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RFLP-based genetic relationships of Einkorn wheats

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Abstract To study the relationships between different species of the Einkorn group, 55 different accessions of *Triticum monococcum, T. boeoticum, T. urartu, T. sinskajae, T. thaoudar* and *T. aegilopoides* were analyzed. Fifteen anonymous probes and four clones corresponding to storage protein genes were used for detecting restriction fragment length polymorphisms (RFLPs). The DNA was restricted with the restriction enzymes *Alu*I, *Hae*III, *Rsa*I and *Taq*I. The 25 probe/enzyme combinations employed yielded a total of 488 polymorphic fragments. Statistical analyses were performed using Jaccard's coefficient of similarity and principal coordinate analysis. Different values of similarity within the three main taxa, *monococcum, boeoticum* and *urartu,* were obtained; the grouping at the species level was quite well reflected by the RFLP analysis done here. The coincidence between RFLP data and the subspecies classification of the *T. monococcum* group was only partial. One *T. urartu* accession is clearly different from all of the other 54 accessions. The need for an RFLP based revision of the Einkorn taxonomy is evident.

Key words Diploid wheat · Einkorn · RFLP Taxonomy

Introduction

Wild diploid wheat species (2n=2x=14) have been investigated to elucidate their contribution to the phylogeny of tetraploid and hexaploid wheats. Cytogenetical studies support *Triticum monococcum* L. as the putative donor of the A genome (reviewed by Kerby and Kuspira 1987), but recent investigations suggest that *T. urartu*, another member of the Einkorn group, may be an alternative donor of this genome (Dvorak et al. 1988; Tsunewaki et al. 1991).

The Einkorn wheats have been assigned to several taxa. Kimber and Feldman (1987) subdivided the species *monococcum* into var. '*urartu*' and var. '*boeoticum*'. *T. boeoticum* was further divided into ssp. *aegilopoides* and ssp. *thaoudar.* The classifications still in use in germplasm collections differ greatly. For instance, *monococcum, boeoticum* and *urartu* are often elevated to the level of species and then further subdivided into several subspecies (Gorham et al. 1991; Rafi et al. 1992). In some collections the taxa *aegilopoides* and *thaoudar* as well as *sinskajae*, a free-threshing form morphologically similar to *T. monococcum*, are considered to be species.

Additional studies of the variability between and within taxa of *T. monococcum* have been based on morphological traits (Sharma et al. 1981), storage protein electrophoretic variants (Waines and Payne 1987), isozymes (Smith-Huerta et al. 1989; Nishikawa et al. 1992) and molecular markers (Vierling and Nguyen 1992; Tsunewaki et al. 1991). In this paper 55 different Einkorn accessions have been characterized by restriction fragment length polymorphism (RFLP) analysis in order to assess the level of coincidence between present-day adopted taxonomical subdivisions and variation observed at the DNA level.

Materials and methods

Plant material

Fifty-five genotypes were chosen from a total of 1393 diploid wheats obtained from several germplasm collections. Of these 55 accessions 45 were classified as *T. monococcum* and subdivided into 13 different subspecies. Three *T. boeoticum* spp., 3 *T. urartu*, 2 *T. sinskajae*, 1 *T. thaoudar* and 1 *T. aegilopoides* were also analyzed (Table 1).

Leaf material was harvested from 3 to 10 plants per accession, pooled, freeze dried and stored at –70°C.

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Table 1 List of accessions used here (*MPI* Max Planck Institut, Köln, Germany, *IDG* Istituto del Germoplasma, Bari, Italy, *INTA* Instituto Nacional de Tecnologia Agropecuaria, Argentina, *AFRC* Cambdrige Laboratory, Norwich, England, *ALB* University of Alberta, Edmonton, Canada, *IGK* Institut für Genetik und Kulturpflanzenforschung, Gatersleben, Germany, *RAC* Recherches Agronomiques de Changins, Nyon, Switzerland)

