0.331	0.317	0.421	0.470	0.401
16	0.416	0.408	0.236	0.335	0.393	0.393	0.390	0.245	0.440	0.353	0.393	0.343	0.415	0.443	0.478	0.424
17	0.379	0.422	0.401	0.433	0.326	0.390	0.349	0.328	0.337	0.368	0.377	0.390	0.349	0.341	0.365	0.422
18	0.393	0.409	0.287	0.368	0.378	0.403	0.373	0.318	0.373	0.298	0.328	0.291	0.385	0.381	0.429	0.435
19	0.477	0.470	0.355	0.411	0.394	0.444	0.348	0.321	0.373	0.311	0.419	0.331	0.385	0.365	0.401	0.375
20	0.461	0.415	0.280	0.380	0.400	0.388	0.410	0.308	0.460	0.379	0.388	0.338	0.447	0.489	0.461	0.419
21	0.444	0.436	0.363	0.325	0.520	0.470	0.430	0.385	0.393	0.393	0.470	0.395	0.430	0.497	0.519	0.465
22	0.370	0.367	0.357	0.357	0.377	0.365	0.425	0.329	0.400	0.388	0.365	0.415	0.438	0.377	0.400	0.390
23	0.429	0.421	0.335	0.335	0.480	0.467	0.440	0.370	0.403	0.452	0.455	0.330	0.464	0.468	0.465	0.475
24	0.372	0.388	0.231	0.300	0.508	0.395	0.343	0.372	0.393	0.361	0.395	0.395	0.380	0.398	0.472	0.491
25	0.380	0.384	0.237	0.343	0.403	0.378	0.385	0.267	0.447	0.348	0.366	0.316	0.410	0.406	0.442	0.422
26	0.393	0.363	0.321	0.380	0.361	0.414	0.392	0.324	0.424	0.346	0.420	0.348	0.392	0.374	0.424	0.420
27	0.299	0.326	0.788	0.419	0.312	0.427	0.380	0.340	0.266	0.348	0.414	0.401	0.380	0.298	0.360	0.317
28	0.316	0.349	0.388	0.463	0.433	0.433	0.436	0.347	0.430	0.337	0.426	0.408	0.436	0.347	0.396	0.471
29	0.413	0.437	0.328	0.385	0.481	0.557	0.453	0.420	0.440	0.371	0.532	0.355	0.478	0.550	0.457	0.482
30	0.354	0.401	0.317	0.348	0.421	0.483	0.390	0.348	0.452	0.390	0.396	0.371	0.427	0.462	0.484	0.434

