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RESEARCH PAPER

Identification of variation in adaptively important traits and genome-wide analysis of trait–marker associations in *Triticum monococcum*

Hai-Chun Jing[1], Dmitry Kornyukhin[1,2], Kostya Kanyuka[1], Simon Orford[3], Anastasiya Zlatska[1,4], Olga P. Mitrofanova[2], Robert Koebner[3] and Kim Hammond-Kosack[1,*]

[1] *Centre for Sustainable Pest and Disease Management, Department of Plant Pathology and Microbiology, Rothamsted Research, Harpenden, Herts AL5 2JQ, UK*

[2] *N. I. Vavilov Research Institute of Plant Industry, St Petersburg, 190000, Russian Federation*

[3] *Department of Crop Genetics, John Innes Centre, Norwich Research Park, Colney, Norwich NR4 7UH, UK*

[4] *Ukrainian Institute for Plant Variety Examination, Centre for Certification Trials, 15 Henerala Rodimtseva Street, Kyiv 03041, Ukraine*

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Abstract

Einkorn wheat *Triticum monococcum* (2*n*=2*x*=14, A^mA^m) is one of the earliest domesticated crops. However, it was abandoned for cultivation before the Bronze Age and has infrequently been used in wheat breeding. Little is known about the genetic variation in adaptively important biological traits in *T. monococcum*. A collection of 30 accessions of diverse geographic origins were characterized for phenotypic variation in various agro-morphological traits including grain storage proteins and endosperm texture, nucleotide-binding site (NBS) domain profiles of resistance (*R*) genes and resistance gene analogues (*RGA*s), and germination under salt and drought stresses. Forty-six SSR (single sequence repeat) markers from bread wheat (*T. aestivum*, 2*n*=6*x*=42, AABBDD) A genome were used to establish trait–marker associations using linear mixed models. Multiple significant associations were identified, some of which were on chromosomal regions containing previously known genetic loci. It is concluded that *T. monococcum* possesses large genetic diversity in multiple traits. The findings also indicate that the efficiency of association mapping is much higher in *T. monococcum* than in other plant species. The use of *T. monococcum* as a reference species for wheat functional genomics is discussed.

Key words: Association mapping, biological and agronomic traits, disease resistance, genetic variation, grain storage proteins, grain texture, salt and drought tolerance, *T. monococcum*.

Introduction

The diploid species *Triticum monococcum* (2*n*=2*x*=14, A^mA^m), commonly known as einkorn wheat coined from the German expression of 'one grain', was widely cultivated during the pioneering human farming activities in the Fertile Crescent. It was domesticated from its wild progenitor *T. boeoticum* near the Karacadag mountains in southeast Turkey (Heun *et al*., 1997). Domesticated einkorn wheat differs from the wild *T. boeoticum* in three major traits: larger and plumper seeds, a tough rachis which prevents spikelets falling apart at maturity, and relatively easy threshing (Salamini *et al*., 2002). Although dominating Neolithic agriculture, einkorn wheat was less favoured after the Bronze Age when the cultivation of high-yielding polyploid wheat species began. It has since been literally left untouched growing in its natural habitats for thousands of years and has not been exposed to intensive human selection (Zohary and Hopf, 1993). Thus, *T. monococcum* may retain its ancient level of genetic diversity and provide an ideal cereal model to study diversity of important traits and genetic diversity after domestication.

* To whom correspondence should be addressed. E-mail: kim.hammond-kosack@bbsrc.ac.uk

The A^m genome of *T. monococcum* and the A^u genome of *T. urartu* are closely related and diverged 0.5–1 million years ago (Huang *et al*., 2002; Dvorak *et al*., 2004). *Triticum urartu* has been the dominant A genome donor of the most important polyploid wheat species including the durum or macaroni wheat *T. turgidum* (AABB), *T. timopheevii* (AAGG), and common wheat *T. aestivum* (AABBDD). In contrast, *T. monococcum* has only been used for the generation of *T. zhukovskyi* (A^mA^mAAGG) (Dvorak *et al*., 1993; Dubcovsky *et al*., 1995; Baum and Bailey, 2004). Thus, the A^m genome is under-represented in hexaploid wheat, and the exploitation of genetic diversity in *T. monococcum* and discovery of novel variant alleles may provide opportunities for further wheat genetic improvement. Indeed, *T. monococcum* has been used for improving various traits of polyploid wheat species (Valkoun, 2001). For example, bread-making quality was improved by introgression of genes encoding the high molecular weight (HMW) glutenin subunits from *T. boeoticum* and *T. monococcum* (Rogers *et al*., 1997; Tranquilli *et al*., 2002*a*). The incorporation of additional copies of *Pina* and *Pinb* genes from *T. monococcum* into the cultivar Chinese Spring resulted in significantly softer grains than those of the progenitor cultivar, improving the biscuit- and cake-making quality (Tranquilli *et al*., 2002*b*; See *et al*., 2004). Various einkorn wheat genetic loci were successfully introgressed into the hexaploid triticale and bread wheat to provide resistance to leaf rust and powdery mildew (Shi *et al*., 1998; Vasu *et al*., 2001; Sodkiewicz and Strzembicka, 2004), prevent pre-harvest sprouting (Sodkiewicz, 2002), and increase zinc uptake efficiency (Cakmak *et al*., 1999). A durum wheat cultivar with *Nax1* and *Nax2* genes introgressed from *T. monococcum* exhibited greatly enhanced salt exclusion ability (James *et al*., 2006). These studies, albeit focused on only a few traits, suggest that *T. monococcum* is useful for wheat genetic improvement. Fregeau-Reid and Abdel-Aal (2005) recently noted variation in numerous traits in the diploid einkorn wheat worthy of exploitation, such as dietary fibre, milling characteristics, and lutein content.

The large genome size and the co-existence of three homoeologous genomes in hexaploid wheat present a huge challenge for the genetic dissection of phenotype–genotype relationships. It is more feasible to use a relatively smaller diploid genome such as that of *T. monococcum* for genetic studies. Also wheat has biological questions which cannot be studied using unrelated model plant species. One particular example is the interaction between wheat and a fungal pathogen *Mycosphaerella graminicola*, which is an exclusive pathogen of some *Triticum* species causing *Septoria tritici* leaf blotch disease (Keon *et al*., 2007). It is unlikely that the *bona fide* resistance mechanisms can be defined by studying resistance in non-host model species such as rice, *Arabidopsis*, barley, or *Brachypodium distachyon*. *Triticum monococcum* is a host of this pathogen and can be used as an alternative route to study the genetics of resistance (H-C Jing and K Hammond-Kosack, unpublished data). It has been convincingly demonstrated that *T. monococcum* is a good model for assisting with the cloning of genes from hexaploid wheat and for gene function studies (Stein *et al*., 2000; Feuillet *et al*., 2003; Yan *et al*., 2003; Yahiaoui *et al*., 2004; Uauy *et al*., 2006).

Our research aims to develop *T. monococcum* into a reference species for wheat genetics and genomics. Here, the variation in several important morphological and agronomic traits was characterized in *T. monococcum*, including plant growth- and yield-related components, various grain features, the profiles of resistance (*R*) gene and resistance gene analogues (*RAG*s), as well as germination under salt and drought stresses. Genetic segregation and association analyses were performed to define simple sequence repeat (SSR) markers (micro-satellites) linked with multiple important biological traits. This is the first report on genome-wide trait–marker associations in *T. monococcum*. The potential to explore novel variation in *T. monococcum* for modern wheat improvement and to use *T. monococcum* as a model for wheat genetics and genomes is discussed.

Materials and methods

The T. monococcum *accessions*

In total 30 *T. monococcum* accessions were used in this study (Table 1). These included 26 accessions from the NI Vavilov Research Institute of Plant Industry (VIR), St Petersburg, Russia, three accessions from the John Innes Centre (JIC, Norwich, UK), and one accession (DV92, referred to as MDR308 in our collection) from Professor Jorge Dubcovsky, University of California at Davis. The 26 VIR accessions were selected based on their resistance/susceptibility to important Russian wheat pathogens such as powdery mildew, leaf rust, and aphids (Lebedeva and Peusha, 2006). MDR050 (V97031) is from Victor Vallega, Italy which has a large grain size and had been used for studying starch biosynthesis in cereal endosperm (Kay Denyer, JIC, personal communication). This genotype has been selected from the progeny of a cross between *T. monococcum* and *T. sinskajae* (Korzun *et al*., 1998*b*) and is characterized by short and compact ears which lack awns and easy threshing. The accession DV92 had been used for generation of a genetic restriction fragment length polymorphism (RFLP) map, construction of a bacterial artificial chromosome (BAC) library, and isolation of a number of genes (Dubcovsky *et al*., 1996; Lijavetzky *et al*., 1999; Yan *et al*., 2003; Yahiaoui *et al*., 2004; Uauy *et al*., 2006).

Evaluation of morphological and agronomic traits

Seeds were germinated and seedlings were vernalized at 4–6 °C for 8 weeks and then grown to maturity in temperature-controlled glasshouse compartments equipped with supplementary lighting. Five plants from each *T. monococcum* accession were arranged in a randomized block design and, at harvest, traits were measured. Produced seeds were dried to ~10% water content and stored for 6 months at 6 °C and 20% relative humidity prior to grain hardness measurements and germination tests.

