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Microsatellite DNA polymorphism divergence in *Triticum dicoccoides* accessions highly resistant to yellow rust

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Abstract Stripe rust (yellow rust), caused by *Puccinia striiformis* f. sp. *tritici*, is one of the most important diseases of wheat throughout the world. Wild emmer wheat, *Triticum dicoccoides*, the progenitor of cultivated wheat, was found to be a valuable source for novel stripe-rust-resistance genes. The objective of the present study was to estimate the extent of genetic diversity among the wild emmer wheat accessions, previously identified as highly resistant to stripe rust, in order to select suitable parents for genetic-mapping studies. Twenty three wheat microsatellite (WMS) markers were used to detect DNA polymorphism among 21 accessions of *T. dicoccoides*, which included 19 resistant and two susceptible accessions originating mainly from the center of origin and diversity in the Upper Galilee and Hermon Mountain in northern Israel. In addition, two *Triticum durum* and one *Triticum aestivum* lines were also included in the analysis. The 23 WMS markers used were located on 23 chromosome arms, representing all 14 chromosomes of genomes A and B of wheat, and revealed a total of 230 alleles. The number of alleles ranged from 5 to 18, with an average of ten alleles per WMS. Genetic dissimilarity values between genotypes, calculated by the WMS-derived data, were used to produce a dendrogram of the relationships among accessions using the unweighted pair-group method with arithmetic averages (UPGMA). The results showed that all of the wild emmer wheat accessions could be distinguished. Most of the resulting groups were strongly related to the ecogeographical origin of the accessions, indicating that the genetic diversity of *T. dicoccoides* is correlated with geographic distribution. The three major groups were the Rosh Pinna group (north of the Sea of Galilee), the Mount Hermon group (north of the Golan Heights) and Mount Kena'an group (Upper Galilee). The genetic similarity (GS) of the 21 *T. dicoccoides* accessions based on WMS results averaged 0.31. As expected, the *T. durum* and *T. aestivum* lines were grouped separately from the *T. dicoccoides* accessions. The results obtained suggest that a relatively small number of microsatellites can be used for the estimation of genetic diversity in wild material of *T. dicoccoides*. These results will be useful in the identification of suitable parents for the development of mapping populations for tagging yellow-rust resistance genes derived from *T. dicoccoides*. Furthermore, future work could test the adaptive evolutionary significance of microsatellites in natural populations of wild emmer wheat.

Key words Genetic diversity · Microsatellite markers · Wheat · Stripe rust · *Triticum dicoccoides*

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Introduction

Stripe rust (yellow rust), caused by *Puccinia striiformis* Westend. f. sp. *tritici* Eriks., is one of the most devastating diseases of wheat throughout the world. The breeding of resistant varieties offers an effective approach to eliminate the use of fungicides and to minimize crop losses due to this disease. However, most of the described major genes for resistance to stripe rust in cultivated wheat have become ineffective to one or more of the known pathogenic races when acting singly (Stubbs 1985). In all wheat-growing areas of the world, the virulence of the stripe-rust races has been shown to

increase step by step (Stubbs 1985), often breaking down even combinations of resistance genes.

The depletion of effective genes for resistance to stripe rust in cultivated wheat led to a search for new resistance genes among wild wheats. A particularly promising source for yellow-rust resistance is wild emmer wheat, *Triticum dicoccoides* Korn, the progenitor of all cultivated wheat (Gerechter-Amitai and Stubbs 1970). This wild species, discovered in northern Israel by Aaronsohn (1910), harbors extensive genetic resources for wheat improvement (Nevo 1983, 1989, 1995), including a potential source of genes for rust resistance (Gerechter-Amitai and Stubbs 1970; Nevo et al. 1986; The et al. 1993).

A collection of about 850 samples from wild emmer populations in Israel were tested for resistance to stripe rust. Of these about 10% proved to be resistant to Israeli test isolates of stripe rust (Van Silfhout et al. 1989a, b). Further tests with 28 isolates from 19 countries in America, Africa, Asia, Australia and Europe revealed that 30 selections were resistant to more than 25 isolates, while the other 38 selections were in various ways susceptible to one or more isolates. Based on a Person-analysis of the results it was estimated that at least 11 different factors for resistance are present in the tested wild emmer selections (Van Silfhout et al. 1989a).