Appendix 1 continued

Pop ID	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48
31	0.391	0.376	0.391	0.410	0.446	0.446	0.464	0.361	0.403	0.341	0.458	0.346	0.477	0.437	0.447	0.370
32	0.429	0.401	0.354	0.460	0.396	0.446	0.378	0.361	0.477	0.390	0.470	0.371	0.415	0.462	0.522	0.421
33	–	0.440	0.317	0.385	0.383	0.408	0.353	0.348	0.489	0.403	0.396	0.308	0.328	0.487	0.434	0.364
34	0.822	–	0.376	0.395	0.457	0.470	0.464	0.434	0.464	0.451	0.431	0.393	0.476	0.409	0.458	0.393
35	1.150	0.978	–	0.404	0.337	0.424	0.403	0.389	0.279	0.359	0.386	0.386	0.378	0.323	0.346	0.323
36	0.954	0.929	0.907	–	0.293	0.393	0.365	0.282	0.390	0.421	0.430	0.430	0.365	0.431	0.415	0.342
37	0.959	0.783	1.089	1.228	–	0.625	0.522	0.591	0.472	0.416	0.450	0.388	0.534	0.530	0.451	0.400
38	0.896	0.756	0.859	0.935	0.470	–	0.534	0.554	0.435	0.404	0.588	0.450	0.522	0.581	0.502	0.489
39	1.041	0.769	0.910	1.007	0.651	0.627	–	0.463	0.420	0.370	0.559	0.398	0.889	0.435	0.470	0.435
40	1.056	0.834	0.945	1.265	0.526	0.592	0.771	–	0.400	0.556	0.415	0.340	0.450	0.419	0.378	0.364
41	0.715	0.769	1.278	0.941	0.751	0.833	0.868	0.916	–	0.420	0.460	0.348	0.407	0.461	0.508	0.524
42	0.910	0.796	1.024	0.865	0.877	0.907	0.993	0.587	0.868	–	0.391	0.391	0.395	0.398	0.395	0.341
43	0.927	0.841	0.951	0.844	0.799	0.532	0.582	0.879	0.777	0.938	–	0.525	0.547	0.441	0.489	0.527
44	1.176	0.934	0.951	0.844	0.948	0.799	0.923	1.080	1.056	0.938	0.644	–	0.435	0.340	0.489	0.502
45	1.114	0.742	0.974	1.007	0.627	0.651	0.118	0.798	0.898	0.929	0.604	0.833	–	0.448	0.458	0.435
46	0.719	0.894	1.131	0.843	0.635	0.543	0.831	0.871	0.775	0.922	0.818	1.080	0.803	–	0.494	0.403
47	0.835	0.781	1.062	0.879	0.796	0.690	0.754	0.973	0.678	0.929	0.715	0.715	0.782	0.706	–	0.644
48	1.011	0.934	1.131	1.073	0.916	0.716	0.831	1.010	0.647	1.077	0.641	0.690	0.831	0.908	0.440	–
49	1.150	0.866	0.923	1.021	0.709	0.709	0.194	0.898	0.974	1.041	0.659	0.967	0.122	0.843	0.792	0.800
50	1.031	0.889	1.095	1.040	0.488	0.300	0.712	0.739	0.910	1.080	0.597	0.828	0.687	0.509	0.703	0.652
51	1.153	0.854	1.041	1.077	0.651	0.699	0.175	0.916	0.993	0.929	0.699	0.923	0.104	0.891	0.782	0.775
52	1.290	1.039	0.858	1.002	0.690	0.571	0.955	0.634	1.134	1.154	0.948	0.982	0.891	0.817	0.883	0.869
53	0.896	0.783	0.802	0.904	0.450	0.450	0.699	0.879	0.833	1.038	0.771	0.856	0.675	0.762	0.947	0.855
54	0.938	0.897	1.056	1.169	0.874	0.735	0.910	0.961	0.910	1.114	0.816	0.967	0.850	0.904	0.662	0.772
55	1.023	0.981	1.105	0.948	0.898	0.755	1.001	0.722	1.001	0.919	0.703	0.898	1.035	1.175	0.674	0.852
56	0.832	0.948	1.052	0.999	0.851	0.662	0.814	0.875	0.786	0.873	0.637	0.712	0.814	1.099	0.598	0.671
57	1.059	0.981	1.143	1.204	0.898	0.809	0.874	0.955	0.761	0.984	0.653	0.838	0.874	1.022	0.674	0.568
58	1.023	0.947	1.183	0.948	0.838	0.838	0.904	0.891	0.904	0.919	0.809	1.140	1.001	0.921	1.064	0.913
59	1.296	1.009	1.099	1.008	1.058	0.955	0.898	0.797	1.311	1.121	1.058	1.095	0.929	1.254	1.134	1.007
60	1.027	0.902	1.089	1.071	0.744	0.575	0.751	0.820	0.954	1.190	0.916	1.204	0.725	0.790	0.884	0.797
61	0.941	0.885	1.007	0.974	1.021	0.892	1.027	1.050	0.839	0.993	0.923	0.987	0.993	1.134	0.898	0.861
62	0.767	0.948	1.114	0.910	1.092	0.892	1.176	1.086	1.062	0.993	0.954	0.751	1.136	1.095	0.898	0.988
63	1.007	0.885	1.041	0.974	1.021	1.021	0.757	0.981	0.868	1.027	0.923	0.987	0.731	1.134	0.809	0.861
64	0.927	1.001	1.018	0.788	0.886	0.856	1.130	1.044	0.987	0.971	0.827	0.827	1.056	1.043	0.915	0.797
65	1.032	0.923	1.124	1.015	0.961	1.065	0.874	0.955	0.967	0.904	1.102	0.929	0.874	1.067	0.843	0.882
66	1.302	0.947	0.998	1.124	1.029	1.140	0.788	0.955	1.272	1.389	1.065	1.102	0.788	1.261	1.223	1.013
67	1.071	0.877	1.008	0.958	1.040	1.040	1.102	1.108	1.180	0.994	1.005	0.876	1.180	1.254	0.890	0.972
68	1.024	0.869	1.059	0.957	1.190	1.111	1.099	1.143	1.176	0.961	1.004	0.877	1.099	1.040	1.012	1.154
69	1.121	0.987	1.056	0.819	1.127	1.127	1.114	1.121	1.007	0.974	0.951	0.919	1.041	1.064	1.147	1.019
70	1.368	1.121	1.008	0.806	1.282	1.328	1.140	1.322	1.180	1.140	1.040	1.077	1.140	1.254	1.175	1.200
71	1.140	0.936	1.222	0.929	1.240	1.198	1.163	1.325	1.050	1.163	1.080	1.008	1.124	1.246	1.089	1.083
Mean	1.021	0.924	1.052	0.966	0.929	0.877	0.907	1.034	0.954	1.010	0.901	0.981	0.896	0.973	0.888	0.905

Pop ID	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64
1	0.304	0.303	0.266	0.253	0.349	0.317	0.288	0.318	0.375	0.338	0.377	0.312	0.316	0.403	0.328	0.399
2	0.460	0.499	0.440	0.437	0.474	0.385	0.394	0.426	0.444	0.381	0.371	0.386	0.303	0.390	0.353	0.374
3	0.435	0.398	0.403	0.298	0.474	0.335	0.444	0.387	0.406	0.494	0.283	0.449	0.328	0.341	0.353	0.374
4	0.359	0.275	0.339	0.375	0.325	0.233	0.370	0.354	0.269	0.320	0.347	0.325	0.288	0.251	0.314	0.300
5	0.316	0.277	0.333	0.303	0.329	0.303	0.312	0.317	0.361	0.299	0.351	0.304	0.358	0.346	0.321	0.391

