Theor Appl Genet (2000) 101:309–316

S.G. Hegde · J. Valkoun · J.G. Waines

Genetic diversity in wild wheats and goat grass

Received: 12 September 1999 / Accepted: 10 November 1999

Abstract The genetic structure of 35 populations of wild relatives of cultivated wheats, all collected in Syria and Lebanon, was assessed using ten isozymes. The populations consisted of diploid goat grass, *Aegilops speltoides*, diploid wild wheats, *Triticum monococcum* spp. *aegilopoides* and *T. urartu*, and tetraploid wild wheat, *T. turgidum* ssp. *dicoccoides*. The majority of the populations were polymorphic (P=0–70%) having low within-population mean genetic diversity (H_{ep}=0.05–0.10) and relatively high within-species genetic diversity (H_{es}=0.14–0.31). The linkage between loci did not seem to be one of the causes for the observed polymorphism. All four species showed significant inbreeding at both the population (0.31–0.64) and species (0.77–0.96) levels, and the extent of inbreeding did not correlate with mating systems. Despite their apparent common ecological and evolutionary history, between-population or between-species level genetic identity was low (I=0.43–0.86). Among the diploid species, populations of *Ae. speltoides* clustered distinctly from those overlapping clusters of *T. monococcum* ssp. *aegilopoides* and *T. urartu*. The tetraploid species *T. turgidum* ssp. *dicoccoides* had relatively less genetic diversity (H_{es}=0.14) and was highly homozygous (F=0.96). The results suggest that these wild progenitors of cultivated wheats have undergone extensive local differentiation and inbreeding. We discuss the implications of our results on the management of wild wheat and goat grass populations.

Keywords Genetic diversity · Wild wheat · Goat grass · Isozyme · Conservation

Communicated by P.M.A. Tigerstedt

S.G. Hegde · J.G. Waines (✉)
Department of Botany and Plant Sciences,
University of California, Riverside, CA 92521, USA
e-mail: waines@ucracl.ucr.edu

J. Valkoun
Genetic Resources Unit, International Center for Agricultural Research in the Dry Areas, P.O. Box 5466, Aleppo, Syria

Introduction

The *Aegilops-Triticum* assemblage is one of the most important grass groups because of its historical and agricultural importance. The various forms of wheat represent almost 30% of the world's grain production, and it is estimated that by year 2020 the global wheat requirement will double the current production level (Rosegrant 1995). As there is growing concern among wheat breeders that a lack of genetic diversity might limit future breeding advances (Rejesus et al. 1996), there is an urgent need to explore the genetic diversity in natural populations of wheats and their relatives. Most of the existing germplasm collections of land races and natural populations of wild wheats around the globe were made mainly on a morphological basis, which is an imprecise indicator of the genetic potential of the genotypes. Hence their use in contemporary breeding programs has sometimes met with failure, thereby stressing the importance of biochemical and molecular markers to evaluate natural populations (Tanksley and McCouch 1997).

There are three major categories of wheats: diploid, tetraploid and hexaploid. Diploid species comprise three taxa – the cultivated einkorn wheat *T. monococcum* ssp. *monococcum* (AA genome) and its immediate wild relative *T. monococcum* ssp. *aegilopoides*, and wild *T. urartu* (AA genome). The tetraploid group is represented by a complex of two species and several subspecies in which the most important types are wild and domesticated emmer wheat, *T. turgidum* ssp. *dicoccoides* (BBAA), and durum wheat, *T. turgidum* ssp. *durum* (BBAA). Both these have other interfertile cultivated forms. Hexaploid wheat, *T. aestivum* (BBAADD), is known only in cultivation and has not been found in the wild (see Zohary and Hopf 1993 for detailed discussion). The tetraploid *T. turgidum* was derived from the hybridization of a wild goat grass (BB) and another diploid species carrying the A genome. *Aegilops speltoides* (SS) is considered a likely candidate for the B genome (for detailed references see Hancock 1992). Both genetic (Heun et al. 1997) and archeological (Zohary and Hopf 1993; de Moulins 1993)

evidence suggests that both wild and domesticated einkorn and emmer are present at early agricultural sites in the northern Fertile Crescent of southeast Turkey and northern Syria (Harlan and Zohary 1966; van Slageren 1994). Similarly, *Aegilops* is characterized as a Mediterranean-Western Asiatic element with its center of diversity in the Mediterranean and Irano-Turanian regions (Takhtajan 1986). Natural populations of wild diploid and tetraploid progenitors of the cultivated wheats occur in and around the center of origin (Blumler 1994).

From the perspective of germplasm conservation, understanding the genetic structure of natural populations of wheat is very important (Frankel and Bennett 1970). Little information is available on the nature and extent of genetic variation in the wild progenitors of cultivated wheats. The few available reports (Nevo et al. 1982; Nevo and Beiles 1989; Medlinger and Zohary 1995) on wild tetraploid emmer wheat (*T. turgidum* ssp. *dicoccoides*) and goat grass species (*Aegilops*) are mainly from Palestine, which is on the southwestern edge of the fertile crescent, whereas the center of diversity of the *Aegilops* and *Triticum* group lies in northern Syria and southeastern Turkey. Although, Yaghoobi-Saray (1979) and Smith-Huerta et al. (1989) assayed wild wheats from these areas, the materials were reproduced once or twice in southern California before they were subjected to isozyme analysis – on that account their results may not reflect the actual diversity of populations in their natural habitat. Further, none of the earlier investigations considered both diploid and tetraploid wheats and goat grass together in their analysis, which would provide information on: (1) the extent and pattern of genetic diversity among diploid and tetraploid wild progenitors, and (2) the process of evolution in these species. In the study described here we investigated allozyme variation in original populations of diploid and tetraploid wild wheats and diploid goat grass collected from Syria and Lebanon. We addressed three questions: first, what is the extent of genetic variation in the natural populations of diploid goat grass, *Aegilops speltoides*, diploid wild wheats, *T. monococcum*, ssp. aegilopoides and *T. urartu*, and tetraploid wild wheat *T. turgidum*, ssp. *dicoccoides*; second, what is the relative magnitude of variation between diploids and tetraploids; third, what is the genetic relationship among diploids.