One of the highly resistant accessions, *T. dicoccoides* G-25, has been well characterized (Gerechter-Amitai and Stubbs 1970; Gerechter-Amitai and Grama 1974), and further studies showed that this resistance was conferred by one dominant gene, designated as *Yr15* (Gerechter-Amitai et al. 1989). Using monosomic mapping, McIntosh et al. (1996) located *Yr15* on chromosome 1B of wheat. In a previous study, we have used the near-isogenic lines (NILs) approach to identify two molecular markers, $OPB13_{1420}$ and *Nor1*, flanking the *Yr15* gene of wheat at a distance of 27.1 cM and 11.0 cM, respectively (Sun et al. 1997). Further genetic mapping studies are required to characterize the additional *T. dicoccoides*-derived resistance genes and identify their chromosomal location. The objective of the present work was to estimate the extent of genetic diversity among the wild emmer wheat accessions, previously identified as highly resistant to stripe rust, in order to select suitable parents for genetic-mapping studies.

Simple-sequence repeats (SSRs), also known as microsatellites, are a relatively new class of molecular markers based on tandem repeats of short (2–6 bp) DNA sequences (Litt and Luty 1989). These repeats are highly polymorphic, even among closely related cultivars, due to mutations causing variation in the number of repeating units. This kind of polymorphism at specific loci is easily detected using specific primers in the flanking regions of such loci and subsequent amplification via the polymerase chain reaction (Litt and Luty 1989; Weber and May 1989). The high level of polymorphism, combined with a high interspersion rate, makes them an abundant source of genetic markers. Extremely high-density molecular maps based on microsatellite DNA markers have been constructed recently, both in human (Dib et al. 1996) and mouse (Dietrich et al. 1996) genomes. Microsatellites appear to be a major source for, and to act as "tuning knobs" in, molecular adaptive evolution, including the evolutionary control of the mutation process itself (Kashi et al. 1997; King et al. 1997). Recently, we have shown an association between microsatellite and ecogeographic variation in wild barley, *Hordeum spontaneum* (Forster et al. unpublished data).

The usefulness of microsatellites 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) and *Arabidopsis* (Bell and Ecker 1994). These studies indicated that microsatellites 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 microsatellite markers was also demonstrated for self-pollinating species with a relatively low level of intraspecific polymorphism, such as hexaploid wheat, *Triticum aestivum* (Plaschke et al. 1995; Röder et al. 1995), and cultivated barley, *Hordeum vulgare* (Saghai Maroof et al. 1994; Liu et al. 1996).

In the present report, we demonstrate the application of wheat microsatellite (WMS) markers for the differentiation and estimation of genetic diversity among different accessions of *T. dicoccoides* originating primarily from northern Israel and selected for their high resistance to yellow rust.

Materials and methods

Ecological background of *T. dicoccoides*

Wild emmer wheat, *T. dicoccoides* (genomic constitution AABB), is the tetraploid, predominantly self-pollinated, wild progenitor from which modern tetraploid and hexaploid cultivated wheats were derived (Zohary 1970). Wild emmer is distributed over the Near East Fertile Crescent, in Israel, Jordan, Lebanon, Syria, east Turkey, north Iraq and west Iran (Harlan and Zohary 1966). The center of distribution and diversity of *T. dicoccoides* is found in the catchment area of the upper Jordan Valley in Israel and its vicinity (Nevo and Beiles 1989). Wild emmer ranges over a wide altitudinal amplitude. Robust, early maturing, phenotypes grow in the winter-warm slopes facing the sea of Galilee, as low as 100 m below sea level. More slender and late-flowering types occur in higher and cooler elevations, reaching 1500 m on Mount Hermon (Zohary 1970).

Plant material

Twenty one *T. dicoccoides* accessions, two *T. durum* lines and one *T. aestivum* line were used in this study. The *T. dicoccoides* accessions were kindly provided by Z. Gerechter-Amitai, Agricultural Research Organization, Bet-Dagan, Israel. The *T. dicoccoides* and *T. durum* accessions used are all tetraploid (AABB); the *T. aestivum* line is a hexaploid (AABBDD). The *T. dicoccoides* accessions were selected

for their high resistance to yellow rust (Van Silfhout et al 1989a). All of the accessions and cultivars used in this work, along with their geographic origin and yellow-rust resistance, are listed in Table 1.

Wheat microsatellite (WMS) analysis

DNA samples were isolated from whole wheat grains as described by Plaschke et al. (1995). The microsatellite primers used are as described by Plaschke et al. (1995; 1996); Röder et al. (1995) and Röder et al. (unpublished data). Twenty three primer pairs representing wheat microsatellites (WMSs) which amplify the expected fragments (according to sequence data) in *T. aestivum* cv 'Chinese Spring' ('CS') were chosen for the analysis. WMS designation, fragment sizes in 'CS', the range of allele size, and the chromosome-arm location of the amplified loci are presented in Table 2. For WMS 18, 95, 120, and 186, the respective data have been already published by Röder et al. (1995). PCR amplifications were performed as described in Röder et al. (1995). The PCR-amplified fragments were detected on an automated laser fluorescence (A.L.F.) sequencer (Pharmacia). To allow for this, one primer of each pair was labelled at the 5′ end with fluorescein. Gel running conditions are as described by Plaschke et al. (1995). Fragment sizes were calculated in the computer program Fragment Manager (Pharmacia) by comparison with internal size standards, which were added to each lane in the loading buffer.