Table 1. *The* T. monococcum *accessions used in this study for phenotype evaluation*

Accession	Variety	Country of origin	Year of collection	Growth habit	Donors	Resources
MDR001	*flavescens*	Algeria	–	Spring	JIC	Transformable[a]
MDR002	*atriaristatum*	Balkans	–	Spring	JIC	Transformable, mapping population
MDR024	*hornemannii*; *flavescens*	Chechen	1904	Spring	VIR	
MDR025	*macedonicum*; *pseudoflavescens*	Crimea, Ukraine	1923	Spring	VIR	
MDR026	*pseudomacedonicum*	Crimea, Ukraine	1923	Spring	VIR	
MDR027	*monococcum*; *macedonicum*	Azerbaijan	1927	Spring	VIR	
MDR028	*flavescens*	Germany	1927	Intermediate	VIR	
MDR029	*flavescens*	Spain	1927	Spring	VIR	
MDR030	*monococcum*	Spain	1927	Spring	VIR	
MDR031	*monococcum*; *macedonicum*	Turkey	1927	Spring	VIR	
MDR032	*vulgare*	Italy	1927	Spring	VIR	
MDR033	*atriaristatum; vulgare*	Yugoslavia	1928	Spring	VIR	
MDR034	*hornemannii; vulgare*	Armenia	1928	Spring	VIR	
MDR035	*flavescens*; *vulgare*	Austria	1930	Spring	VIR	
MDR036	*monococcum*; *pseudovulgare*	Czechoslovakia	1932	Spring	VIR	
MDR037	*macedonicum*	Armenia	1934	Spring	VIR	
MDR038	*monococcum*	Armenia	1934	Spring	VIR	
MDR039	*hornemannii*	Georgia	1934	Spring	VIR	
MDR040	*vulgare*; *macedonicum*	Bulgaria	1940	Spring	VIR	Mapping population[b]
MDR041	*nigricultum*; *flavescens*	Albania	1950	Spring	VIR	
MDR042	*flavescens*; *macedonicum*	Balkans	1950	Spring	VIR	
MDR043	*vulgare*	Greece	1950	Spring	VIR	Mapping population
MDR044	*hornemannii*	Turkey	1965	Spring	VIR	Mapping population[b]
MDR045	*vulgare*	Denmark	1970	Spring	VIR	
MDR046	*atriaristatum*; *macedonicum*	Romania	1970	Spring	VIR	
MDR047	*macedonicum*; *vulgare*	Hungary	1970	Winter	VIR	
MDR048	*vulgare*	Sweden	–	Spring	VIR	
MDR049	*pseudohornemannii*	Iran	–	Winter	VIR	
MDR050	[d]	Italy	–	Spring	JIC	EMS-mutagenized population[c]
MDR308 (DV92)	–	Titograd Montenegro, Italy	–	Spring	Dr Jorge Dubcovsky	BAC library, genetic map; EST library, mapping populations

[a] H Jones, RRes, unpublished.
[b] These mapping populations were not used in this study.
[c] K Denyer, John Innes Centre, UK, unpublished.
[d] Progeny from a cross between *T. monococcum* and *T. sinskajae* (Korzun *et al*., 1998).

Analysis of grain

Electrophoretic profiles of seed storage proteins were generated as described (Shewry *et al*., 2006). Each profile was verified by comparison of three independent extractions. The gel images were analysed for polymorphism using Totallab image analysis software (Nonlinear Dynamics, Newcastle, UK).

Endosperm hardness was assessed using a single kernel characterization system (Perten SKCS 4100, Perten Instruments AB, Huddinge, Sweden). The weight, length, diameter, and moisture content of 250 individual grains per accession were measured. Grain texture was visualized by scanning electron microscopy (SEM). For this purpose, mature seeds were quench-frozen in liquid nitrogen, transferred to a Cryo SEM preparation chamber (Gatan Alto 2100), fractured, etched by sublimation at 85 °C for 2 min, sputter coated with gold, and finally examined at 5–15 kV in a JEOL JSM-6360 LV scanning electron microscope.

NBS (nucleotide-binding site) profiling

NBS profiling was carried out essentially as described (van der Linden *et al*., 2004) with some modification. Briefly, 200 ng of genomic DNA were subjected to *Mse*I restriction and ligation in 30 µl of buffer containing 10 mM TRIS acetate, pH 7.5, 10 mM magnesium acetate, 50 mM potassium acatate, 5 mM dithiothreitol, 1.5 µg of bovine serum albumin, 1 mM ATP, 5 U of *Mse*I, 0.5 U of T4 DNA ligase, and 0.025 nmol adaptor primers. The amplification of NBS-specific fragments involved a two-step procedure with the second using [γ-^{33}P]ATP-end-labelled NBS-specific primers. PCR products were separated on 6% polyacrylamide gels and imaged with a Typhoon 8600 Variable Mode Imager (Amersham). The images were processed using the Totallab image analysis software (Nonlinear Dynamics, Newcastle, UK).

Seed germination test

Seeds (three lots of 25 seeds per accession) were surface-sterilized with bleach containing 1% sodium hypochloride for 5 min, rinsed vigorously five times with deionized water for 5 min, and imbibed on two layers of Whatman filter paper soaked with 8 ml of deionized water, 150 mM NaCl solution, or 135 g kg^{-1} PEG-6000 solution (–0.8 MPa), respectively. Petri dishes were sealed with Parafilm and maintained at 25 °C with a 16 h/8 h day/night cycle. The germination percentage was scored daily for 7 d.

Generation of mapping populations and genetic segregation analysis

Accessions MDR002, MDR308, and MDR043 were used to generate two mapping populations. Anthers from female plants

were emasculated using a fine pair of tweezers ~15 d after ear emergence, and pollination was carried out 2–3 d after emasculation. To increase the rate of success, a single anther was used to pollinate a single floret. The pollinated ears were then covered with cheesecloth pockets to prevent cross-pollination and allowed to set seed. The electrophoretic profiles of the endosperm tips from the resulting F_1 seeds were compared with parental lines to confirm their authenticity. F_2 progeny were grown in greenhouses for assessing segregation of various growth and morphological traits.

Microsatellite genotyping

Genomic DNA was isolated from the second leaf of 2-week-old seedlings using a QIAgene DNA mini-kit. To test intra-accession genetic variation, DNA was extracted from five seedlings per accession.

Primer sequences for microsatellites mapped to the bread wheat A genome were obtained from the Graingenes database (http://wheat.pw.usda.gov/GG2/index.shtml) and used to amplify genomic DNA templates from *T. monococcum* accessions. Each 10 µl reaction contained 50 ng of template DNA, 1.5 mM Mg^{2+}, 1.5 mM of dNTPs, 1.5 µM of each primer, 1 µl of 10× PCR buffer, and 1.25 U of *Taq* DNA polymerase (Promega). The PCR conditions were 2 min at 95 °C, followed by 30 cycles of 94 °C/30 s, T_m/30 s, and 72 °C/60 s, ending with an extension of 72 °C/5 min. The T_m varied between 50 °C and 63 °C depending on the SSR markers (Table 2). The PCR products of 46 microsatellites were analysed using either an ABI 3730 DNA analyser or a 3% agarose gel consisting of one-third of Nusieve® 3:1 agarose and two-thirds of MetaPhor® agarose (Cambrex Bio Science, Rockland, ME, USA).

Table 2. *The 46 microsatellites used for assessing genetic diversity in* Triticum monococcum