Appendix 1 continued

Pop ID	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64
6	0.291	0.227	0.296	0.265	0.304	0.229	0.274	0.253	0.237	0.224	0.263	0.267	0.259	0.284	0.272	0.292
7	0.305	0.266	0.311	0.241	0.331	0.206	0.276	0.293	0.314	0.326	0.240	0.306	0.249	0.298	0.323	0.356
8	0.335	0.278	0.303	0.342	0.417	0.335	0.350	0.305	0.275	0.363	0.340	0.368	0.303	0.365	0.303	0.417
9	0.365	0.352	0.333	0.360	0.460	0.341	0.330	0.367	0.368	0.355	0.332	0.298	0.370	0.370	0.370	0.398
10	0.344	0.269	0.324	0.296	0.374	0.280	0.228	0.309	0.253	0.343	0.319	0.297	0.286	0.299	0.299	0.348
11	0.378	0.327	0.346	0.316	0.429	0.328	0.262	0.329	0.324	0.374	0.326	0.342	0.346	0.358	0.346	0.391
12	0.287	0.355	0.273	0.343	0.400	0.299	0.351	0.357	0.351	0.364	0.391	0.338	0.398	0.373	0.348	0.475
13	0.461	0.418	0.435	0.470	0.513	0.436	0.439	0.395	0.351	0.376	0.341	0.425	0.373	0.298	0.335	0.375
14	0.406	0.384	0.405	0.405	0.424	0.445	0.463	0.412	0.361	0.323	0.351	0.360	0.342	0.355	0.279	0.398
15	0.343	0.419	0.329	0.388	0.370	0.381	0.383	0.357	0.383	0.371	0.334	0.395	0.405	0.291	0.393	0.370
16	0.416	0.379	0.452	0.298	0.393	0.441	0.431	0.445	0.394	0.431	0.308	0.417	0.452	0.341	0.365	0.430
17	0.350	0.395	0.349	0.383	0.454	0.376	0.430	0.424	0.404	0.404	0.407	0.454	0.375	0.451	0.438	0.594
18	0.374	0.427	0.398	0.397	0.391	0.411	0.317	0.405	0.354	0.329	0.357	0.403	0.323	0.311	0.323	0.353
19	0.386	0.468	0.385	0.311	0.431	0.374	0.320	0.446	0.420	0.370	0.347	0.394	0.360	0.298	0.286	0.356
20	0.449	0.424	0.460	0.292	0.338	0.449	0.439	0.427	0.389	0.470	0.334	0.463	0.410	0.323	0.360	0.425
21	0.419	0.470	0.430	0.376	0.445	0.681	0.447	0.422	0.484	0.396	0.361	0.483	0.393	0.405	0.330	0.420
22	0.445	0.420	0.438	0.403	0.390	0.357	0.284	0.321	0.334	0.322	0.324	0.352	0.425	0.325	0.363	0.340
23	0.478	0.505	0.464	0.386	0.430	0.354	0.369	0.406	0.381	0.431	0.333	0.380	0.353	0.279	0.328	0.318
24	0.369	0.400	0.380	0.363	0.357	0.494	0.447	0.454	0.434	0.396	0.361	0.445	0.343	0.393	0.418	0.445
25	0.399	0.351	0.435	0.270	0.316	0.436	0.430	0.443	0.417	0.430	0.281	0.416	0.422	0.323	0.385	0.453
26	0.406	0.399	0.392	0.354	0.440	0.419	0.310	0.361	0.336	0.375	0.391	0.387	0.294	0.359	0.346	0.348
27	0.381	0.368	0.367	0.421	0.427	0.381	0.364	0.422	0.339	0.364	0.328	0.376	0.367	0.355	0.355	0.363
28	0.394	0.457	0.411	0.408	0.445	0.344	0.302	0.409	0.453	0.377	0.317	0.433	0.305	0.368	0.393	0.408
29	0.435	0.532	0.440	0.405	0.468	0.423	0.464	0.394	0.400	0.438	0.351	0.494	0.390	0.365	0.377	0.443
30	0.410	0.426	0.403	0.345	0.433	0.398	0.416	0.403	0.353	0.353	0.381	0.446	0.365	0.316	0.303	0.383
31	0.472	0.413	0.452	0.332	0.470	0.360	0.378	0.365	0.328	0.403	0.431	0.396	0.316	0.365	0.390	0.408
32	0.410	0.375	0.378	0.320	0.421	0.398	0.391	0.416	0.378	0.428	0.318	0.383	0.291	0.341	0.365	0.371
33	0.317	0.357	0.316	0.275	0.408	0.391	0.359	0.435	0.347	0.359	0.274	0.358	0.390	0.464	0.365	0.396
34	0.421	0.411	0.426	0.354	0.457	0.408	0.375	0.388	0.375	0.388	0.365	0.406	0.413	0.388	0.413	0.368
35	0.398	0.334	0.353	0.424	0.449	0.348	0.331	0.349	0.319	0.306	0.333	0.337	0.365	0.328	0.353	0.361
36	0.360	0.353	0.341	0.367	0.405	0.311	0.388	0.368	0.300	0.388	0.365	0.343	0.378	0.403	0.378	0.455
37	0.492	0.614	0.522	0.502	0.638	0.417	0.408	0.427	0.408	0.433	0.347	0.475	0.360	0.335	0.360	0.413
38	0.492	0.741	0.497	0.565	0.638	0.480	0.470	0.516	0.445	0.433	0.385	0.563	0.410	0.410	0.360	0.425
39	0.824	0.491	0.840	0.385	0.497	0.403	0.368	0.443	0.418	0.405	0.408	0.472	0.358	0.309	0.469	0.323
40	0.408	0.478	0.400	0.530	0.415	0.383	0.486	0.417	0.385	0.410	0.451	0.440	0.350	0.338	0.375	0.352
41	0.378	0.403	0.370	0.322	0.435	0.403	0.368	0.456	0.467	0.405	0.270	0.385	0.432	0.346	0.420	0.373
42	0.353	0.340	0.395	0.316	0.354	0.328	0.399	0.418	0.374	0.399	0.326	0.304	0.370	0.370	0.358	0.379
43	0.517	0.551	0.497	0.387	0.463	0.442	0.495	0.529	0.520	0.445	0.347	0.400	0.398	0.385	0.398	0.438
44	0.380	0.437	0.398	0.375	0.425	0.380	0.408	0.491	0.433	0.320	0.334	0.300	0.373	0.472	0.373	0.438
45	0.886	0.503	0.901	0.410	0.509	0.427	0.355	0.443	0.418	0.368	0.395	0.485	0.370	0.321	0.482	0.348
46	0.431	0.601	0.410	0.442	0.467	0.405	0.309	0.333	0.360	0.398	0.285	0.454	0.322	0.335	0.322	0.352
47	0.453	0.495	0.458	0.414	0.388	0.516	0.510	0.550	0.510	0.345	0.322	0.413	0.408	0.408	0.445	0.401
48	0.449	0.521	0.461	0.419	0.426	0.462	0.427	0.511	0.567	0.401	0.365	0.451	0.423	0.372	0.423	0.451
49	–	0.517	**0.947**	0.437	0.567	0.441	0.394	0.445	0.406	0.419	0.396	0.492	0.341	0.303	0.489	0.330
50	0.659	–	0.503	0.585	0.601	0.505	0.413	0.465	0.451	0.464	0.377	0.500	0.340	0.327	0.377	0.373
51	**0.054**	0.687	–	0.423	0.534	0.427	0.368	0.443	0.430	0.380	0.395	0.460	0.333	0.296	0.457	0.323
52	0.828	0.536	0.861	–	0.514	0.399	0.503	0.395	0.299	0.350	0.417	0.451	0.385	0.360	0.322	0.375
53	0.567	0.509	0.627	0.665	–	0.467	0.382	0.503	0.458	0.408	0.423	0.438	0.410	0.410	0.398	0.488