Statistical analysis

The presence or absence of each single fragment was coded by 1 or 0, respectively, and scored for a binary data matrix. Genetic distance was calculated for each pair of lines using the percentage difference in the program NCLAS of the computer package SYN-TAX IV (Podani 1990), according to the equation: $PD = 1 - 2Nij/(Ni + Nj)$, where Nij is the number of fragments common to accessions i and j, and (Ni + Nj) is the total number of fragments in both accessions. This value lies between 0 and 1, with a score of 0 indicating that all fragments are in common, and 1 indicating no common fragments. The unweighted pair-group method with arithmetic average (UPGMA) was chosen as a clustering method. The dendrogram was designed using DENDPLOT from the same computer package. Genetic similarity (GS) was calculated according to Nei and Li (1979): $GS = 2Nij/(Ni + Nj) = 1 - PD$.

Results

Wheat microsatellites in wild emmer wheat

The code number, country of origin, collection location and the reaction to yellow rust of each of the accessions used in this work are listed in Table 1. Nineteen of the total twenty one *T. dicoccoides* accessions included were from different locations in Northern Israel, the primary distribution site and center of origin and diversity of *T. dicoccoides* (Nevo and Beiles 1989). One accession was from a location in Lebanon and one from Turkey. In addition, two *T. durum* lines and one *T. aestivum* line were also included in this analysis.

In total, 230 alleles were detected with the 23 WMSs, located on 14 different chromosomes and 23 different chromosome arms of the A and B genomes of wheat (Table 2). All of the WMS markers used in this study yielded polymorphic fragments among the 21 *T. dicoccoides* accessions and the three cultivated wheat lines.

Table 1 Wild and cultivated wheat accessions, their geographic origin, and their resistance to stripe rust

No.	Code /name	Species	Country of origin	Site	Resistance to stripe rust
1.	D447	*T. durum*	USA	–	S[a]
2.	Langdon	*T. durum*	USA	–	S
3.	G4-1-3-2-2	*T. dicoccoides*	Israel	Rosh Pinna	R[b]
4.	G25-78-35	*T. dicoccoides*	Israel	Rosh Pinna	R
5.	G40-1-2B-1	*T. dicoccoides*	Israel	Mt. Beatitudes	R
6.	G84-1M-8-10	*T. dicoccoides*	Israel	Rosh Pinna	R
7.	G90-1-1B-15	*T. dicoccoides*	Israel	Rosh Pinna	R
8.	G94-1M-1BM	*T. dicoccoides*	Israel	Almagor	S
9.	G121-1-3-1-1	*T. dicoccoides*	Israel	Bet Qeshet	R
10.	G148-1-2M-21	*T. dicoccoides*	Israel	Meron	R
11.	G149-9	*T. dicoccoides*	Israel	Rosh Pinna	R
12.	G194-3M-37-10	*T. dicoccoides*	Lebanon	Rashaya	R
13.	G201M-1M-6-1	*T. dicoccoides*	Turkey	Karakadaj	S
14.	G283-10-2-9-1	*T. dicoccoides*	Israel	Majdal Shams	R
15.	G288-3-5M-9-1	*T. dicoccoides*	Israel	Mt. Kena'an	R
16.	G303-1M-4-3-2	*T. dicoccoides*	Israel	Dishon	R
17.	G312-10-5B-4	*T. dicoccoides*	Israel	Majdal Shams	R
18.	G313-9-1-1M	*T. dicoccoides*	Israel	Majdal Shams	R
19.	G316-2-5-4-20	*T. dicoccoides*	Israel	Mt. Hermon	R
20.	G340-3-3B-3-6	*T. dicoccoides*	Israel	Mt. Kena'an	R
21.	G344-1-1-3-2	*T. dicoccoides*	Israel	Dalton	R
22.	G348-10-BW-3	*T. dicoccoides*	Israel	Kefar Shamai	R
23.	G485-5M	*T. dicoccoides*	Israel	Majdal Shams	R
24.	Chinese Spring	*T. aestivum*	China	–	S

[a] S = susceptible to Israeli test isolates; the *T. dicoccoides* and *T. durum* accessions are all tetraploid (AABB), the *T. aestivum* line is a hexaploid (AABBDD)

[b] R = resistant to more than 25 stripe rust isolates from all over the world (Van Silfhout et al. 1989 a, b)