SSR marker	Genome location	Forward primer	Reverse primer	Annealing temperature (°C)
BARC83	1AL	AAGCAAGGAACGAGCAAGAGCAGTAG	TGGATTTACGACGACGATGAAGATGA	58
BARC287	1AL	CGGATGGGTTACTTACTTAGGATG	CGCAACTCCATTTCAGAATCATT	50
DUPW038	1AL	ATTAGACACGACCAAACGGG	TCAAACAAACAACAGCCAGC	60
PSP3003	1AL	GATCGACAAGGCTCTAATGC	CAGGAGGAGAGCCTCTTGG	63
PSP3027	1AL	GATCGTGACATCTCAAGAAC	ATAAATGCTGCTACATTTCCC	61
BARC263	1AS	GGAAGCGCGTCAGCACTAGGCAAC	GGCTTCTAGGTGCTGCGGCTTTTGTC	55
GWM164	1AS	ACATTTCTCCCCCATCGTC	TTGTAAACAAATCGCATGCG	56
BARC309	2AL	GCGAAAGCCCTAAAGTTACAA	AAGCCGCAGAGAAGGTCAGC	55
GWM356	2AL	AGCGTTCTTGGGAATTAGAGA	CCAATCAGCCTGCAACAAC	56
BARC5	2AS	GCGCCTGGACCGGTTTTCTATTTT	GCGTTGGGAATTCCTGAACATTTT	52
GWM636	2AS	CGGTAGTTTTTAGCAAAGAG	CCTTACAGTTCTTGGCAGAA	50
WMC177	2AS	AGGGCTCTCTTTAATTCTTGCT	GGTCTATCGTAATCCACCTGTA	55
GWM674	3AC	TCGAGCGATTTTTCCTGC	TGACCGAGTTGACCAAAACA	60
BARC1060	3AL	GCGTCTATTTTTGCCATTTCCATTCA	GCGATGTTCTGTAGTTCTTAGTGTTCTTT	55
BARC57	3AS	GCGACCACCTCAGCCAACTTATTATGT	GCGGGGAGGCACATTCATAGGAGT	55
BARC45	3AS	CCCAGATGCAATGAAACCACAAT	GCGTAGAACTGAAGCGTAAAATTA	52
GWM369	3AS	CTGCAGGCCATGATGATG	ACCGTGGGTGTTGTGAGC	60
GWM002	3AS	CTGCAAGCCTGTGATCAACT	CATTCTCAAATGATCGAACA	50
BARC1047	4AL	GCGCAGACCGTACCCAACCAGATAG	CATGCCTTGCCCTTGGTTTCA	55
BARC52	4AL	GCGCCATCCATCAACCGTCATCGTCATA	GCGAGGAAGGCGGCCACCAGAATGA	60
BARC70	4AL	GCGAAAAACGATGCGACTCAAAG	GCGCCATATAATTCAGACCCACAAAA	55
DUPW004	4AL	GGTCTGGTCGGAGAAGAAGC	TGGGAGCGTACGTTGTATCC	60
GWM165	4AS+4BL+4DL	TGCAGTGGTCAGATGTTTCC	CTTTTCTTTCAGATTGCGCC	60
GWM186	5AL	GCAGAGCCTGGTTCAAAAAG	CGCCTCTAGCGAGAGCTATG	56
GWM179	5AL	AAGTTGAGTTGATGCGGGAG	CCATGACCAGCATCCACTC	56
WMC415	5AL	AATTCGATACCTCTCACTCACG	TCAACTGCTACAACCTAGACCC	56
BARC56	5AS	GCGGGAATTTACGGGAAGTCAAGAA	GCGAGTGGTTCAAATTTATGTCTGT	55
GWM293	5AS	TACTGGTTCACATTGGTGCG	TCGCCATCACTCGTTCAAG	56
BARC180	5AS	GCGATGCTTGTTTGTTACTTCTC	GCGATGGAACTTCTTTTTGCTCTA	52
GWM156	5AS	CCAACCGTGCTATTAGTCATTC	CAATGCAGGCCCTCCTAAC	50
GWM129	5AS (+2BL)	TCAGTGGGCAAGCTACACAG	GTTTCTTAAAACTTAGTAGCCGCGT	55
GWM205	5AS+5DS	CGACCCGGTTCACTTCAG	AGTCGCCGTTGTATAGTGCC	58
BARC122	5AS/(5AL)	CCCGTGTATATCCAGGAGTG	CAGCCCTTGTGATGTGATG	52
BARC1055	6AL	GCCAGACGCACAGGGACAAGATACACTA	GCCGTACCCTGGTTATTGTTG	55
GWM570	6AL	TCGCCTTTTACAGTCGGC	ATGGGTAGCTGAGAGCCAAA	60
DUPW167	6AL	CGGAGCAAGGACGATAGG	CACCACACCAATCAGGAACC	60
GWM570	6AL	TCGCCTTTTACAGTCGGC	ATGGGTAGCTGAGAGCCAAA	63
PSP3152	6AL	AAGGAAAAACCCGTAGAAAAAGA	ACTCCACCACCAAATCAAGAA	58
PSP3029	6AL+2AL	CCATCGATGAGGATCTCCTCGGGCA	GCAACAGGACCATGGTCG	63
BARC3	6AS	TTCCCTGTGTCTTTCTAATTTTTTTT	GCGAACTCCCGAACATTTTTAT	52
BARC146	6DS+6B+6A	AAGGCGATGCTGCAGCTAAT	GGCAATATGGAAACTGGAGAGAAAT	52
GWM332	7AL	AGCCAGCAAGTCACCAAAAC	AGTGCTGGAAAGAGTAGTGAAGC	62
DUPW254	7AL	TTAACCATGCAGCAACTTCG	GTGTGTACTAACGGCTACGGC	58
WMC346	7AL	CTGAAGTTCCAGCCAACACA	ATTCCCTCATCCCGTTGC	58
GWM130	7AS	AGCTCTGCTTCACGAGGAAG	CTCCTCTTTATATCGCGTCCC	62
PSP3001	7AS	GCAGAGAGATGAGGGCACC	CTCTGCTCCCTTAACTTCTG	63

The microsatellite profiles were scored for clustering and association analyses.

Linkage and association statistical analysis

GenStat™ (release 9.2 2007, Lawes Agricultural Trust, Rothamsted Research) was used to perform statistical analyses. Genetic diversity of *T. monococcum* accessions was assessed by clustering analysis, in which a Jaccard similarity matrix was generated using the microsatellite banding data, and the UPGMA (unweighted pair group mean average) method was used for generating clustering dendrograms.

Linkages between SSR markers and two morphological traits, awn colour and leaf pubescence, were analysed using the marker regression function of the software Map Manager QTX (http://www.mapmanager.org/mmQTX.html). For association mapping, linear mixed models using residual maximum likelihood (REML) were employed to identify associations between SSR markers and genetic loci controlling traits. For each trait, the following linear model was fitted using each SSR marker with the directive REML:

$$y_{ij} = \mu + MK_i + Accession_j + e_{ij}$$

where, y_{ij} is the logit transformation of the proportion, MK_i represents the fixed effect of the ith SSR marker, $Accession_j$ represents the random effect of the jth accession, and e_{ij} represents the random residual. In this model, *Accession* was considered random and residuals were considered independent for simplicity. An association is considered significant if the probability value is equal to or less than α=0.005.

Results

Genetic purity and diversity of T. monococcum *accessions as assessed by SSR analysis*

Wheat A genome-specific SSR markers were used to assess the genetic diversity within the *T. monococcum* accessions. Out of 101 primer pairs tested, 73 amplified products effectively from *T. monococcum* templates following some minor adjustment to the PCR conditions, giving a transferability rate of >70%. Forty-six SSR markers were selected to assess the genetic purity of the accessions based on their genome coverage (Table 2).

The VIR accessions were collections of various landraces, and therefore genetic variation within these accessions was expected. Amongst the 26 accessions examined, seven showed genetic heterogeneity (data not shown), suggesting that VIR landraces are fairly homogenous genetically. For these seven accessions, the dominant genotype was selected and multiplied to produce a pure line.

The same set of 46 SSR markers was also used to assess genetic diversity of the 26 *T. monococcum* accessions from the Vavilov Institute, and three accessions requested from the JIC and DV92 (Table 1). In total, 293 polymorphic bands were identified, and this gave an average of six polymorphic bands per marker. A Jaccard similarity matrix was generated using these polymorphic bands, which was then used to construct a phylogenetic tree deciphering the genetic relationships of the 30 accessions (Fig. 1). The minimal similarity was <0.3, suggesting an overall high genetic variation in these accessions. MDR050 clustered well with other *T. monococcum* accessions, confirming the notion that *T. sinskajae* was generated from a spontaneous mutation in *T. monococcum* (Korzun *et al*., 1998*b*). The clustering analysis also clearly indicates that the genetic variation only partially correlates with the geographic origin. For instance, accessions MDR025 and MDR026 from the Ukraine and accessions MDR034, MDR037, and MDR038 from Armenia were in the same clads, while accession MDR001 from Algeria was distantly related to all the other accessions. However, the two accessions from Turkey, MDR031 and MDR044, were split into different clads. Accession MDR308 (DV92) from Italy was clustered together with MDR043 from Greece, but was distantly related to another Italian accession MDR032. The SSR clustering also only partially correlated with subspecies classification. These results were not anticipated and were in contrast to those obtained for barley, where a good correlation between geographic origin and SSR marker clustering was discovered (Malysheva-Otto *et al*., 2006). To explore the genetic diversity and geographic location association in greater detail, an additional 66 *T. monococcum* and 13 *T. boeoticum* accessions were genotyped using the same set of SSR markers (see Supplementary Table S1 at *JXB* online). These results again indicate that only a partial correlation exists between SSR marker clustering and the geographic origin, although *T. monococcum* was reasonably well separated from *T. boeoticum* (see Supplementary Fig. S1 at *JXB* online).

Variation in morphological and agronomic traits

Table 3 shows a large variation in 11 scored morphological traits of agronomic relevance. Several significant correlations were evident (Table 4). As could be expected, the numbers of tillers were negatively correlated with plant height, peduncle length, and spikelet numbers. The grain weight was positively correlated with many traits including seed volume, ear length, peduncle length, and plant height, but was negatively correlated with spikelet numbers.

Variation in grain features

Figure 2 shows the banding patterns for both gliadin and glutenin subunits. In the experimental system used, both HMW and low molecular weight (LMW) glutenin and the ω and γ fractions of gliadin were detected. Highly polymorphic bands were observed for the gliadin fractions and the LMW glutenin subunits, whereas the HMW glutenin subunits were rather monomorphic. The 30 accessions showed discrete electrophoretic profiles.