Appendix 1 continued

Pop ID	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64
54	0.819	0.684	0.850	0.919	0.761	–	0.506	0.508	0.519	0.406	0.396	0.492	0.452	0.489	0.390	0.467
55	0.932	0.885	1.001	0.687	0.961	0.681	–	0.556	0.472	0.459	0.462	0.508	0.455	0.442	0.368	0.571
56	0.811	0.767	0.814	0.929	0.687	0.677	0.587	–	0.607	0.428	0.367	0.401	0.481	0.418	0.431	0.491
57	0.901	0.797	0.844	1.206	0.782	0.656	0.751	0.499	–	0.371	0.348	0.395	0.430	0.455	0.418	0.508
58	0.871	0.769	0.967	1.049	0.898	0.901	0.778	0.848	0.991	–	0.361	0.571	0.343	0.355	0.368	0.395
59	0.926	0.976	0.929	0.875	0.861	0.926	0.772	1.004	1.055	1.020	–	0.398	0.307	0.383	0.357	0.435
60	0.709	0.693	0.777	0.797	0.827	0.709	0.678	0.913	0.929	0.561	0.923	–	0.385	0.447	0.335	0.450
61	1.077	1.080	1.099	0.955	0.892	0.794	0.788	0.732	0.844	1.071	1.180	0.954	–	0.383	0.469	0.509
62	1.193	1.118	1.216	1.023	0.892	0.715	0.816	0.873	0.788	1.035	0.961	0.805	0.961	–	0.420	0.646
63	0.715	0.974	0.784	1.134	0.923	0.941	1.001	0.843	0.874	1.001	1.029	1.092	0.757	0.868	–	0.422
64	1.108	0.985	1.130	0.982	0.719	0.761	0.561	0.712	0.678	0.929	0.832	0.799	0.675	0.437	0.862	–
65	0.964	1.070	0.967	1.250	0.929	0.706	0.725	0.749	0.806	0.725	0.952	0.867	0.874	0.844	0.709	0.678
66	0.731	1.016	0.761	0.913	1.102	1.032	0.864	1.161	1.137	1.137	0.919	0.995	1.467	1.001	1.001	0.961
67	1.157	1.102	1.222	1.118	0.939	0.765	0.800	0.857	0.858	1.020	0.766	0.939	0.782	0.418	0.867	0.684
68	0.974	1.043	1.062	1.154	0.847	0.850	1.127	0.903	0.919	1.089	0.795	1.150	1.176	0.706	0.993	0.791
69	0.987	0.971	1.041	1.019	1.018	0.954	0.964	1.070	1.105	1.032	0.926	0.984	1.041	0.822	0.974	0.802
70	1.008	1.224	1.140	1.200	1.195	1.240	1.055	1.175	1.131	1.020	0.979	0.939	1.222	0.961	1.029	1.005
71	1.102	1.144	1.163	1.161	1.080	1.265	1.134	1.179	0.988	1.216	1.016	1.157	1.050	0.885	1.050	0.942
Mean	0.892	0.905	0.914	1.002	0.862	0.936	0.943	0.905	0.950	0.971	1.034	0.923	1.015	0.997	0.995	0.906