There are conflicting reports concerning the amount of genetic diversity in diploid wild wheat populations. Here we report only those studies whose inferences were based on a meaningful number of allozymes. Yaghoobi-Sary (1979) studied five diploid wild species from the *Aegilops-Triticum* complex, and he reported higher levels of allozyme diversity in *T. monococcum* ssp. *aegilopoides* and *T. urartu*. However, Smith-Huerta et al. (1989) observed uniformly low genetic variability when they studied the same two *Triticum* species from Turkey, Iraq and Lebanon. According to the latter authors, this discrepancy could be due to the different enzyme systems used between these two studies. Medlinger and Zohary (1995) found significant genetic diversity in *Ae. speltoides* populations from Palestine. Most populations of *Ae. speltoides*, regardless of location or population size, showed similar levels of genetic variation. Nevo et al. (1982) studied allozyme genetic diversity and environmental associations in 12 populations of wild tetraploid wheat, *T. turgidum* ssp. *dicoccoides* in Palestine. They observed very low average genetic variation within populations; however, those populations held distinct genetic differences over short geographic distances.

Materials and methods

Sampling

In 1994, populations of *Ae. speltoides* Tausch (SS=BB), *T. monococcum* L. ssp. *aegilopoides* (Link) Bois. (AA), *T. urartu* Tum. ex Gand. (AA) and *T. turgidum* L. ssp. *dicoccoides* (Korn ex. Asch. & Graebn.) Thell. (BBAA) were collected along a transect running eastwards from Abeen and Der Jamal, Aleppo province, to Ain Diwar on the Dicle (Tigris) River in Hasake province, northern Syria (Table 1, and Fig. 1). One *T. urartu* population was also collected from Sha'af in the Jebel Druz, Sweida province, in southern Syria. The wild wheat populations were also collected from the Bekka Valley in Lebanon (Table 1, Fig. 1). One spike was randomly collected from each of 30 or more plants in each population.

Electrophoresis

From each spike a seed was randomly chosen from a spikelet for electrophoresis. The seeds were soaked overnight in an aqueous solution of 600 ppm gibberellic acid to achieve uniform germination and sown in potting soil; the seedlings were raised in a germination tray in a glasshouse. Leaves from 10-day-old seedlings were crushed in 0.1 *M* TRIS-HCl buffer pH 8.0 and 0.1 *M* 2-mercaptoethanol, and the extract was absorbed on paper wicks. The gel and electrode buffer systems and other electrophoretic procedures were similar to those described by Smith-Huerta et al. (1989).

The populations were scored for the following ten isozymes: acid phosphatase (ACP), isocitrate dehydrogenase (IDH), malate

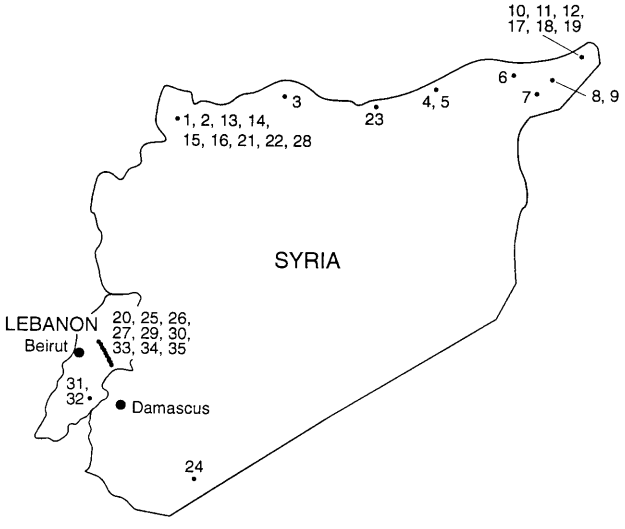

Fig. 1 The collection sites from Syria and Lebanon (refer to Table 1 for population descriptions)

Table 1 The population locations of *Aegiloops speltoides*, *Triticum monococcum* ssp. *aegilopoides*, *T. urartu* and *T. turgidum* ssp. *dicoccoides* in Syria and Lebanon