Grain texture measurement indicated that all the *T. monococcum* accessions examined had a hardness index

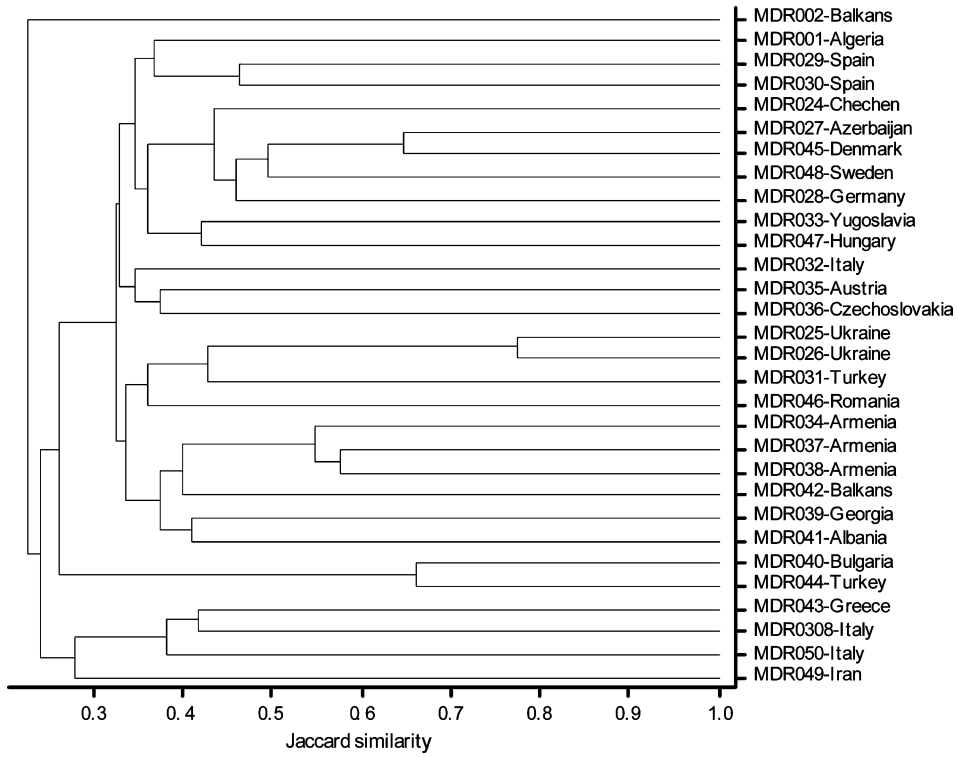

Fig. 1. A dendrogram generated using 46 SSR markers deciphering the relationships of 30 *T. monococcum* accessions based on the UPGMA (unweighted pair group mean average) method and the Jaccard similarity matrix.

<35, a minimal threshold level of hard endosperm suggesting that overall *T. monococcum* has a soft grain texture (Fig. 3A). Interestingly, the 30 *T. monococcum* accessions fell into two groups. While the majority of the accessions showed a minus value of hardness index, four accessions, MDR001, MDR002, MDR047, and MDR308, exhibited a hardness index over +10, suggesting that these four accessions may be the 'hard grains' in *T. monococcum*. SEM examination (Fig. 3B) showed that the MDR308 grain cryofracture images were similar to the representative hard-grain wheat Mercia, while those of MDR040 resembled the representative soft-grain wheat Riband. Thus, these *T. monococcum* accessions can be used to explore the genetic controls of grain texture.

Polymorphism in disease R *genes and* RGAs in T. monococcum

Figure 4 shows representative NBS profiles obtained for the *T. monococcum* accessions in comparison with those for hexaploid wheat. Multiple novel polymorphic bands were observed in einkorn wheat NBS profiles which were absent in hexaploid wheat using primers specifically targeting NBS2 and NBS5 domains. However, the NBS3 profiles in *T. monococcum* were monomorphic (data not shown). Thus, there exists a high level of polymorphism in *R* genes and/or *resistance gene analogue* (*RGA*) genes in *T. monococcum*. Further exploitation may allow the identification of novel variant alleles conferring high disease resistance to important wheat pathogens.

Responses of T. monococcum *to salt and drought stresses*

The *T. monococcum* accessions were explored for tolerance to salt and drought stresses in a germination assay (Fig. 5). Seeds of the tested accessions could reach a total germination of >90% and there were no significant differences in germination rates and total germinations when seeds were imbibed in water (data not shown). However, variation in germination rate and total germination was observed when seeds were imbibed in the presence of 150 mM NaCl or –0.8 MPa. Seeds of MDR0001, MDR033, MDR034, MDR037, MDR038, MDR047, and MDR308 exhibited slow germination rates and could not reach full germination under salt stress, while seeds of MDR001, MDR037, MDR038, MDR047, and MDR049 showed poor germination under drought stress. Thus, *T. monococcum* accessions possess different levels of tolerance to salt and drought stresses.

Identification of SSR markers associated with genetic loci controlling awn colour and leaf pubescence

Awn colour and leaf pubescence are two prominent traits in wheat. In the *T. monococcum* accessions, MDR002 has strong leaf pubescence and black awns, whereas most of

Table 3. *Comparison of morphological and agronomical traits in* T. monococcum *accessions*

Values are given ±SD.

Accession	Tiller no.	Height (cm)	Awn length (cm)	Peduncle length (cm)	Ear to flag leaf length (cm)	Spikelet no.	Ear length (cm)	1000-seed weight (g)	1000-seed volume (ml)	Awn colour	Stem[a]
MDR001	29.60±9.61	148.80±9.01	9.25±1.26	57.47±4.19	36.17±3.08	30.13±3.48	16.37±0.92	34.20±2.56	47.13±6.58	Black	Empty
MDR002	41.80±6.38	132.80±4.09	8.60±0.55	45.00±5.76	25.53±6.02	28.40±0.60	17.23±0.35	26.86±2.11	34.10±3.45	Black	Empty
MDR024	40.20±5.59	157.40±7.83	5.50±0.58	54.07±6.87	32.17±6.61	40.40±2.24	15.53±1.16	23.58±2.88	31.59±0.57	Yellow	Empty
MDR025	69.80±10.26	137.60±3.36	3.40±1.52	46.90±3.07	29.27±2.99	30.40±1.38	11.50±0.76	28.38±2.69	41.31±5.92	Black	Empty
MDR026	55.80±7.79	140.20±6.10	3.75±0.50	47.10±3.24	29.47±3.03	29.87±1.45	11.00±0.59	32.33±3.50	43.23±5.91	Black	Empty
MDR027	38.40±5.03	151.20±5.76	4.40±0.55	55.87±3.81	36.33±4.42	37.20±1.10	13.10±0.58	25.35±0.68	37.17±1.62	Yellow	Empty
MDR028	29.00±3.67	165.40±4.45	11.25±4.03	55.90±6.86	32.67±7.92	37.60±2.85	18.03±1.99	28.90±4.41	43.92±4.21	Yellow	Semi-full
MDR029	27.00±9.54	138.00±2.65	5.33±0.58	54.83±4.09	36.00±3.42	29.33±0.67	14.22±0.69	31.45±1.80	43.53±2.64	Black	Semi-full
MDR030	45.00±6.00	145.00±8.46	6.40±0.89	59.00±8.88	40.67±8.71	29.73±0.76	14.67±0.31	33.49±5.73	49.61±3.29	Yellow	Empty
MDR031	53.60±8.93	141.60±13.52	7.50±0.58	59.70±5.34	40.00±4.71	27.47±4.25	15.37±2.34	35.30±1.59	49.08±5.41	Yellow	Empty
MDR032	38.25±5.74	162.50±9.47	3.67±0.58	47.75±4.13	29.71±3.48	35.17±3.00	12.46±0.57	20.68±7.86	35.85±5.86	Yellow	Empty
MDR033	41.20±4.27	160.20±13.66	7.50±0.58	63.43±10.00	42.73±9.39	27.33±1.33	16.37±1.22	42.55±11.93	47.20±11.91	Yellow	Semi-full
MDR034	58.60±10.88	157.20±5.12	7.33±1.53	54.00±5.54	33.93±4.72	33.33±1.56	14.53±0.65	30.32±3.40	46.30±5.46	Yellow	Empty
MDR035	60.20±8.98	152.60±5.55	5.00±1.41	54.63±4.76	34.90±4.24	28.67±1.76	13.37±0.93	42.14±2.16	53.98±4.06	Yellow	Empty
MDR036	39.20±4.97	148.40±2.30	4.60±0.89	53.87±3.48	34.87±2.46	36.67±0.82	14.70±1.60	27.60±2.56	28.14±0.91	Yellow	Empty
MDR037	46.20±10.26	170.00±6.86	6.80±1.79	58.17±7.51	37.37±6.52	36.00±1.25	15.67±2.17	35.52±2.05	49.73±3.94	Yellow	Empty
MDR038	41.00±6.24	164.00±4.00	7.20±1.30	56.10±4.47	34.03±3.49	36.13±1.91	15.77±1.67	38.65±2.91	45.42±2.56	Yellow	Empty
MDR039	37.80±2.77	166.60±4.28	6.80±1.30	61.07±4.75	38.13±3.81	34.40±1.01	17.03±1.17	31.83±0.63	46.26±4.04	Yellow	Empty
MDR040	61.80±10.28	151.60±8.08	4.25±1.26	58.70±4.61	37.75±3.34	30.00±1.05	13.50±1.42	33.89±0.96	46.26±2.51	Yellow	Empty
MDR041	35.60±9.04	158.60±9.96	5.50±0.58	56.00±3.00	33.37±2.00	36.67±1.49	16.03±1.23	37.51±2.08	52.36±8.10	Black	Empty
MDR042	49.80±8.61	150.60±3.21	4.25±1.71	55.93±0.92	37.23±1.21	35.07±1.61	14.10±0.88	26.77±1.39	39.46±3.28	Yellow	Empty
MDR043	56.60±10.26	145.40±4.04	7.00±0.82	49.23±2.88	30.37±3.22	33.60±1.46	15.80±1.03	30.40±3.05	45.57±3.13	Yellow	Empty
MDR044	31.40±5.86	154.60±4.51	8.00±1.41	61.83±4.04	41.43±4.21	30.93±1.61	18.60±0.67	31.37±3.32	49.03±4.56	Yellow	Empty
MDR045	53.00±8.72	147.20±6.18	4.50±1.00	55.03±6.60	34.17±5.91	35.60±1.01	13.57±1.01	31.11±1.80	33.31±2.57	Yellow	Semi-full
MDR046	52.00±19.34	132.60±6.84	5.67±0.58	53.83±7.34	36.37±5.83	27.87±1.85	12.57±0.78	24.16±4.86	42.65±10.35	Black	Empty
MDR07	48.80±4.87	160.60±3.13	5.20±0.45	60.47±2.14	40.07±1.34	30.93±1.12	13.13±0.64	34.09±2.97	44.17±1.67	Yellow	Empty
MDR048	33.80±6.76	156.40±3.85	6.60±0.89	51.47±4.16	31.23±4.17	38.93±1.61	16.03±0.82	27.01±3.50	38.63±0.95	Yellow	Empty
MDR049	28.60±5.03	170.80±10.23	6.25±1.71	60.87±4.67	36.33±2.83	40.40±1.67	15.5±2.27	35.91±2.65	44.02±4.41	Yellow	Empty
MDR050	69.00±20.72	142.60±5.68	1.40±0.89	53.60±3.23	31.63±3.23	28.67±1.25	8.40±0.98	44.47±1.21	60.50±4.09	Yellow	Empty
MDR308	51.20±11.73	145.20±9.07	4.00±0.00	50.43±5.86	31.00±5.71	36.00±1.56	13.47±0.73	29.33±3.60	42.91±6.64	Yellow	Empty
LSD (0.05)	10.06	7.62	1.40	6.00	5.48	2.19	1.41	4.66	6.11		