The number of alleles per WMS ranged from 5 to 18 (Table 2). On average, ten alleles were detected per locus. Out of 483 marker-accession combinations, only four (WMS99-G303, WMS99-G201, WMS340-G316, WMS397-G312) failed to amplify a product in *T. dicoccoides*. The most polymorphic microsatellite was WMS443 with 18 alleles. Figure 1 shows the distribution of the 18 fragments amplified with WMS443 among the 21 *T. dicoccoides* accessions, the two *T. durum* lines and the one *T. aestivum* line. Most of the *T. dicoccoides* accessions show two peaks per accession. The sizes of the 18 alleles observed ranged from 127 to 229 bp. WMS443 is a complex microsatellite with a motif $(CA)_{20}(GA)_{22}$ in 'CS' (M. Röder, unpublished). Hence, because 'CS' has a repetitive motif of 84 bp and amplified a fragment of 134 bp, it is assumed that the length of the repetitive motif in the *T. dicoccoides* accessions used here ranged from 78 to 180 bp.

Genetic diversity

The wild emmer wheat collections included a total of 21 accessions, which can be divided as follows: 19 from Israel, one from Turkey and one from Lebanon. The collections from Israel included 11 locations, namely, Rosh Pinna (5), Mt. Beatitude (1), Almagor (1), Bet-Qeshet (1), Meron (1), Majdal Shams (4), Mt. Kena'an (2), Dishon (1), Mt. Hermon (1), Dalton (1) and Kefar Shamai (1). Pairwise comparisons were made between all accessions and the average dissimilarity values were calculated based on WMS product data. A matrix of dissimilarity values for all 24 wild and cultivated wheat accessions is presented in Table 3. The genetic distances for all 276 pairs ranged from 0.018 to 0.968

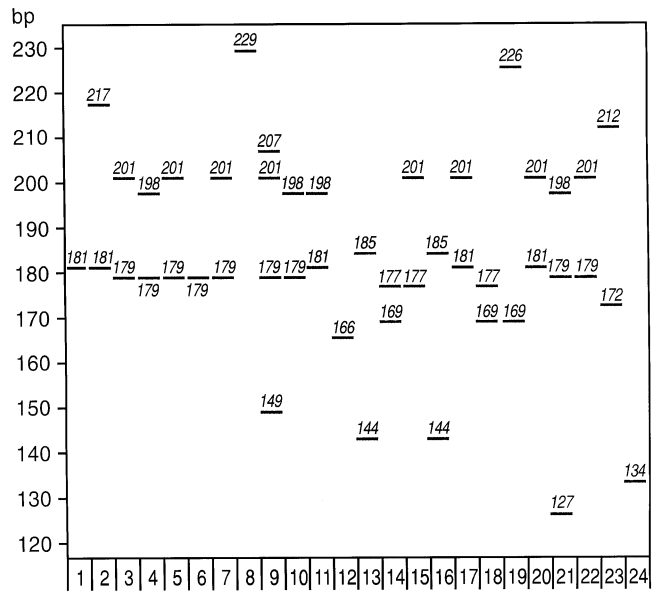

Fig. 1 WMS443-PCR products of 24 wild and cultivated wheat accessions displayed by the fluorogram-method of the Fragment Manager program after separation on an A.L.F. sequencer. *Accession numbers* correspond to those in Table 1. The sizes of the 18 alleles observed ranged from 127 to 229 bp. WMS443 is a complex microsatellite with the repetitive motif $(CA)_{20}(GA)_{22}$

Table 2 Wheat microsatellite designation, chromosomal and chromosomal-arm locations, and the number of alleles for the WMS employed

Designation[a]	Chromosomal location	Number of alleles	Fragment size in 'CS' (bp)	Range of allele size (bp)
WMS18	1BS	6	186	178–207
WMS60	7AS	12	139, 204	139–227
WMS95	2AS	7	119	102–124
WMS99	1AL	10	107, 125	92–162
WMS120	2BL	8	139	127–156
WMS169	6AL	11	188	176–244
WMS186	5AL	13	137	87–163
WMS218	3AS	11	153	129–223
WMS219	6BL	7	181	148–181
WMS251	4BL	12	75, 100, 106	70–106
WMS257	2BS	5	192	181–194
WMS265	2AL	5	150, 200	150–200
WMS332	7AL	11	194, 231	183–231
WMS340	3BL	15	122, 161	103–213
WMS357	1AL	7	123	115–137
WMS361	6BS	6	126	124–134
WMS389	3BS	10	129	100–171
WMS397	4AL	11	188, 199	180–229
WMS400	7BS	10	139	138–164
WMS403	1BL	10	133	124–145
WMS408	5BL	10	175,198	144–198
WMS443	3AL(?)	18	134	127–229
WMS459	6AS	15	146, 198	60–394

[a] WMS marker designation and chromosomal location are according to Röder et al. (1995), Plaschke et al. (1995, 1996) and M. Roder (unpublished). The chromosomal location of WMS443 is currently uncertain

and averaged 0.739. For the 210 pairs of *T. dicoccoides* accessions the genetic distance ranged from 0.018 to 0.964 and averaged 0.69 (Table 3).