Genus-species	Population ID	Location
Ae. speltoides	1	Abeen (population I), Halab, Syria
	2	Abben (population II), Halab, Syria
	3	2 km N Nahda, Sandi, Syria
	4	16 km E Ras El Ain road to Derbasieh, Syria
	5	32 km E Ras El Ain road to Derbasieh, Syria
	6	6 km E Amouda road to Kamishli 3 km before Omarabia, Syria
	7	7 km E Kakhtanieh road Jawadieh, Syria
	8	5 km E Roumelan, Syria
	9	11 km Roumelan road to Malkie, Syria
	10	4 km E Malkie toward Ain Diwar, Syria
	11	500 m off Ain Diwar road to Malkie, Syria
	12	2 km SW of Ain Diwar in the fields Syria
T. monococcum ssp. *aegilopoides*	13	Abeen (population I), Halab, Syria
	14	Abeen (population II), Halab, Syria
	15	Der Jamal (population I), Syria
	16	Der Jamal (population II), Syria
	17	4 km E Malkie toward Ain Diwar, Syria
	18	500 m off Ain Diwar road to Malkie, Syria
	19	2 km SW of Ain Diwar in the fields, Syria
	20	2 km before Yanta road from Aita Al Foukhar, Lebanon
T. urartu	21	Der Jamal (population I), Syria
	22	Der Jamal (population II), Syria
	23	51 km Bab El Hadjar, Syria
	24	Sha'af, Sweida, Syria
	25	2 km before Yanta road from Aita el Foukhar, Lebanon
	26	3 km W of Aita road to Der Al Ahmar, Lebanon
	27	2 km W of Aita road to Der Al Ahmar, Lebanon
T. turgidum ssp. *dicoccoides*	28	Der Jamal, Syria
	29	Karadum, Lebanon
	30	Between Kframechki and Jeb Farah, Lebanon
	31	2 km before Rachiya, Lebanon
	32	1 km before Kfar Qouq from Rachaiya, Lebanon
	33	2 km E of Aita el Foukhar, Lebanon
	34	2 km before Yanta road from Aita el Foukhar, Lebanon
	35	2 km before Ain Arab road from Yanta, Lebanon

dehydrogenase (MDH-1 and MDH-2), phosphoglucose isomerase (PGI), 6-phosphogluconate dehydrogenase (6PGD), phosphoglucomutase (PGM), shikimate dehydrogenase (SKDH) and triosephosphate isomerase (TPI-1 and TPI-2). The staining procedure, the number of loci per enzyme and their alleles were inferred from past electrophoretic studies of *Triticum* and *Aegilops* (Nevo et al. 1982; Smith-Huerta et al. 1989; Medlinger and Zohary 1995). For convenience, the two genomes of tetraploid wheat were designated as slow (S) and fast (F) genomes based on their mobility on the electrophoretic gel. Expression of these two genome on the gel may not necessarily be the exclusive product of the two distinct genomes (BBAA) of the tetraploid, for each genome may possess a locus or alleles that is capable of migrating slower or faster on the gel. For that reason, we did not make any attempt to associate these two genomes with the conventional A and B genomes of tetraploid wheat.

Genetic diversity parameters

The following genetic variability parameters were calculated at the population and species levels: alleles per locus (A), percentage polymorphic loci (P), observed heterozygosity (H_0), expected heterozygosity (H_e=Hardy-Weinberg expected heterozygosity, Nei 1987) and fixation index [F=1–(H_0/He), a measure of heterozygote deficiency or excess, Wright 1978]. The population (p) and species (s) level genetic variability parameters were calculated according to Hamrick and Godt (1989). In each population, all polymorphic loci were tested for Hardy-Weinberg equilibrium (Levene 1953), and chi-square (χ^2) and likelihood ratio (G^2) tests were used to assess significant deviations. Further, to account for the multiple comparisons Bonferroni corrections (Rice 1995) were employed. The linkage disequilibrium between loci was estimated (Weir 1979). The gene flow (Nm) between populations within a species was calculated [Fst=0.25 (1-Fst)/Fst, Slatkin and Barton 1989]. The genetic identity (I) values were calculated between populations within a species and between species (Nei 1972). The genetic distance values (D) were used to generate an unweighted pair-group clustering based on the arithmetic averages (UPGMA) phenogram (Felsenstein 1993).

Results

Genetic diversity

Genetic diversity parameters such as mean number of alleles per locus (A), percentage of polymorphic loci (P), observed heterozygosity per locus (H_0), diversity index (H_e) and fixation index (F) are presented in Table 2.

Except for 1 *Ae. speltoides* population (population number 8, Table 1), which was monomorphic for all the ten loci, the remaining 34 populations from the four species showed polymorphism for several loci (data not shown). The mean number of alleles per locus (A) did

Table 2[a] Summary of genetic diversity based on mean of ten loci for populations of *Aegilops speltoides. T. monococcum* ssp. *aegilopoides, T. urartu,* and *T. turgidum* ssp. *dicoccoides*