[a] The stem filling was observed at the base of the main tillers.

Table 4. *Correlation matrix of morpho-agronomical traits in* T. monococcum

	1000-seed weight (g)	1000-seed volume (ml)	Spikelet no.	Tiller no.	Awn length (cm)	Ear length (cm)	Ear to flag leaf length (cm)	Plant height (cm)	Peduncle length (cm)
1000-seed weight (g)	1								
1000-seed volume (ml)	0.733**	1							
Spikelet no.	–0.348**	–0.359**	1						
Tiller no.	0.16	0.143	–0.42**	1					
Awn length (cm)	–0.012	0.017	0.047	–0.411**	1				
Ear length (cm)	0.32**	0.295**	0.042	–0.188	0.388**	1			
Ear to flag leaf length (cm)	0.282**	0.216	–0.099	–0.189	0.167	0.155	1		
Height (cm)	0.247**	0.142	0.48**	-0.396**	0.266**	0.251	0.419**	1	
Peduncle length (cm)	0.368**	0.29	0.039	–0.262**	0.228	0.331**	0.943**	0.567**	1

** Correlation significant at P <0.01 in a two-sided test of null correlations.

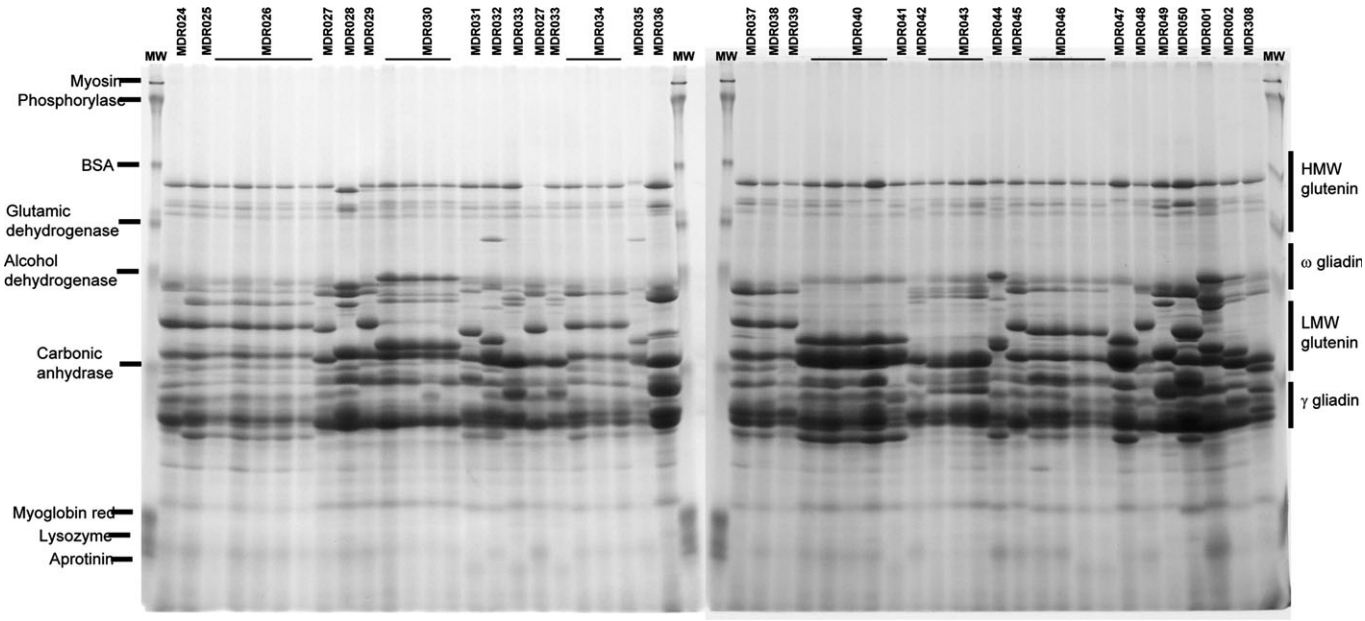

Fig. 2. Electrophoretic patterns of gliadin and glutenin in different *T. monococcum* accessions. The reduced extracts of seed storage proteins were separated by 10% SDS–PAGE and the bands were visualized using colloidal Coomassie blue staining. For ease of comparison, samples from some accessions were loaded several times.

the accessions have glabrous leaves and yellow awns (Fig. 6). MDR002 was crossed with MDR043 and MDR308 to study the inheritance of these two traits. The leaf pubescence and black awn phenotypes were evident in F_1 hybrids of both crosses (22 and 18 F_1 seeds were examined for crosses MDR002×MDR043 and MDR308×MDR002, respectively). In MDR308×MDR002 F_2 populations, these two traits segregated in a 3:1 ratio (79 black awn:25 yellow awn, χ^2=0.0513; 73 pubescence:23 glabrousness, χ^2=0.0556). These results confirm that both traits are controlled by single dominant genes. Genetic loci controlling glume colour and leaf pubescence have been previously mapped in hexaploid wheat (*T. aestivum*) to 1AS and 4B and 7B, respectively (Borner *et al.*, 2002; Taketa *et al.*, 2002). Therefore, 94 F_2 individuals from the MDR308 and MDR002 cross were genotyped using SSR markers in the vicinity of these loci and it was found that awn colour was associated with the SSR marker *Xwmc336* locus on $1A^m$ (P <0.00001) and leaf pubescence with the *Xcfd39* locus on $5A^m$ (P=0.00002) (Table 5).