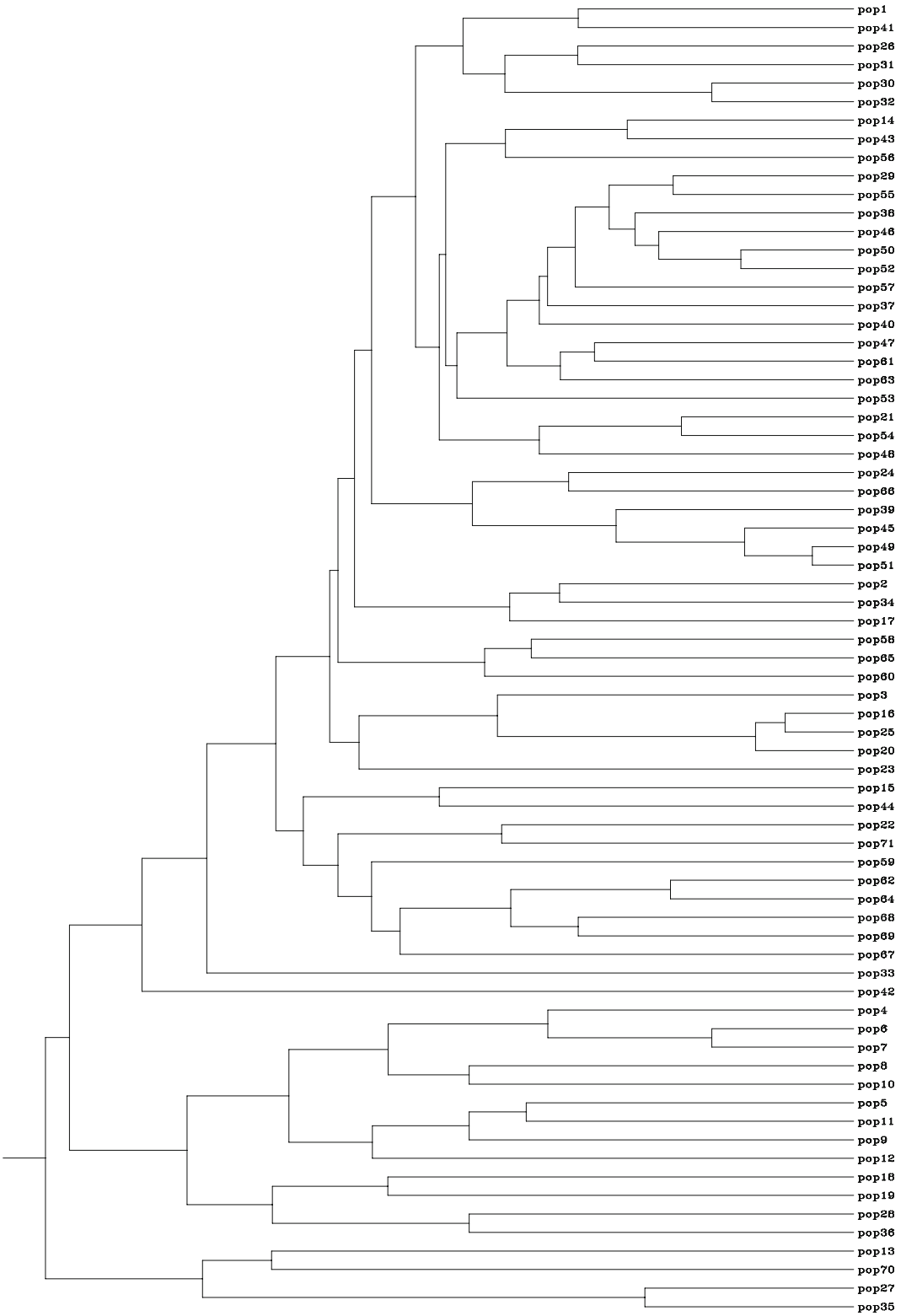
Pop ID	65	66	67	68	69	70	71	Mean
1	0.294	0.288	0.415	0.433	0.398	0.428	0.357	0.336
2	0.263	0.319	0.365	0.396	0.348	0.315	0.345	0.397
3	0.338	0.356	0.390	0.372	0.422	0.428	0.320	0.386
4	0.285	0.307	0.312	0.357	0.396	0.389	0.330	0.336
5	0.318	0.262	0.332	0.420	0.403	0.370	0.363	0.355
6	0.355	0.262	0.257	0.259	0.279	0.307	0.313	0.291
7	0.345	0.364	0.284	0.249	0.330	0.322	0.327	0.324
8	0.288	0.300	0.384	0.440	0.391	0.396	0.370	0.35
9	0.318	0.256	0.370	0.469	0.440	0.408	0.438	0.372
10	0.295	0.240	0.287	0.432	0.398	0.416	0.328	0.341
11	0.318	0.262	0.357	0.482	0.415	0.395	0.375	0.364
12	0.351	0.326	0.379	0.379	0.436	0.379	0.453	0.369
13	0.351	0.301	0.366	0.342	0.424	0.404	0.365	0.385
14	0.294	0.323	0.331	0.374	0.340	0.318	0.269	0.376
15	0.358	0.268	0.289	0.329	0.362	0.354	0.333	0.367
16	0.406	0.406	0.365	0.316	0.348	0.315	0.270	0.387
17	0.507	0.391	0.394	0.432	0.401	0.394	0.380	0.393
18	0.326	0.329	0.262	0.304	0.296	0.211	0.252	0.347
19	0.288	0.320	0.290	0.311	0.312	0.316	0.302	0.364
20	0.395	0.389	0.379	0.335	0.380	0.347	0.302	0.403
21	0.421	0.321	0.405	0.393	0.350	0.291	0.309	0.408
22	0.309	0.271	0.324	0.550	0.508	0.489	0.506	0.378
23	0.369	0.356	0.352	0.291	0.311	0.289	0.270	0.391
24	0.497	0.409	0.373	0.349	0.344	0.310	0.284	0.381
25	0.401	0.367	0.388	0.329	0.358	0.312	0.277	0.38
26	0.389	0.290	0.292	0.424	0.367	0.351	0.337	0.383
27	0.320	0.300	0.379	0.386	0.362	0.379	0.308	0.367
28	0.377	0.384	0.323	0.361	0.394	0.405	0.410	0.39