Population ID	*n*	Alleles/locus (A)	Percentage of polymorphic loci (P)	Heterozygotes/ individual (H_0)	Diversity index (H_e)	Fixation index (F)
1	60	1.5	40	0.05	0.07	0.29
2	50	1.4	40	0.06	0.11	0.45
3	60	1.6	60	0.03	0.08	0.38
4	60	1.2	20	0.003	0.03	0.90
5	60	1.6	40	0.08	0.16	0.50
6	60	1.7	50	0.04	0.07	0.57
7	20	1.4	40	0.13	0.12	–0.08
8	60	1	0	0.00	0.00	0.00
9	60	1.6	40	0.07	0.11	0.36
10	28	2.00	50	0.09	0.22	0.59
11	60	1.8	60	0.09	0.18	0.50
12	60	1.5	40	0.05	0.07	0.71
Population (p)[b]	**53.17**	**1.53**	**40**	**0.06**	**0.10**	**0.43**
Species (s)[b]	**638**	**2.90**	**90**	**0.05**	**0.22**	**0.77**
13	60	1.5	50	0.03	0.04	0.25
14	30	1.5	50	0.01	0.10	0.90
15	26	1.4	30	0.02	0.03	0.33
16	18	1.2	20	0.00	0.08	1.00
17	30	1.3	20	0.01	0.05	0.80
18	40	1.1	10	0.02	0.02	0.00
19	60	2.00	70	0.01	0.16	0.94
20	40	1.9	70	0.05	0.11	0.45
Population (p)	**38**	**1.49**	**40**	**0.02**	**0.07**	**0.58**
Species (s)	**304**	**2.80**	**100**	**0.02**	**0.21**	**0.90**
21	34	1.1	10	0.006	0.006	0.00
22	32	1.5	40	0.00	0.10	1.00
23	28	1.2	20	0.04	0.06	0.33
24	28	1.70	60	0.08	0.11	0.27
25	38	1.4	30	0.01	0.04	0.75
26	60	1.5	40	0.01	0.07	0.14
27	32	1.1	10	0.04	0.03	–0.33
Population (p)	**36**	**1.36**	**30**	**0.03**	**0.06**	**0.31**
Species (s)	**252**	**2.70**	**100**	**0.02**	**0.31**	**0.94**
28	60	1.45	35.29	0.01	0.08	0.88
29	70	1.18	11.76	0.01	0.01	0.00
30	60	1.12	11.76	0.00	0.01	1.00
31	32	1.41	41.18	0.01	0.17	0.94
32	24	1.06	5.88	0.00	0.01	1.00
33	54	1.18	11.76	0.01	0.02	0.50
34	56	1.24	17.65	0.01	0.06	0.83
35	60	1.12	5.88	0.004	0.004	0.00
Population (p)	**52**	**1.22**	**17.65**	**0.007**	**0.05**	**0.64**
Species (s)	**416**	**2.12**	**70.59**	**0.005**	**0.14**	**0.96**

[a] Individual population allele frequencies for the ten isozymes are available from the authors on request
[b] p, Population averages, s, species' estimates. See Hamrick and Godt (1989) for explanations

not differ significantly between species (A_s range between species=2.12–2.90). Although both diploid wheats and goat grass exhibited very high within-species polymorphism (P_s=70.59 to 100), there were many monomorphic loci within populations. For instance, only 40% of the loci were polymorphic within the *Ae. speltoides* and *T. monococcum* ssp. *aegilopoides* populations, 30% in the case of *T. urartu*, and 17% in the case of *T. turgidum* ssp. *dicoccoides.* Observed heterozygosity (H_0) and the genetic diversity index (H_e) were uniformly low across the species and ploidy level, and tetraploid wheat possessed less than half the observed heterozygosity and diveristy than the diploids. *Aegilops speltoides* populations showed the highest observed heterozygosity and diversity. The population estimates for H_{0p} and H_{ep} were 0.06 and 0.10, respectively, for *Ae. speltoides*, 0.02 and 0.07 for *T. monococcum* ssp. *aegilopoides*, 0.03 and 0.06 for *T. urartu* and 0.007 and 0.05 for *T. turgidum* ssp. *dicoccoides*. However, with respect to the H_0 and H_e values of individual populations, there were no differences among the diploid species. For instance, range values for H_0 are 0.00–0.13 in *Ae. speltoides*, 0.00–0.05 in *T. mono-*

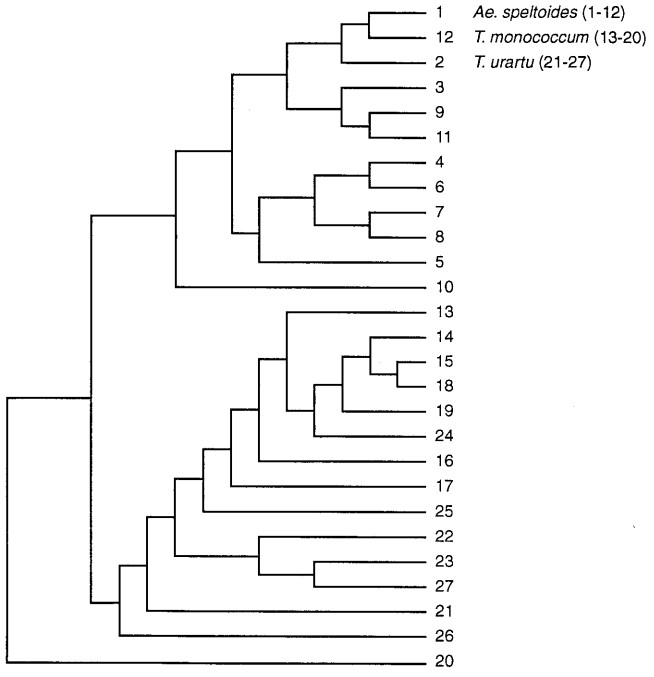

Fig. 2 UPGMA phenogram based on genetic distances within taxa and between taxa for goat grass and diploid wheats (refer to Table 1 for population descriptions)

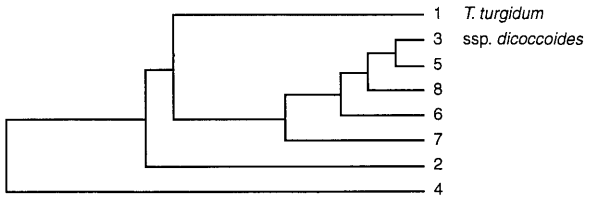

Fig. 3 UPGMA phenogram based on genetic distances within taxa (refer to Table 1 populations 28–35, respectively, for population descriptions)

coccum ssp. *aegilopoides* and 0.00–0.08 in *T. urartu.* But H_0 values were almost identical (0.00–0.01) among tetraploid populations. The general tendency for low observed heterozygosity or diversity values among diploids and the tetraploid were also indirectly reflected in their higher inbreeding (F) estimates (population average: 0.31–0.64). On average, tetraploid populations showed higher estimates of inbreeding (F_p=0.64) than diploids (F_p=0.31–0.58).