Associations between SSR markers and other traits in T. monococcum

To analyse further the genetic basis of the variation in the 14 other traits examined, their associations with 46 SSR markers were tested (Table 5). Strong linkages with SSR markers were found for quantitative traits such as grain hardness, seed germination under salt and drought stresses, and various yield components, as well as the traits which had qualitative scores such as seed storage protein profiles and NBS profiles. Interestingly,

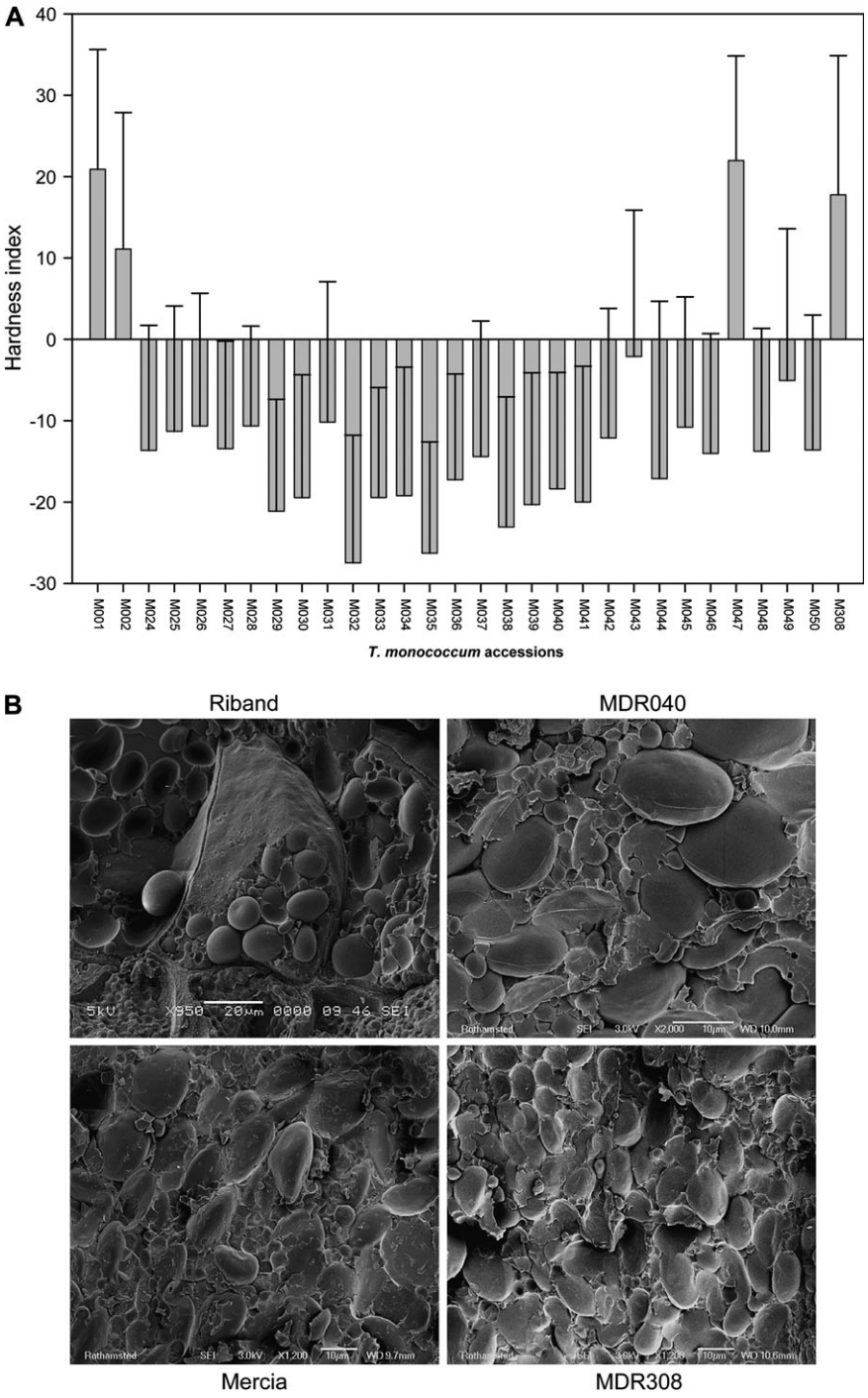

Fig. 3. (A) Comparison of the endosperm texture of 30 *T. monococcum* accessions. The measurements were performed using the single kernel characterization system as described in Materials and methods. For each accession, ~200 grains were measured, and data are shown as mean ±SD. (B) Stereoscan electron microscopy of a freeze-fractured soft grain of the hexaploid wheat cultivar Riband and *T. monococcum* accession MDR040, and a hard grain of the hexaploid wheat cultivar Mercia and *T. monococcum* accession MDR308.

a few SSR markers were linked to multiple traits. For example, SSR markers BARC52 and GWM179 were associated with several yield-related components, grain hardness as well as germination under salt tolerance. On the other hand, variation in one trait could be linked to SSR markers from various chromosomal regions as exemplified by grain moisture content, ear length, grain hardness, and germination under salt stresses. Some of the associations were on chromosomal regions containing previously known genetic loci in hexaploid wheat. This is particularly true for the associations between SSR markers and NBS profiles. Comparing these with map

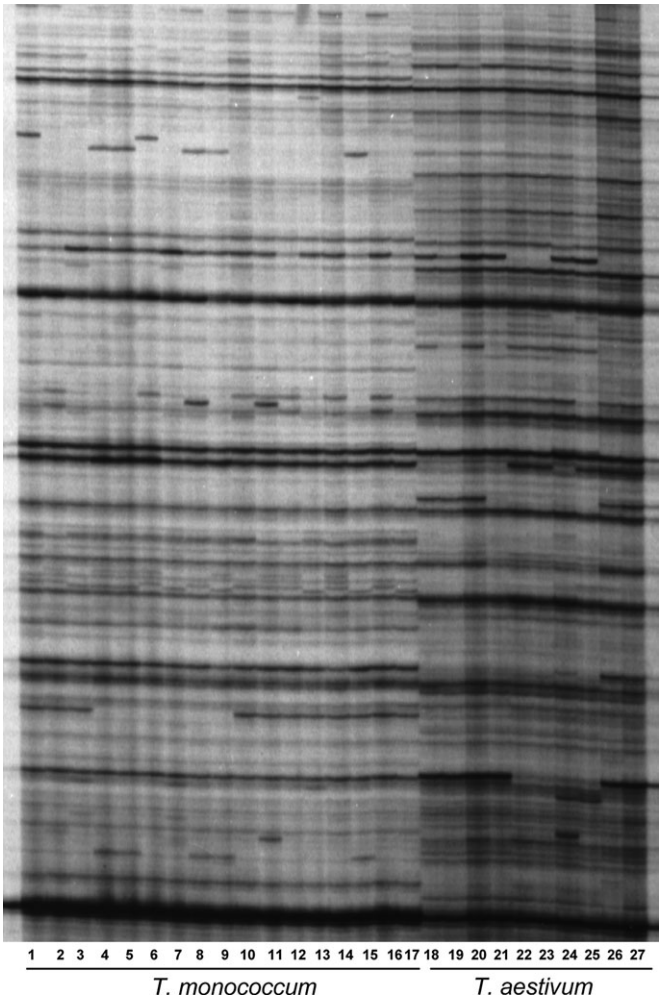

Fig. 4. NBS profiling of *T. monococcum* accessions. The genomic DNA from each accession was fragmented with the *Mse*I restriction enzyme, and PCR amplified using a linker primer and a ^{33}P-labelled NBS domain-specific degenerated primer (see Materials and methods for details). After electrophoresis, the polymorphic bands were visualized by autoradiography. Shown is part of a representative NBS2 autoradiograph. Each lane represents a single *T. monococcum* or *T. aestivum* accession. Many common bands are seen, but polymorphic bands are also obvious.

locations of *RGA* expressed sequence tags (ESTs) in bread wheat (McFadden *et al*., 2006), it was found that *Xgwm164* was estimated to be only 0.7 cM away from *RGA10* on chromosome 1A, while *Xgwm293* and *Xgwm129* were tightly linked to *RGA71* on chromosome 5A. Furthermore, a quantitative trait locus (QTL) controlling germination under salt and drought stresses was linked to *Xwgm179*, which is within a 10 cM distance to the *Nax2* gene (Byrt *et al*., 2007). The present data also indicate that a relatively high map resolution can be achieved by association mapping in *T. monococcum*. For instance, ear length is linked to *Xgwm636* and *Xwmc177* which are only ~4 cM apart on 2A; hardness to *Xwmc177* and *Xbarc5* loci which are also 7 cM apart on 2A; and an NBS2 band to *Xgwm293* and *Xgwm129* loci which are <0.5 cM apart on 5A. These data imply that association mapping is a powerful tool to identify trait–marker links in *T. monococcum*.

Discussion

Triticum monococcum is one of the most ancient small grain cereals which turned Stone Age nomads into farmers. Various agriculturally important traits were analysed and their associations with SSR markers in *T. monococcum* were examined. Several important trait–marker associations were identified efficiently using only a small core collection. The results demonstrate that *T. monococcum* possesses genetic variation in 16 useful traits and is a good model for wheat genetic study.

Genetic diversity and trait variation in T. monococcum

Using microsatellites spanning the genome, a genetic similarity as low as 0.3 was found in the *T. monococcum* collection examined. A similar estimation was reported in an earlier study on 26 *T. monococcum* accessions using 20 microsatellites (Korzun *et al*., 1998*b*). Studies using RFLP and rapid amplification of polymorphic DNA (RAPD) indicated that the genetic diversity in *T. monococcum* was lower than that of *T. boeoticum* and *T. urartu*, and there is high genetic diversity in the three A genome diploid wheat species (Vierling and Nguyen, 1992; Castagna *et al*., 1994). Interestingly, the microsatellite clustering of *T. monococcum* accessions correlated only to a limited degree with the geographical origins. This implies that *T. monococcum* has been widely spread after domestication, but has not undergone significant genetic changes during the past 10 000 years (Zohary and Hopf, 1993). Furthermore, the cultivation and spread of *T. monococcum* had already declined before the Bronze Age ~7000 years ago, far earlier than the start of human crop breeding activities, which would narrow the genetic diversity of local landraces but increase the genetic distance for accessions with different geographical origins. In line with this argument, the correlation between geographical origin and microsatellite clustering is high in barley which has been intensively selected for over centuries (Malysheva-Otto *et al*., 2006).