Appendix 1 continued

Pop ID	65	66	67	68	69	70	71	Mean
29	0.362	0.324	0.371	0.371	0.391	0.332	0.357	0.404
30	0.400	0.316	0.330	0.359	0.314	0.293	0.307	0.384
31	0.450	0.416	0.368	0.396	0.326	0.355	0.320	0.397
32	0.388	0.291	0.330	0.384	0.326	0.305	0.295	0.391
33	0.356	0.272	0.343	0.359	0.326	0.255	0.320	0.365
34	0.398	0.388	0.416	0.419	0.373	0.326	0.392	0.399
35	0.325	0.369	0.365	0.347	0.348	0.365	0.295	0.355
36	0.363	0.325	0.384	0.384	0.441	0.447	0.395	0.383
37	0.382	0.357	0.353	0.304	0.324	0.278	0.289	0.405
38	0.345	0.320	0.353	0.329	0.324	0.265	0.302	0.428
39	0.418	0.455	0.332	0.333	0.328	0.320	0.313	0.415
40	0.385	0.385	0.330	0.319	0.326	0.267	0.266	0.363
41	0.380	0.280	0.307	0.309	0.365	0.307	0.350	0.39
42	0.405	0.249	0.370	0.383	0.378	0.320	0.313	0.367
43	0.332	0.345	0.366	0.367	0.386	0.353	0.340	0.415
44	0.395	0.332	0.416	0.416	0.399	0.341	0.365	0.379
45	0.418	0.455	0.307	0.333	0.353	0.320	0.325	0.42
46	0.344	0.284	0.285	0.353	0.345	0.285	0.288	0.386
47	0.430	0.294	0.411	0.364	0.318	0.309	0.337	0.418
48	0.414	0.363	0.378	0.316	0.361	0.301	0.339	0.41
49	0.381	0.481	0.315	0.378	0.373	0.365	0.332	0.421
50	0.343	0.362	0.332	0.352	0.379	0.294	0.319	0.415
51	0.380	0.467	0.295	0.346	0.353	0.320	0.313	0.413
52	0.287	0.401	0.327	0.316	0.361	0.301	0.313	0.374
53	0.395	0.332	0.391	0.429	0.361	0.303	0.340	0.428
54	0.494	0.356	0.465	0.427	0.385	0.289	0.282	0.4
55	0.484	0.421	0.449	0.324	0.381	0.348	0.322	0.396
56	0.473	0.313	0.425	0.405	0.343	0.309	0.308	0.41
57	0.447	0.321	0.424	0.399	0.331	0.323	0.372	0.393
58	0.484	0.321	0.361	0.337	0.356	0.361	0.297	0.382
59	0.386	0.399	0.465	0.452	0.396	0.376	0.362	0.359
60	0.420	0.370	0.391	0.317	0.374	0.391	0.315	0.403
61	0.418	0.231	0.458	0.309	0.353	0.295	0.350	0.367
62	0.430	0.368	0.658	0.494	0.440	0.383	0.413	0.375
63	0.492	0.368	0.420	0.370	0.378	0.357	0.350	0.373
64	0.508	0.382	0.505	0.453	0.449	0.366	0.390	0.409
65	–	0.371	0.430	0.368	0.350	0.354	0.322	0.378
66	0.991	–	0.373	0.312	0.344	0.335	0.309	0.339
67	0.843	0.985	–	0.470	0.459	0.452	0.438	0.372
68	1.001	1.166	0.754	–	0.625	0.633	0.588	0.382
69	1.050	1.068	0.778	0.469	–	0.660	0.658	0.381
70	1.037	1.092	0.794	0.457	0.415	–	0.603	0.354
71	1.134	1.174	0.825	0.532	0.418	0.506	–	0.347
Mean	0.984	1.095	1.002	0.979	0.979	1.058	1.077	–