Cluster analysis was then carried out using the UPGMA method and resulted in the phenogram shown in Fig. 2. This phenogram discriminates all of the accessions and lines tested. Six major groups can be distinguished (Fig. 2). The phenogram obtained shows that the pattern of clustering of most of the *T. dicoccoides* accessions is related to the geographic distribution of the wild wheat genotypes. Group 1 is a cluster of accessions from the central and eastern Upper Galilee Mountains, north-west of the Sea of Galilee. The genetic variability within this group is lower than within the other groups. This group consists of nine accessions, five from one site at Rosh Pinna (G4, G90, G25 G84 and G149) and four from different sites at Meron (G148), Dalton (G344), Mt. Beatitude (G40) and Kefar Shamai (G348), located within a short distance (about 20 km) from the Sea of Galilee. The next outer branch connects this group with group 2 which includes three accessions, two from Mt. Kena'an (G288 and G340), in the eastern Upper Galilee, and one from Majdal Shams (G312), on the slope of Mount Hermon. Group 4 consists of four accessions from the Mt. Hermon area, north of the Golan Heights (altitude 1100–1500 m). Accession G316 is from Mt. Hermon (1500 m), while accessions G283, G313, and G485 are from Majdal

Table 3 Dissimilarity matrix of 24 accessions of wild and cultivated wheats

Line/accession[a]	1	2	3	4	5	6	7	8	9	10	11	12
1. D447	0.000											
2. Langdon	0.508	0.000										
3. G4	0.931	0.895	0.000									
4. G25	0.967	0.867	0.263	0.000								
5. G40	0.966	0.897	0.236	0.241	0.000							
6. G84	0.966	0.931	0.309	0.241	0.393	0.000						
7. G90	0.932	0.931	0.091	0.310	0.321	0.250	0.000					
8. G94	0.828	0.930	0.889	0.930	0.927	0.891	0.855	0.000				
9. G121	0.690	0.771	0.731	0.714	0.618	0.765	0.765	0.851	0.000			
10. G148	0.966	0.930	0.370	0.228	0.418	0.127	0.345	0.889	0.761	0.000		
11. G149	0.931	0.895	0.407	0.263	0.491	0.164	0.345	0.926	0.821	0.111	0.000	
12. G194	0.860	0.893	0.887	0.964	0.963	0.963	0.889	0.849	0.909	0.962	0.962	0.000
13. G201	0.810	0.871	0.763	0.742	0.867	0.733	0.733	0.797	0.806	0.729	0.729	0.793
14. G283	0.862	0.860	0.889	0.825	0.855	0.855	0.891	0.926	0.821	0.852	0.852	0.811
15. G288	0.875	0.873	0.500	0.556	0.574	0.475	0.475	0.867	0.863	0.533	0.500	0.898
16. G303	0.841	0.903	0.763	0.742	0.833	0.733	0.733	0.831	0.861	0.695	0.695	0.862
17. G312	0.862	0.860	0.519	0.649	0.564	0.600	0.564	0.926	0.821	0.667	0.630	0.925
18. G313	0.864	0.862	0.855	0.793	0.821	0.821	0.857	0.927	0.794	0.818	0.818	0.815
19. G316	0.860	0.893	0.887	0.893	0.926	0.852	0.852	0.849	0.909	0.887	0.849	0.846
20. G340	0.793	0.825	0.556	0.649	0.564	0.636	0.564	0.815	0.821	0.667	0.630	0.849
21. G344	0.938	0.905	0.433	0.333	0.475	0.344	0.443	0.900	0.753	0.333	0.367	0.966
22. G348	0.931	0.895	0.333	0.404	0.200	0.527	0.418	0.926	0.672	0.556	0.593	0.962
23. G485	0.806	0.836	0.724	0.738	0.797	0.763	0.763	0.862	0.831	0.793	0.793	0.930
24. 'CS'	0.844	0.841	0.967	0.968	0.967	0.869	0.934	0.933	0.808	0.867	0.867	0.932