The genetic diversity observed in *T. monococcum* is well reflected by the variation in multiple biological traits. For instance, several-fold differences were found in yield-related components. *Triticum monococcum* is generally considered as a soft grain owing to the control of *Pina* and *Pinb* genes (Luo *et al*., 2005). However, grain hardness indexes between –20 and +20 were found in the accessions tested. In a Danish *T. monococcum* collection, grain hardness indexes between –7.3 and +27.2 were reported (Loje *et al*., 2003). *Triticum monococcum* therefore can be exploited for genetic variation in grain

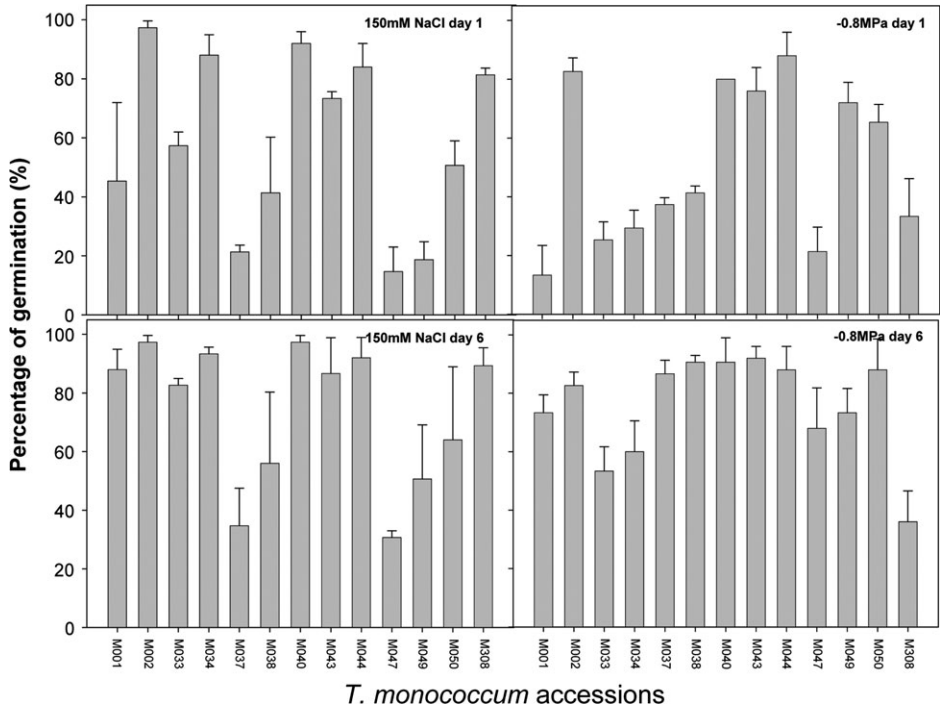

Fig. 5. Percentage of seed germination of 13 *T. monococcum* accessions under the indicated salt and drought stresses. Bars represent the mean ±SE of three replicates of 25 seeds. The other 17 accessions examined reached full germination under the stressed conditions and the data are not shown.

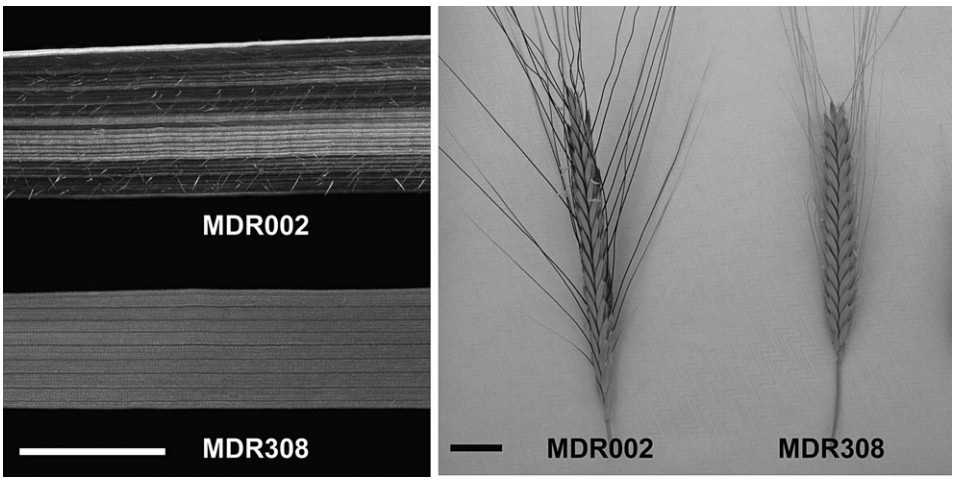

Fig. 6. Leaf pubescence and awn colour phenotypes in *T. monococcum*. The left panel shows that MDR002 has hairy leaves and MDR308 has glabrous leaves. The right panel shows that MDR002 has black awns and MDR308 has yellow awns. The scale bars in the left and right panels represent 0.5 cm and 1 cm, respectively.

hardness. This information may then be used to alter the grain hardness for various purposes in polyploid wheat species, which lost the *Pin* genes during polyploidization (Shewry and Halford, 2002). Over a dozen different electrophoretic profiles of grain storage proteins and a high level of diversity in the ω gliadin and LMW glutenin exist in the 30 *T. monococcum* accessions examined, which may provide new sources for bread wheat improvement (An *et al.*, 2006).

Global climate changes are predicted to bring new biotic and abiotic stresses to the wheat crop and impose further impacts on water and other natural resources (Reynolds and Borlaug, 2006). These changes may render the current elite wheat cultivars and/or cropping systems inappropriate. Plant *R* genes and *RGA*s are prominent components in induced defence responses conferring resistance in either a race-specific or race-non-specific manner (Hammond-Kosack and Parker, 2003; Chisholm *et al.*, 2006). The NBS

Table 5. *Associations between SSR loci and important traits in* T. monococcum

Traits	SSR markers	FDR[a]
Agro-morphological traits		
Plant height (cm)	*Xgwm2 (72.0cM, 3A), Xbarc70 (186.58cM, 4A), Xgwm179 (96.0cM, 5A), Xpsp3029 (95.09cM, 6A)*	0.361
Peduncle length (cm)	*Xgwm369 (35.88cM, 3A), Xbarc45 (73.0cM, 3A), Xpsp3152 (80.66cM, 6A), Xpsp3001 (3B, 1A, 7A)*	0.289
Hairy leaf	*Xcfd39 (83.19cM, 5A)*	–
Black awn	*Xwmc336 (21.52cM, 1A)*	–
Yield components		
Grain weight (mg)	*Xbarc45 (73.0cM, 3A), Xbarc52 (161.54cM, 4A), Xdupw38 (1A)*	0.482
Grain length (mm)	*Xbarc52 (161.54cM, 4A), Xgwm179 (96.0cM, 5A), Xdupw38 (1A)*	0.482
Grain diameter (mm)	*Xbarc52 (161.54cM, 4A), Xgwm179 (96.0cM, 5A), Xdupw38 (1A)*	0.482
Grain moisture (%)	*Xwmc177 (22.04cM, 2A), Xbarc309 (60.0cM, 2A), Xbarc57 (0.0cM, 3A), Xpsp3152 (80.66cM, 6A), Xpsp3001 (3B, 1A, 7A)*	0.241
Spike numbers	*Xgwm369 (35.88cM, 3A), Xgwm2 (72.0cM, 3A), Xbarc209 (?)*	0.482
Ear length (cm)	*Xgwm636 (17.7cM, 2A), Xwmc177 (22.04cM, 2A), Xgwm165 (35.46cM, 4A), Xgwm205 (7.44cM, 5A), Xgwm130 (26.0cM, 7A), Xwmc346 (7A), Xpsp3001 (3B, 1A, 7A)*	0.206
Grain features		
Storage protein	*Xbarc309 (60.0cM, 2A), Xgwm570 (117.64cM, 6A)*	0.208
Hardness	*Xwmc177 (22.04cM, 2A), Xbarc5 (29.0cM, 2A), Xbarc309 (60.0cM, 2A), Xgwm369 (35.88cM, 3A), Xgwm186 (52.58cM, 5A), Xgwm179 (96.0cM, 5A), Xpsp3152 (80.66cM, 6A), Xpsp3001 (3B, 1A, 7A)*	0.131
NBS2 profile	*Xgwm164 (57.31cM, 1A), Xbarc57 (0.0cM, 3A), Xbarc52 (161.54cM, 4A), Xbarc70 (186.58cM, 4A), Xbarc180 (11.59cM, 5A), Xgwm293 (26.97cM, 5A), Xgwm129 (27.34cM, 5A), Xbarc1055 (89.53cM, 6A), Xgwm570 (117.64cM, 6A), Xbarc1055 (6A), Xdupw167 (6A), Xpsp3001 (3B, 1A, 7A)*	0.038
Germination		
Salt stress	*Xgwm636 (17.7cM, 2A), Xbarc45 (73.0cM, 3A), Xbarc52 (161.54cM, 4A), Xdupw4 (4A), Xgwm205 (7.44/39.33cM, 5A), Xbarc180 (11.59cM, 5A), Xgwm179 (96.0cM, 5A)*	0.145