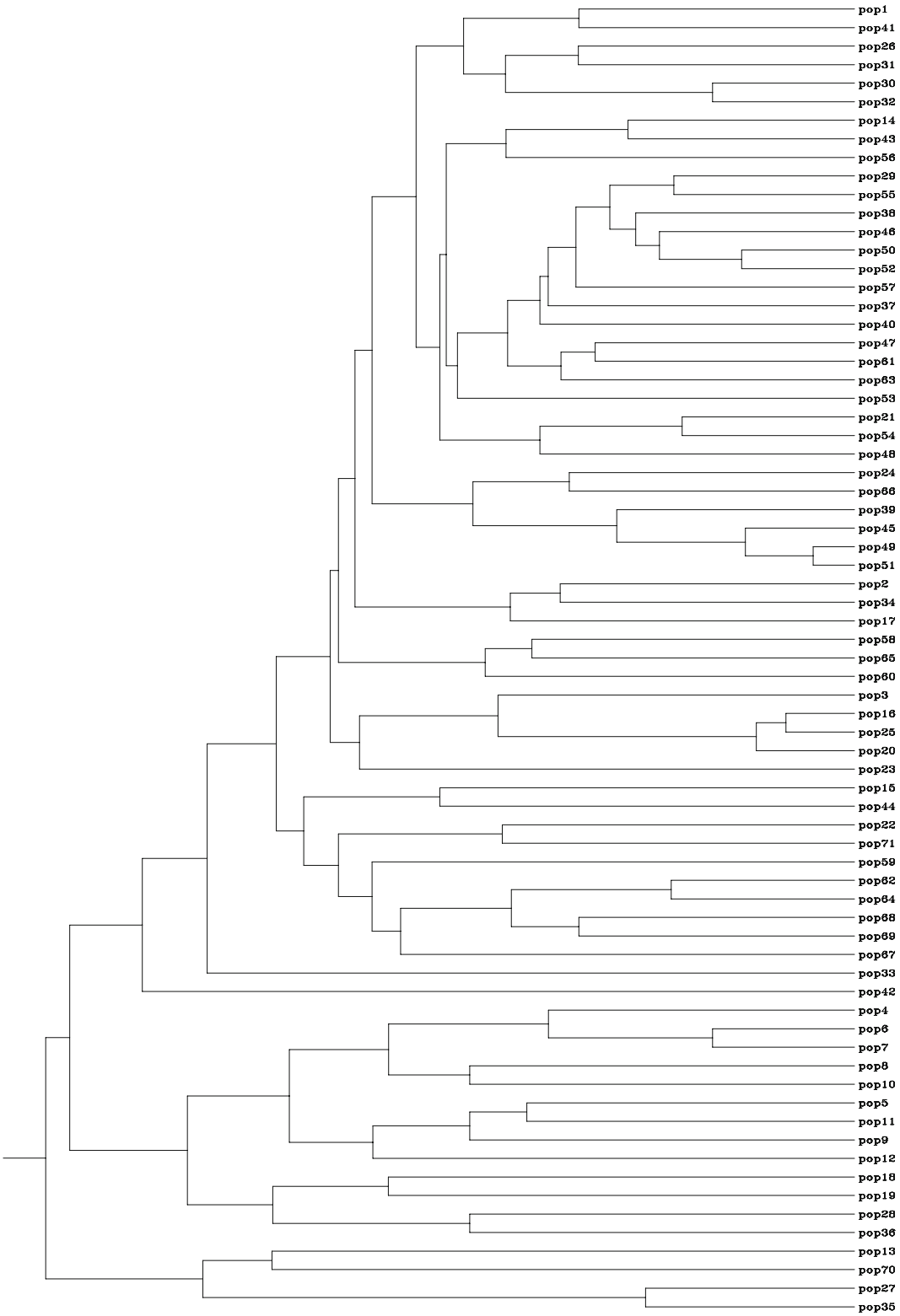
[a] Nei's genetic identity (above diagonal) and genetic distance (below diagonal)