Diploid species showed significantly more genetic diversity than the tetraploid species. The highest genetic diversity was observed in *T. urartu* (H_{es}=0.31) and the lowest in *T. turgidum* ssp. *dicoccoides* (H_{es}=0.14). All the diploid and tetraploid species showed very high inbreeding at the species level (F_s=0.77–0.96), and the Hardy-Weinberg test revealed that all four species had excess homozygous individuals, which is typical for self-pollinating species. At the species level, among polymorphic loci, 38.70% were in Hardy-Weinberg equilibrium in *Ae. speltoides*, 34.60% in *T. monococcum* ssp. *aegilopoides*, 41.17% in *T. urartu* and only 20% in *T. turgidum* ssp. *dicoccoides*.

Linked gene systems are invoked to explain polymorphism that persists in inbreeders (Tolbert et al. 1979; Jain 1983). To test this hypothesis we calculated pairwise linkage estimates (Weir 1979) between polymorphic loci in all possible combinations for each population. None of the 12 *Ae. speltoides* or seven *T. urartu* populations showed linkage disequilibria between any pairs of polymorphic loci. However, population 13 and 19 of *T. monococcum* ssp. *aegilopoides*, and populations 28, 29 and 30 of *T. turgidum* ssp. *dicoccoides* had a few loci which were in significant linkage disequilibria, although there was no consistent pattern among these populations. For instance, in the case of population 13 two alleles of locus *Pgi* were in linkage disequilibria with two alleles of *Skdh*, but in population 19 only one allele of *Pgi* was in nonequilibrium with one allele of *Skdh*, and two alleles each of *Pgm* and *Acp*. Therefore, it seems that the presence or maintenance of polymorphic loci in these diploid and tetraploid wheats and goat grass was not due to significant linkage between the loci.

Genetic identity

The genetic similarity (I) between populations within a species were relatively low and, except for *T. urartu* (0.70), these estimates were nearly identical for the species *Ae. speltoides* (0.86), *T. monococcum* ssp. *aegilopoides* (0.84) and *T. turgidum* ssp. *dicoccoides* (0.88). Among the diploid species, genetic similarity estimates were significantly low between species. *Aegilops speltoides* was relatively more closely related genetically with *T. monococcum* ssp. *aegilopoides* (0.60) than with *T. urartu* (0.54). *Triticum monococcum* ssp. *aegilopoides* was genetically more similar to *T. urartu* (0.70). Interestingly, genetic similarity values within populations of *T. urartu* (0.70) were identical to those observed between *T. urartu* and *T. monococcum* ssp. *aegilopoides*. Among all the similarity estimates, the one between, *Ae. speltoides* and *T. urartu* was the least (0.54).

Using genetic distance values we constructed a phenogram to visualize the relative genetic relatedness among diploids and within tetraploid taxa. (Figs. 2 and 3). The diploid taxa formed two distinct clusters; one exclusively among goat grass populations and another with diploid wild wheats (Fig. 3). In the diploid wheat cluster, populations of *T. monococcum* and *T. urartu* were interspersed among themselves. The population numbers (Table 1) for goat grass and diploid wheats were assigned sequentially based on their location within a transect. For instance, except for population number 24 (T. urartu from Sha'af in Sweida Province in southern Syria) all the Syrian goat grass and diploid wheat populations were collected in an east to west direction in northern Syria, covering approximately 500 km. Likewise, the tetraploid wheat populations were collected in the Bekka Valley of Lebanon in a west to east direction, covering around 15–20 km. The pattern of associations among populations within a taxon

for goat grass and diploid wheats appear to be related to their geographical distances (Figs. 1 and 2) as genetic distances between any 2 populations nearly reflected their geographical distances. Such a pattern was absent among the tetraploid populations (Figs. 1 and 3).

Discussion

Except for a few rare alleles, there were no species-specific marker alleles in either the diploids or the tetraploid. This could be possible if there were significant gene flow between species, or if all the species had evolved from one another or from a common ancestor. The gene flow estimates among the diploid taxa were insignificant (Nm=0.07). Of the three diploid species only *Ae. speltoides* is a partial outcrosser, and the remaining two, *T. monococcum* ssp. *aegilopoies* and *T. urartu*, are selfers. Although all three species occur sympatrically over several locations in their range of distribution (Johnson 1975; Gandilyan 1998), natural hybrids are rarely, if ever, observed, and even artificial crossing results in sterile hybrids (Johnson and Dhaliwal 1976; Waines and Barnhart 1992). Therefore, it is unlikely that the observed allelic pattern is due to significant gene flow between the present-day diploids. There are a few suggestions regarding sympatric speciation for *T. monococcum* and *T. urartu* (Johnson and Dhaliwal 1976) and a common heritage for *Ae. speltoides* and the diploid wheats based on crossability and genetic evidence (Stebbins 1956; Bowden 1959; Morris and Sears 1967). *Triticum monococcum* and *T. urartu* are morphologically very similar (Johnson 1975) and, despite F_1 sterility between them (Johnson and Dhaliwal 1976), hybrid plants show regular intergenomic pairing at meiosis. According to these authors, reproductive isolation between these two species was probably effected through accumulated cryptic chromosomal differences. There are several reports in plants and animals where a single or a very few gene differences resulted in speciation (Knowlton 1993). Further, since these two species also overlap in their geographical distribution they might not have had an opportunity to diverge significantly from one another after their evolutionary diversification from a common ancestor. That may be why both the diploid wheat species show so much genetic similarity between them. In contrast, *Ae. speltoides* is relatively different in certain morphological traits compared with diploid wheats (Waines 1994) and, furthermore, this diversification in morphological traits together with a variation in seed dispersal enabled this species to adapt to the diverse environments around the Fertile Crescent (van Slageren 1994). This might explain the divergent allele frequencies that we observed in *Ae. speltoides* compared to the diploid wheats. However, the lack of any species-specific allele may be due to a common ancestor between *Aegilops* and *Triticum* and their common ecological and geographical distributions.