Table 3 Continued

Line/accession[a]	13	14	15	16	17	18	19	20	21	22	23	24
13. G201	0.000											
14. G283	0.797	0.000										
15. G288	0.815	0.800	0.000									
16. G303	0.156	0.763	0.754	0.000								
17. G312	0.864	0.852	0.367	0.797	0.000							
18. G313	0.767	0.018	0.770	0.733	0.818	0.000						
19. G316	0.828	0.585	0.797	0.793	0.887	0.593	0.000					
20. G340	0.797	0.852	0.300	0.763	0.296	0.818	0.849	0.000				
21. G344	0.785	0.867	0.545	0.754	0.633	0.836	0.898	0.667	0.000			
22. G348	0.864	0.889	0.600	0.831	0.556	0.855	0.925	0.593	0.367	0.000		
23. G485	0.778	0.724	0.813	0.778	0.828	0.729	0.684	0.793	0.656	0.724	0.000	
24. 'CS'	0.908	0.933	0.909	0.908	0.933	0.934	0.898	0.900	0.879	0.933	0.844	0.000

[a] Line/accession codes correspond to those in Table 1. Genetic distances were calculated for each pair of lines using the percentage difference in the program NCLAS of the computer package SYN-TAX IV

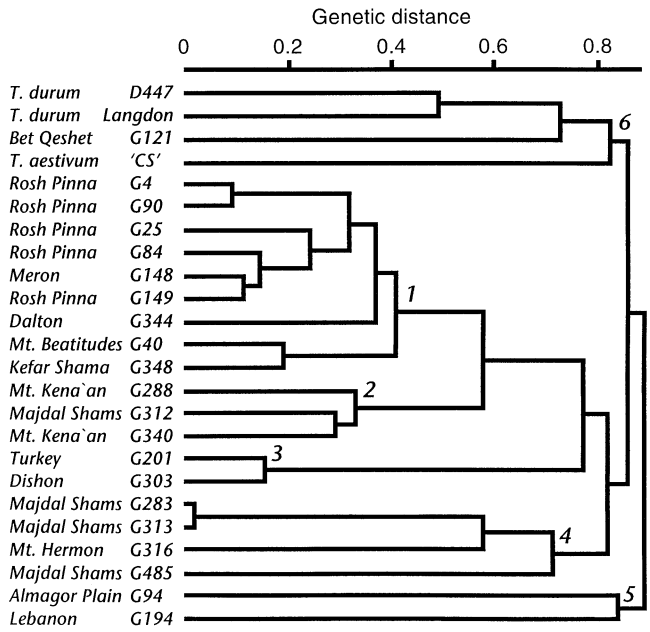

Fig. 2 Dendrogram of 24 wild and cultivated wheat accessions, based on the genetic distance (percentage difference) calculated from data of 23 wheat microsatellites, using the UPGMA as the clustering method. Marked groups (1–6) are discussed in the results section. *Accession codes* correspond to those in Table 1

Shams (1100–1300 m). The closest similarity between two accessions collected at Majdal Shams (G283 and G313) appears to be interesting. These two lines differed only in WMS60, with a genetic distance of 0.018.

Groups 3 and 5 are heterogeneous and are well separated from the other groups. They each consist of a pair of *T. dicoccoides* accessions, collected from diverse locations but grouped close to each other. The accessions in group 3 are from Karakadaj in Turkey (G201) and Dishon in Israel (G303), while the accessions in group 5 are from Rashaya, Lebanon (G194) and Almagor, Israel (G94). The most distinct *T. dicoccoides* accessions were G194 (Rashaya, Lebanon), G94 (Almagor, Israel) and G121 (Bet Qeshet, Israel) which show the highest dissimilarity to the other wild wheat accessions of this collection, with average genetic distances of 0.90, 0.88 and 0.79, respectively, from the remaining 20 *T. dicoccoides* accessions. Accession G194 from Rashaya (Lebanon) and G344 from Dalton (Israel) show the highest dissimilarity among the accessions of *T. dicoccoides* from this collection, with a genetic distance of 0.966. Of a total of 59 DNA fragments amplified by 23 WMS primer pairs, only two (3%) were common in these two accessions, whereas the remaining 57 products (97%) exhibited a different presence-or-absence behavior.

As expected, the *T. durum* and *T. aestivum* lines were grouped separately from the *T. dicoccoides* accessions (group 6). Furthermore, the two *T. durum* tetraploid lines are clearly separated from the hexaploid wheat 'CS', with an average genetic distance of 0.842. Although the *T. durum* line D447 is a derivative of cv Langdon (cv D447 = LD393/2 Langdon ND58-322), the genetic distance between these two lines was relatively high, 0.508. Accession G121 of *T. dicoccoides* from Bet Qeshet is distinctly different from the other *T. dicoccoides* genotypes and was grouped within the cluster of *T. durum* and *T. aestivum* cultivated lines.