Appendix 2



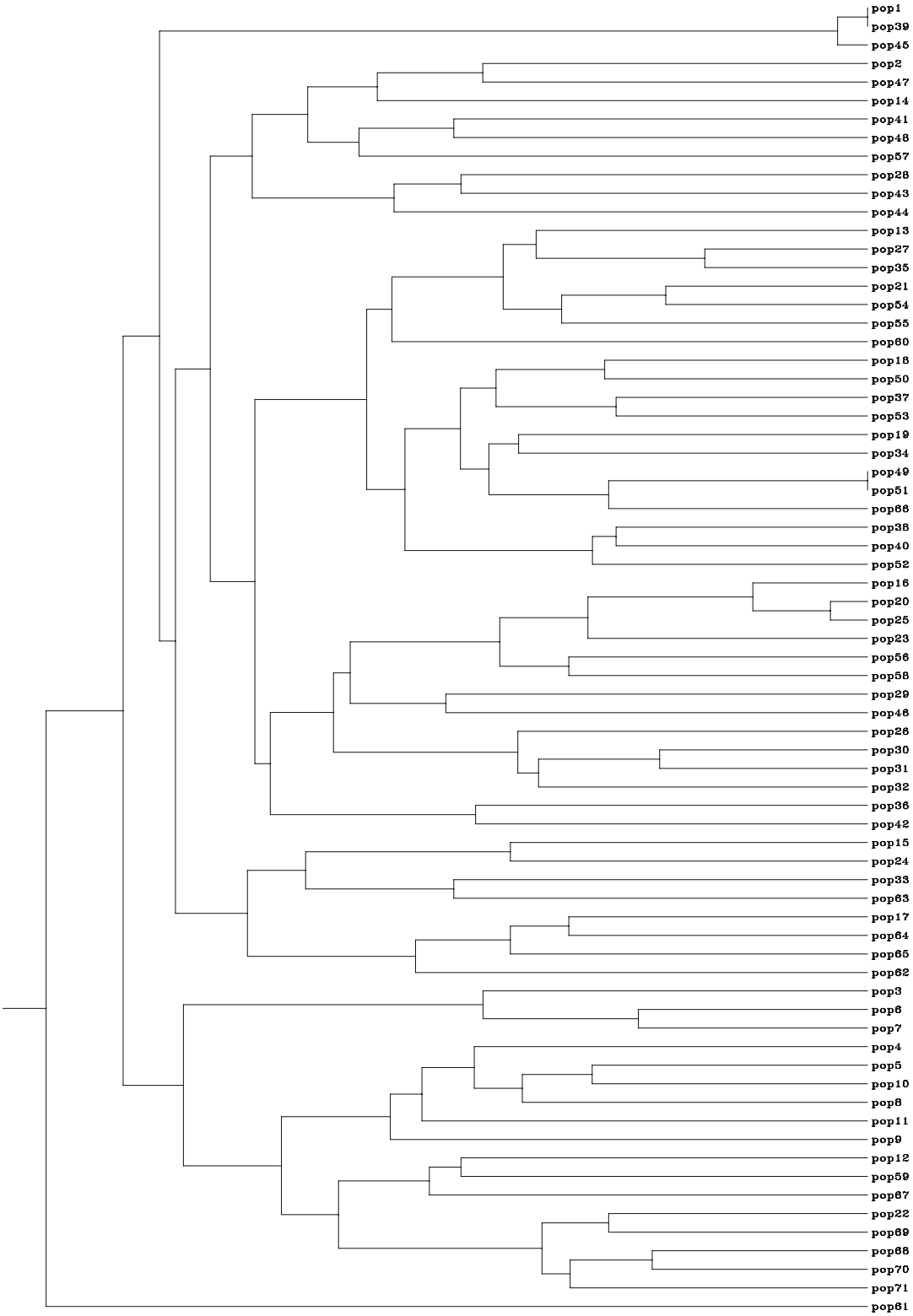
Appendix 2 Dendrogram of 71 wheat genotypes based on the Nei's (1972) original genetic distance calculated from data of 19 SSR loci in the Genome A, using the UPGMA as the clustering method. The population ID is the same as the line ID in Table 1

Appendix 3



Appendix 3 Dendrogram of 71 wheat genotypes based on the Nei's (1972) original genetic distance calculated from data of 28 SSR loci in the Genome B, using the UPGMA as the clustering method. The population ID is the same as the line ID in Table 1

Appendix 4



Appendix 4 Dendrogram of 71 wheat genotypes based on the Nei's (1972) original genetic distance calculated from data of 20 SSR loci in the Genome D, using the UPGMA as the clustering method. The population ID is the same as the line ID in Table 1

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