Like the diploid wheats, tetraploid wheat is highly self-fertile but has the genomes of both a goat grass and a diploid wheat, consequently potentially providing more scope for allelic variation. Of the 18 loci in our study 6 were completely monomorphic for both genomes, and there was also a high degree of monomorphism in the remaining polymorphic loci within populations; in other words, there was relatively more homozygosity and a certain degree of gene silencing within the tetraploid species. Such gene silencing has been observed in polyploid wheats (Galili and Feldman 1983), and it has been described as an evolutionary response of the species to nullify the deleterious effect of large-scale genome duplications (Aragoncillo et al. 1978).

Overall, for both diploids and tetraploids, parameters of genetic variability (A, P) and genetic diversity (H_e) were higher (Table 2) than those of monocots (P=59.2%, H_{es}=0.18) or of annuals (P=50.7%; 0.16) (Hamrick and Godt 1989). All these parameters, however, showed significant variations between populations except for H_0 in the case of tetraploid wheat. We observed low genetic similarity values between populations within species for both diploids and tetraploids (range for I=0.70–0.88) and also between species among diploids (range for I=0.54–070), which were the lowest compared to all previous studies (Table 3). In all four species, the genetic diversity estimates were higher between populations than within individual populations, and also all four species showed nonsignificant gene flow within populations (Nm=0.19 for *Ae. speltoides*, 0.15 for *T. monococcum* ssp. *aegilopoides*; 0.06 for *T. urartu*; 0.10 for *T. turgidum* ssp. *dicoccoides*), making individual populations genetically more differentiated within a species.

The within-population genetic variability and genetic diversity values reported in this study were partially concordant with those from earlier reports on *Ae. speltoides*

Table 3 Average (±SE) genetic identiy (I) within and between species of diploid wheats and goat grass, and within tetraploid wheat

Species	*Ae. speltoides*	*T. monococcum* ssp. *aegilopoides*	*T. urartu*	*T. turgidum* ssp. *dicoccoides*
Ae. speltoides	0.86±0.01 (0.66–1.00)[a]	0.60±0.01 (0.22–0.83)	0.54±0.07 (0.40–0.71)	–
T. monococcum ssp. *aegilopoides*		0.84±0.02 (0.58–1.00)	0.70±0.02 (0.38–0.92)	–
T. urartu			0.70±0.02 (0.56–0.92)	–
T. turgidum ssp. *dicoccoides*				0.88±0.02 (0.70–1.00)

[a] Range values

from Palestine (Medlinger and Zohary 1995), *T. monococcum* ssp. *aegilopoides* and *T. urartu* from Turkey and Lebanon (Smith-Huerta et al. 1989) and *T. turgidum* ssp. *dicoccoides* from Palestine (Nevo et al. 1982). The species level values could not be observed due to a lack of comparable data in those earlier reports. For instance, population averages for parameters like A, P and H_e were similar for *Ae. speltoides* between this study and those of Medlinger and Zohary (1995), but inter-population differences for the same above parameters were found to be very high in our study (range: A=0.00–2.00; P=0–60%; H_0=0.00–0.13; He=0.00–0.22) compared to those of Medlinger and Zohary (range: A=1.81–2.19; P=50–75%; H_0=0.003–0.05; He=0.15–0.32) who found them to be uniform between populations. Likewise, for *T. monococcum* ssp. *aegilopoides* and for *T. urartu* Smith-Heurta et al. (1989) observed values of 19.71% and 18.35% polymorphism, respectively, which were less than half of what we observed in these two species (Table 3). This latter difference between our results and those of Smith-Heurta et al. (1989), where the natural populations were increased once or twice before isozyme electrophoresis, may be due to the way the populations were handled between these two studies. Since all wild wheats show a high degree of inbreeding, even one or two generations of selfing in a small-sampled population could alter the genetic structure of these populations – in particular, it is likely to reduce variation.

Aegilops populations showed very close relationships among themselves compared to diploid wheats. The result was not surprising given that, except for the reproductive isolation, both *T. monococcum* and *T. urartu* have close morphological and taxonomic relationships (Johnson and Dhaliwal 1976; Waines and Barnhart 1992). Interestingly, in all three diploid taxa the genetic distance was closely associated with the geographical distance, implying the differential adaptation of populations to environmental heterogeneity (Levene 1953). However, tetraploid populations did not show any clear association between genetic and geographical distances, probably because seven of the eight populations were collected over very short distances of 5–20 km from each other. Besides, polyploids are thought to undergo less ecological differentiation than diploids because of their ability to buffer genetically against ecological changes (see Hancock 1992 for detailed discussion). It is also possible that if the tetraploid wheat has evolved from a single or few hybridization events between diploid parents, the hybrid populations would capture only a small fraction of the genetic variability of the diploid progenitors.