Contribution of the A and B genomes to the genetic variability of *T. dicoccoides* (AABB)

Out of 23 microsatellite markers used, 12 WMS markers were located on the seven chromosomes of the A genome, and detected 131 alleles; while 11 WMS markers were located on the seven chromosomes of the B genome and detected 99 alleles. The average number of alleles per marker for the A genome was 10.9, while for the B genome it was only nine alleles per marker; i.e. 20% higher for the A genome. In an attempt to estimate the different contribution of each genome to the genetic variation within *T. dicoccoides*, the genetic distances were calculated based on each set of markers separately. The resulting phenogram showed a clustering pattern similar to that obtained with all 23 WMSs (presented in Fig. 2), though with some minor changes, mainly within the heterogeneous groups. However, based on the resulting genetic distances between the *T. dicoccoides* accessions it seems that the A genome is more variable than the B genome. For example, three pairs of accessions which could be distinguished according to the A-genome analysis, were indistinguishable by their B-genome microsatellite analysis.

Discussion

The objective of this study was to estimate the extent of genetic diversity among the wild emmer wheat accessions, previously identified as highly resistant to stripe rust, in order to select suitable parents for genetic-mapping studies. Wheat microsatellites (WMSs) were used to detect DNA polymorphism among 21 accessions of *T. dicoccoides*, which included 19 resistant and two susceptible accessions originating mainly from the Upper Galilee and Mount Hermon in northern Israel. In addition, two *T. durum* lines and one *T. aestivum* line were also included in the analysis.

Wheat microsatellites

This research demonstrates the utility of microsatellite markers in the study of genetic diversity both within and between wild and cultivated wheat germplasm. Regions flanking microsatellite loci are often conserved

between closely related species (Moore et al. 1991), allowing the use of primers to amplify loci in such closely related species. As the microsatellite markers used in this study were derived from hexaploid wheat, *T. aestivum*, it was demonstrated here that there is no technical problem in using these PCR markers across different wheat species, and to apply them successfully for the analysis of wild emmer wheat, *T. dicoccoides*. Out of 483 marker-accession combinations, only four (WMS99-G303, WMS99-G201, WMS340-G316, WMS397-G312) failed to amplify a product in *T. dicoccoides*. These missing amplification products are most likely due to sequence alterations, such as point mutations, deletions or inversions, within the priming sites, as reported by Devos et al. (1995). Plaschke et al. (1995) had already demonstrated the utility of these markers for the tetraploid wheats (AABB) *T. durum* and *T. aethiopicum*. However, of 15 primer pairs that amplified microsatellite sites in wheats, only one pair amplified a microsatellite-containing fragment in several rye and barley accessions (Röder et al. 1995). Hence, it can be concluded that the high efficiency of wheat microsatellite markers is limited to closely related *Triticum* species.

Genetic diversity

In a previous study, using 23 WMS markers, Plaschke et al. (1995) revealed a total of 142 alleles, among 40 cultivated wheat lines; an average of 6.2 alleles per WMS. In the present study, also using 23 WMSs, 230 alleles were revealed among only 24 wild and cultivated wheat accessions; an average of ten alleles per WMS. These results demonstrate the high variation in microsatellite sequences among *T. dicoccoides* accessions compared to the cultivated germplasm, as was earlier demonstrated by allozyme markers (Nevo et al. 1986; Nevo and Beiles 1989). Clearly, genetic diversity was eroded during the domestication process as was also the case in major cereal crops (Nevo 1986).

The dendrogram presented in the present study (Fig. 2) demonstrates clearly the ability of microsatellites, developed based on *T. aestivum* sequences, to detect a large amount of genetic diversity in wild emmer wheat and to identify intergroup differences. All of the *T. dicoccoides* accessions of this collection were distinguishable by the WMS used, even within closely related populations originating from the same geographic location (e.g. the Rosh Pinna group and the Mt. Hermon group). Most of the resulting groups are strongly related to the geographical origin of the accessions, indicating that the genetic diversity of *T. dicoccoides* is correlated with geographic distribution. The three major groups are the Rosh Pinna group (north of the Sea of Galilee), the Mount Hermon group and the Mount Kena'an group (eastern Upper Galilee). However, there were also two heterogeneous groups (3 and 5), well separated from the others. These accessions were collected from diverse locations but were clustered close to each other. Group 5 contains accessions from Rashaya, Lebanon (G194) and Almagor, Israel (G94), that are distinct from each other, though the computer program has grouped them together because no other grouping was possible. The grouping of the Israeli accession, Dishon G303, with the Turkish accession, Karakadaj G201, is an exception. These accessions appeared to share many similarities. The reason for this grouping is not clear; however, it may reflect ecological similarities between these populations.