[a] FDR (false discovery rate) is a prediction of the number of identified loci which may not be associated with the traits of interest. A correction such as Bonferroni was not applied to the data because the analyses were aimed at identifying potential associations for future experimental verification.

domain of R proteins and *RGA*s contains the following characteristic motifs: P loop (phosphate-binding domain), kinase-2 motif, and GLPL-motif (Meyers *et al*., 1999). The NBS profiling in *T. monococcum* indicates that homologues of the potential *R* genes containing *NBS2* and *NBS5* are highly polymorphic. *Triticum monococcum* possesses a high level of resistance to a range of diseases and pests including leaf rust (Hussien *et al*., 1998), stem rust (Bai *et al*., 1998), powdery mildew (Shi *et al*., 1998), cereal aphid (Migui and Lamb, 2004), Russian wheat aphid (Deol *et al*., 1995), and Hessian fly (Sharma *et al*., 1997). The present *T. monococcum* collection contains accessions resistant to soil-borne cereal mosaic viruses and partially resistant to the virus vector *Polymyxa graminis* (Kanyuka *et al*., 2004; Ward *et al*., 2005). All the 30 *T. monococcum* accessions tested exhibited high resistance to *Septoria tritici* blotch under UK wheat production conditions, and *in planta* fungal sporulation was not observed throughout the growing season for four consecutive years (Jing *et al*., 2005; H-C Jing and K Hammond-Kosack, unpublished data).

Tolerance to abiotic stresses is pivotal for the success of crop production (Reynolds and Borlaug, 2006). Durum wheat containing the *Nax1* (Na^+ exclusion) and *Nax2* genes introgressed from *T. monococcum* exhibited greatly enhanced ability for salt exclusion and hence tolerance (James *et al*., 2006). This suggests that *T. monococcum* also has novel genes which can be used to enhance tolerance to abiotic stresses. In the 30 *T. monococcum* accessions, differences in tolerance to salt and drought stress were found using a germination assay. In a salt exclusion assay using young seedlings, a >10 times difference in leaf sodium content has been identified in these accessions (Y Shavrukov and H-C Jing, unpublished data). These results provide a strong basis to explore the genetic control of salt tolerance in *T. monococcum*.

Genetic basis of variation in multiple traits

Two types of genetic analyses were carried out to establish trait–marker associations in *T. monococcum*. First, segregation and linkage analyses were performed to identify genetic loci controlling awn colour and leaf pubescence. In bread wheat, the awn colour and glume colour are suggested to be controlled by the same genetic loci, which are associated with RFLP loci *QRaw.ipk-1A* on 1AS and *QRaw.ipk-1D* on 1D, respectively (Borner *et al*., 2002). It is not clear whether these two traits are linked in *T. monococcum*. The accession MDR002 has black awns but yellow glumes, suggesting that these two traits may be controlled by independent loci. However, the tightly linked SSR locus *Xwmc336* is located at 21.52 cM on chromosome 1A, which is in the vicinity of the bread wheat 1AS genetic locus controlling the black awn and glume trait. Furthermore, the *T. monococcum* black glume trait was previously mapped to a similar region on $1A^mS$ using two different mapping populations, and it was suggested that there is allelic variation in the

black glume locus in *T. monococcum* (Dubcovsky *et al*., 1996). Hence, it is most likely that in *T. monococcum* black awn and black glume are controlled by one single dominant locus. It was found that in *T. monococcum* leaf pubescence is dominant over leaf glabrousness. A tight linkage of the hairy leaf locus with *Xcfd39* at 83.19 cM on chromosome 5AL was found. In hexaploid wheat the hairy leaf loci *Hl1* and *Hl2* have been mapped to 4B and 7B, respectively (Taketa *et al*., 2002). After polyploidization there are serial events of chromosomal translocations amongst 5AL, 4AL, 4AS, and 7BS (Devos *et al*., 1995). It is likely that the mapped hairy leaf locus in *T. monococcum* is allelic to that on 7BS in hexaploid wheat. Interestingly, the hairy leaf loci *Hbs* and *Hp1* have been found in homologous chromosomal regions in barley and rye (Korzun *et al*., 1998*a*, 1999).

Association genetics analyses the variation of particular phenotypes amongst plants to detect and measure the degree of association between molecular markers and traits of interest (Gupta *et al*., 2005). This approach has been successfully used to identify a range of marker–trait associations in hexaploid wheat (Roy *et al*., 2006). The present study points to some interesting marker–trait associations in *T. monococcum*, even though only a limited numbers of 30 accessions were used (Table 5). Remarkably, some of the associations identify chromosomal regions containing previously known genetic loci. For instance, several SSR markers associated with NBS profiles are in the vicinity of mapped *RGA*s (McFadden *et al*., 2006). The association mapping also identified that germination under salt and drought stresses is probably linked to *Nax2*. Both *Nax1* and *Nax2* genes were identified in a seedling salt exclusion assay (James *et al*., 2006). However, it appears that only *Nax2* is involved in salt tolerance during both seed germination and seedling growth. These may correlate with the divergence of the two genes in terms of function. Nax1 works to reduce sodium content in leaf blades (Huang *et al*., 2006), whereas Nax2 removes sodium from xylem in the roots (Byrt *et al*., 2007). In addition to confirming previous marker–trait associations, the association mapping has identified many new associations which merit further study.

In hexaploid wheat, grain texture is mainly controlled by the *Hardness* (*Ha*) locus on 5D consisting of the *Pina-D1*, *Pinb-D1*, and *Gsp-D1* genes (Gautier *et al*., 1994; Sourdille *et al*., 1996). These *Ha*-related genes were shown to be arranged in a highly conserved manner on $5A^m$ (Tranquilli *et al*., 1999). Interestingly, it was not possible to identify associations with the predominant *Ha* locus; none of the identified SSR markers is located on the 5AS region containing *PinA*, *PinB*, and *Gsp* genes. Also the previously known *Ha* locus SSR markers are not linked to variation in hardness in *T. monococcum* (Table 5; Bonafede *et al*., 2007). This may imply that the observed variation in grain hardness in *T. monococcum* is controlled by genetic loci other than the *Hardness* locus. Indeed, there is a report indicating that additional QTLs exist in hexaploid wheat controlling grain texture (Turner *et al*., 2004). Furthermore, our preliminary results showed that the sequences of *Pina* and *Pinb* genes are highly conserved in these *T. monococcum* accessions (M Wilkinson and P Shewry, unpublished data).

A number of other important associations between microsatellites and traits were found in this study, including plant height, peduncle length, grain moisture content, and ear length. Thus, via screening only a small pool of genotypes, possible loci conferring a specific trait are detected in *T. monococcum*, which often requires a large pool of germplasm to be screened in other species (Gupta *et al*., 2005). The findings in this report indicate that the efficiency of association mapping is much higher in *T. monococcum* than in other plant species. Mapping populations are currently being generated and more SSR markers are being applied to construct a high density genetic map with the bulk segregating populations from a cross between MDR308 and MDR002 and to narrow down the linkage intervals.

The genetic diversity of modern hexaploid wheat has been achieved through the introgression of novel genetic materials (Reif *et al*., 2005). The unique characteristics and evolution of *T. monococcum* make it ideal as a reference species for wheat genetics and genomics. Examples exist demonstrating the success of gene cloning using a subgenome approach in *T. monococcum* (Keller *et al*., 2005). Furthermore, useful traits and genic variants can be introgressed into elite wheat varieties using conventional breeding approaches assisted by molecular makers (Korzun, 2002). Over the years, many approaches have been developed to facilitate the introgression of novel traits through conventional breeding (Potgieter *et al*., 1991). These include the utilization of a unique *T. monococcum* accession (PI355520), containing two dominant genetic loci which could help achieve high rates of viable and functional hybrids when crossed to hexaploid wheat (Cox *et al*., 1991). In addition, a range of bridge species have been developed, including a multiploid mutant or amphiploid of durum wheat (Multani *et al*., 1988; Klindworth and Williams, 2003), a synthetic allotetraploid *T. monococcum*/*Secale cereale* (A^mA^mRR) (Kison and Neumann, 1993), or tetraploid and hexaploid triticale (Sodkiewicz and Apolinarska, 2000; Sodkiewicz and Strzembicka, 2004).

In the current post-genomic era, many molecular genetic resources and technology breakthroughs are ready or under development for crop sciences. TILLING (Targeting Induced Local Lesions IN Genomes) and VIGS (virus-induced gene silencing) have been efficiently used for functional genomics in cereals (Hein *et al*., 2005; Scofield *et al*., 2005; Slade *et al*., 2005). All these will be helpful for exploiting *T. monococcum* as a reference

species to establish tight trait–marker associations, and eventually leading to gene function studies using both forward and reverse genetic approaches.

Supplementary material

The supplementary material available at *JXB* online includes the following: (1) Supplementary Table S1 showing the additional 66 *T. monococcum* and 13 *T. boeoticum* accessions used for assessing genetic diversity; (2) Supplementary Fig. S1 showing cluster analysis of the 96 *T. monococcum* and 13 *T. boeoticum* accessions.

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