Despite the appearance of a high percentage of polymorphic loci across the diploid and tetraploid species, the presence of low-level heterozygosity (H_0) in all of them indicates the existence of a high proportion of homozygotes in these species. This is to be expected in species that are typically self-pollinating. Among diploid and tetraploid species in this study only *Ae. speltoides* is a partial outcrosser, whereas the other two diploids and the tetraploid species are largely self-pollinating. But the pattern and extent of genetic variation were not significantly different between these breeding systems. Therefore, the observed pattern in genetic variability, which was nearly identical for both diploids and tetraploids, may be brought about by similar kinds of evolutionary force(s) for which all the species might have had identical responses. Nevo and co-workers (1982) found a strong association between specific isozyme loci and climatic regimes and soil types in tetraploid wheat *T. turgidum* ssp. *dicoccoides*. Based on these observations, the authors infer that strong local and regional differences in genetic variability values in tetraploid wheat in Israel are, at least partly, adaptive. On the contrary, Medlinger and Zohary (1995) did not observe any significant association between environmental factors and allele frequencies. In our study both large-scale morphological similarities and low-level genetic variation among diploid and tetraploid wheats and goat grass suggest that, probably, all these taxa possess similar types of genetic potentials, have experienced similar ecological interactions and, as a consequence, have attained a specialized adaptation to their current niches. Further, the high rate of inbreeding combined with the stochastic and anthropogenic factors might have eliminated many neutral and rare alleles from these populations. The spread of genetic diversity between populations within a taxa could probably be a fine-scale adaptation to microhabitat, such as soil type and climatic regime. On the contrary, one could still argue in favor of other micro/macro evolutionary forces such as meiotic drive and genetic drift, purely random processes, for the observed pattern of variation in these taxa. The probability of the occurrence of such processes to bring a consistent genetic pattern over all the populations and species observed in this study is very small.

The only exception to this relationship was population number 20 of *T. monococcum* ssp. *aegilopoides* from Lebanon. Out of the eight *T. monococcum* ssp. *aegilopoides* populations, seven populations were from Syria and one population (number 20, Table 1) was from Lebanon. This Lebanese *T. monococcum* population was genetically more related to the *T. urartu* populations than to other *T. monococcum* populations from Syria. One of the most probable explanations for this type of genetic divergence between populations of a species is if a small founder population has undergone genetic drift. We were not able to locate any *other T. monococcum* ssp. *aegilopoides* populations around population number 20 – therefore, the Lebanese *T. monococcum* population most probably arrived from Syria. Although, adaptive radiation through selection could also bring such a genetically divergent population within a species, that seems to be a less likely an explanation in this case because there were four *T. urartu* populations, two each from Syria and Lebanon (Table 1), which did not show comparable genetic divergence between these two locations.

Today, the ultimate goal of exploring the genetic potential of natural populations of the ancestors of crop plants is to make an informed decision on their conservation and commercial utilization. Despite the growing concern among wheat breeders about the lack of genetic diversity for future breeding advances, in several countries invest-

ments in strategic germplasm development have declined (McGuire 1997) and the rate of extinction of natural populations has increased (Raven 1987). Therefore, there is a pressing need to make conservation decisions quickly and effectively to save the remaining few natural populations. Diversity analyses have a potential role of designing where and how much germplasm needs to be collected (Tolbert et al. 1979). The extent and nature of genetic diversity in the natural populations of wild wheats and goat grass in our study indicate that a germplasm collection obtained from few populations from one location or from very few locations is ineffective in capturing species-level genetic variability. The only way to maximize the genetic diversity in germplasm is through random sampling of more populations over wider geographical and environmental gradients.

Acknowledgements We thank Isam Abu Maizar, Khaled Obari and Simon Khairella for help with collection, and Norman Ellstrand for his critical comments. J.G.W. received support from NCISE-IARC Pilot Linkage Program during germplasm collection. The experiments comply with the current laws of the country in which the experiments were performed.

References

Aragoncillo C, Rodriguez-Lopperena MA, Salcedo G, Carbonero P, Garcia-Olmedo F (1978) Influence of homoeologous chromosomes on gene-dosage effects in allohexaploid wheat (*Triticum aestivum*). Proc Natl Acad Sci USA 75:1446–1450

Bowden WL (1959) The taxonomy and nomenclature of the wheats, barleys, and ryes and their wild relatives. Can J Bot 37:657–684

Blumler MA (1994) Evolutionary trends in the wheat group in relation to environment, quaternary climatic change and human impacts. In: Millington AC, Pye K (eds) Environmental change in dryland: biogeographical and geomorphological perspectives. John Wiley and Sons, Chichester, pp 253–269

de Moulins D (1993) Agricultural changes at Euphrates and Steppe sites in the mid-8th to the 6th Millennium BC. Br Archeological Rep Int Ser 683

Felsenstein J (1993) PHYLIP (Phylogeny Inference Package) version 3.5c. Department of Genetics, University of Washington, Seattle, WA 98195-7360, USA

Frankel OH, Bennett E (1970) Genetic resources in plants – their exploitation and conservation. Blackwell, Oxford