Our results demonstrate that the DNA polymorphism of wild wheat was correlated with the ecogeographic distribution of the accessions. In addition, the Israeli collection studied here exhibited high inter-population and inter-regional polymorphism. These observations are consistent with previous results obtained with isozymes and different DNA markers for different collections of wild emmer wheat covering a much wider geographical range (Nevo et al. 1982; Nevo 1983; 1989; 1995; Nevo and Beiles 1989). Using RAPD markers, Joshi and Nguyen (1993) found great variation in the similarity index (0.2–1.0) among 20 wild emmer accessions collected from Israel, Turkey, and Jordan. Although the accessions used in their study were collected at different locations from those used for the current study, they also found that accessions from the same locality tend to cluster together. We recently showed microscale edaphic RAPD differentiation in wild emmer wheat (Li et al. unpublished data) and wild barley (Owuor et al. 1997).

Although the results presented here agree with previous studies using isozyme and DNA markers on wild wheat (Nevo et al. 1982; Nevo 1983; 1989; 1995; Nevo and Beiles 1989), it must be emphasized that the present study is confined to only a small proportion of the germ plasm of wild wheat collections. This is not a study on the natural populations, nor a project covering all of, the wild wheat collections. It should be noted that the wild emmer wheat accessions studied here were selected for their high resistance to stripe rust and not by their geographic distribution. Hence, uneven representation of the different wild populations may have contributed to some of the groupings in the phenogram presented in this study, although locality clustering reflects ecogeographical correlates.

Contribution of the A and B genomes to genetic variability

In an attempt to estimate the different contribution of each genome to the genetic variation within *T. dicoccoides*, the genetic distance was calculated based on each set of markers separately. The average number of alleles per marker for the A genome was 10.9, while that for the B genome was only nine alleles per marker;

i.e. 20% higher for the A genome. Based on 12 markers only, representing the A genome, it was still possible to distinguish between all of the 24 accessions used in this study. Based on 11 highly polymorphic markers representing the B genome, most of the accessions were distinguishable, except for three pairs of closely related accessions from the same location which showed an identical fragment pattern. These results indicate that the microsatellite loci of the A genome are more variable than those of the B genome. Nevertheless, these results also indicate that about half of the number of WMS markers used in the present study was sufficient to give a good estimation of the genetic distances within this material. The results obtained suggest that a relatively small number of microsatellites can be used for the estimation of genetic diversity in wild material of *T. dicoccoides*. Plaschke et al. (1995) also mentioned that a small number of markers was sufficient when testing a set of cultivated wheat lines with WMS markers. Clearly, the high polymorphism of WMSs provides a powerful tool for both population genetics and mapping studies in wild and cultivated wheat.

Selection of parents for mapping

Several approaches were used previously to determine whether new collections of wild emmer wheat differ from each other with respect to their resistance genes. These studies resulted in the estimation that several different factors for resistance to yellow rust are present in the tested wild emmer accessions (Van Silfhout et al. 1989a). The stripe-rust resistance harbored by *T. dicoccoides* accession G25 has been characterized. It involves one dominant gene designated *Yr15* (Gerechter-Amitai et al. 1989). Using molecular markers, we previously mapped *Yr15* to chromosome 1B of wheat (Sun et al. 1997). Further genetic mapping studies are required to characterize the additional *T. dicoccoides*-derived resistance genes and identify their chromosomal location.

Genetic dissimilarity values between genotypes, calculated by the WMS-derived data, should be useful in the identification of suitable parents for the development of mapping populations for tagging yellow-rust resistance genes derived from *T. dicoccoides*. The *T. dicoccoides* accession G25 was clustered within the Rosh Pinna group, which contains nine accessions collected from several sites located near the Sea of Galilee. The genetic distances within this group was low relative to other *T. dicoccoides* accessions. Hence, we assume that the probability of identifying novel stripe-rust resistance genes within this group which differ from *Yr15* is not high. The G194 collection from Rashaya (Lebanon) and G344 from Dalton (Israel) showed the highest dissimilarity among accessions of *T. dicoccoides* in the present collection, with a genetic distance of 0.966. Out of a total of 59 amplified products, only 3% were common in these two accessions, whereas the remaining 97% products exhibited different presence or absence behavior. In the future, these genotypes will be used as parents in inter-subspecific hybridization with the most dissimilar durum genotypes.

The strategy of using interspecific crosses for the development of a highly saturated genetic linkage map has proven extremely successful in tomato and other crop species (Tanksley et al. 1989). This approach is especially important for self-pollinating crop species such as wheat. The selection of dissimilar parents from natural populations of wild emmer wheat (for example, G194, G121, G485 and G344) and the cultivated durum wheat group (D447 or Langdon) is likely to provide a large number of segregating WMS markers, suitable for mapping and tagging agronomically important traits, such as resistance to stripe rust.

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