Galli G, Feldman M (1983) Diploidization of endosperm protein genes in polyploid wheats. In: Sakamoto S (ed) Proc 6th Int Wheat Genet Symp Maruzen, Kyoto, Japan, pp 1119–1123

Gandilyan PA (1998) *Triticum urartu* Thum. Ex Gandilyan, and its role in the evolution of the genus *Triticum* L. In: Jaradat AA (ed) Triticeae III. Science Publ, NH, USA, pp 67–76

Hamrick JL, Godt MJW (1989) Allozyme diversity in plant species. In: Brown AHD, Clegg MT, Kahler AL, Weir BS (eds) Plant population genetics, breeding, and genetic resources. Sunderland, Mass., pp 43–65

Hancock JF (1992) Plant evolution and the origin of crop species. Prentice Hall, New Jersey

Harlan JR, Zohary D (1966) Distribution of wild wheat and barley. Science 153:1078

Heun M, Schafer-Pregl R, Klawan D, Castagna R, Accerbi M, Borghi B, Salamini F (1997) Site of einkorn wheat domestication identified by DNA fingerprinting. Science 278:1312–1314

Jain SK (1983) Genetic characteristics of populations. In: Mooney HA (ed) Comparative structural and functional characteristics of natural versus man-modified ecosystems. Springer, Berlin Heidelberg New York, pp 240–257

Johnson BL (1975) Identification of the apparent B-genome donor of wheat. Can J Genet Cytol 17:21–39

Johnson BL, Dhaliwal HS (1976) Reproductive isolation of *Triticum boeoticum* and *Triticum urartu* and the origin of the tetraploid wheats. Am J Bot 63:1088–1094

Knowlton N (1993) Sibling species in the sea. Annu Rev Ecol Syst 24:189–216

Levene H (1953) Genetic equilibrium when more than one niche is available. Am Nat 87:331–333

McGuire S (1997) The effects of privatization on winter-wheat breeding in the UK. Biotechnol Dev Monitor 33:8–11

Medlinger S, Zohary D (1995) The extent and structure of genetic variation in species of the Sitopsis group of *Aegilops*. Heredity 74:616–627

Morris R, Sears ER (1967) The cytogenetics of wheat and its relatives. In. Quisenberry KS, Reitz LP (eds) Wheat and wheat improvement. American Society of Agronomy, Madison, Wis, pp 20–21

Nei M (1987) Molecular evolutionary genetics. Columbia University Press, New York

Nei M (1972) Genetic distance between populations. Am Nat 106: 283–292

Nevo E, Beiles A (1989) Genetic diversity in wild emmer wheat in Israel and Turkey. Theor Appl Genet 77:421–455

Nevo E, Goldberg E, Beiles A, Brown ADH, Zohary D (1982) Genetic diversity and environmental associations of wild wheat *Triticum dicoccoides* in Israel. Theor Appl Genet 78:260–264

Raven PH (1987) The scope of the plant conservation problem world-wide. In: Bramwell D, Hamann O, Heywood V, Synge H (eds) Botanic gardens and the world conservation strategy, Academic Press, London, pp 19–20

Rejesus RM, van Ginkel M, Smale M (1996) Wheat breeder's perspectives of genetic diversity and germplasm use. Wheat Special Report 40, CYMMIT, Mexico

Rice JA (1995) Mathematical statistics and data analysis. Duxbury Press, Belmont, Calif

Rosegrant MW, Agcaoili-Sombilla A, Perez N (1995) Global food projections to 2020. Discussion paper 5. International Food Policy Research Institute (IFFRI), Washington, D.C.

Slatkin M, Barton NH (1989) A comparison of three indirect methods for estimating average levels of gene flow. Evolution 43:1349–1368

Smith-Huerta NL, Huerta AJ, Barnhart D, Waines JG (1989) Genetic diversity in wild diploid wheats *Triticum monococcum* var. *boeoticum* and *T. urartu* (Poaceae). Theor Appl Genet 78:260–264

Stebbins GL (1956) Taxonomy and the evolution of the genera, with special reference to the family Gramineae. Evolution 10:238–240

Takhtajan AL (1986) Floristics regions of the world (English translation by Crovello TJ). University of California Press, Berkeley

Tanksley SD, McCouch SR (1997) Seed banks and molecular maps: unlocking genetic potential for the wild. Science 277:1063–1066

Tolbert DM, Qualset CO, Jain SK, Craddock JC (1979) A diversity analysis of world collection of barley. Crop Sci 19:789–794

van Slageren MW (1994) Wild wheats: a monograph of *Aegilops* L. and *Amblyporum* (Jaub and Spach) Eig (Poaceae). Wageningen Agriculture University Papers, Wageningen Agricultural University, Wageningen, The Netherlands

Waines JG (1994) High temperature stress in wild wheats and spring wheats. Aust J Plant Physiol 21:705–715

Waines JG, Barnhart D (1992) Biosystematic research in *Aegilops* and *Triticum*. Hereditas 116:207–212

Weir B (1979) Inferences about linkage disequilibrium. Biometrics 35:235–254

Wright S (1978) Variability within and among natural populations, vol 4. The University of Chicago Press, Chicago

Yaghoobi-Saray J (1979) An electrophoretic analysis of genetic variation within and between populations of five species in the *Triticum-Aegilops* complex. PhD thesis, Genetics Program, University of California, Davis, Calif

Zohary D, Hopf M (1993) Domestication of plants in the old world: the origin and spread of cultivated plants in West Asia, Euroope, and the Nile Valley. Clarendon Press, Oxford, UK