Code	Species	Accession	Source
1	*T. monococcum*	Einkorn	MPI
2	*T. monococcum*	Winterform	MPI
3	*T. monococcum*	IDG 4242	IDG
4	*T. monococcum*	MG 4278	IDG
5	*T. monococcum*	22553	INTA
6	*T. monococcum*	22796	INTA
7	*T. monococcum*	104002	AFRC
8	*T. monococcum*	104003	AFRC
9	*T. monococcum*	104004	AFRC
10	*T. monococcum* ssp. *monococcum*	PI 94740	ALB
11	*T. monococcum* ssp. *monococcum*	CI 13961	ALB
12	*T. monococcum* ssp. *monococcum*	PI 221413	ALB
13	*T. monococcum* ssp. *monococcum*	PGR 10406	ALB
14	*T. monococcum* ssp. *monococcum*	ATRI 11360/80	IGK
15	*T. monococcum* ssp. *vulgare*	ATRI 618/74	IGK
16	*T. monococcum* ssp. *vulgare*	ASchgt 2/88	IGK
17	*T. monococcum* ssp. *vulgare*	ATRI 617/74	IGK
18	*T. monococcum* ssp. *vulgare*	ATRI 1990/74	IGK
19	*T. monococcum* ssp. *vulgare*	ATRI 1985/74	IGK
20	*T. monococcum* ssp. *vulgare*	ATRI 3637/74	IGK
21	*T. monococcum* ssp. *atriaristatum*	HTRI 2399/74	IGK
22	*T. monococcum* ssp. *atriaristatum*	ATRI 2124/74	IGK
23	*T. monococcum* ssp. *laetissimum*	486	RAC
24	*T. monococcum* ssp. *laetissimum*	1496	RAC
25	*T. monococcum* ssp. *laetissimum*	ATRI 4321/75	IGK
26	*T. monococcum* ssp. *nigricultum*	ASchgt 3/88	IGK
27	*T. monococcum* ssp. *nigricultum*	1498	RAC
28	*T. monococcum* ssp. *nigricultum*	22554	INTA
29	*T. monococcum* ssp. *nigricultum*	22929	INTA
30	*T. monococcum* ssp. *sofianum*	1497	RAC
31	*T. monococcum* ssp. *halbohornemanii*	ATRI 3409/79	IGK
32	*T. monococcum* ssp. *macedonicum*	ATRI 2126/74	IGK
33	*T. monococcum* ssp. *macedonicum*	ATRI 4320/74	IGK
34	*T. monococcum* ssp. *macedonicum*	22928	INTA
35	*T. monococcum* ssp. *pseudomacedonicum*	2372	RAC
36	*T. monococcum* ssp. *flavescens*	ATRI 4309/74	IGK
37	*T. monococcum* ssp. *flavescens*	ATRI 580/74	IGK
38	*T. monococcum* ssp. *hornemanii*	ATRI 2001/74	IGK
39	*T. monococcum* ssp. *hornemanii*	ATRI 4304/74	IGK
40	*T. monococcum* ssp. *hornemanii*	ATRI 895/74	IGK
41	*T. monococcum* ssp. *hornemanii*	ATRI 896/74	IGK
42	*T. monococcum* ssp. *boeoticum*	PGR 10403	ALB
43	*T. monococcum* ssp. *boeoticum*	PGR 11099	ALB
44	*T. monococcum* ssp. *boeoticum*	PGR 10895	ALB
45	*T. boeoticum* ssp. *boeoticum*	HTRI 11164/90	IGK
46	*T. boeoticum* ssp. *rufinigrum*	ASchgt 1/88	IGK
47	*T. boeoticum* ssp. *albinigrescens*	HTRI 10060/74	IGK
48	*T. thaoudar*	102003	AFRC
49	*T. aegilopoides*	103005	AFRC
50	*T. urartu*	HTRI 6734/89	IGK
51	*T. urartu*	HTRI 6735/83	IGK
52	*T. urartu*	PGR 6150	ALB
53	*T. sinskajae*	ATRI 12910/89	IGK
54	*T. sinskajae*	ATRI 11525/76	IGK
55	*T. monococcum* ssp. *sinskajae*	G 4325	ALB

Table 2 List of probes used here

Probe	Chromosome location[a]
K9	1AL, 1BL, 1DL
K32	1AS, 1BS, 1DS
pB11	1HS
pTU 4/97	1AS, 1BS, 1DS, 2A, 2B, 2D, 3A, 3B, 3D
WG 996	2H
pTU 4/16	3AL, 3BL, 3DL
pTU 4/19	3AL, 3BL, 3DL
pTU 4/34	4AS, 4BS, 4DL
WG 114	4HL
WG 464	4HL
WG 622	4HS
WG 364	5H
WG 541	5H
WG 1026	5HL
pTU 1	6AS, 6BS, 6DS
pTU 4/82	7AL, 7BL, 7DL
WG 420	7HL
WG 669	7HS
WG 719	7HS

[a] According to: Bartels et al. (1986) for the K32 clone, Forde et al. (1985) for the pB11 clone, Heun et al. (1991) for the WG clones, Tanzarella (personal communication) for the pTU clones, Thompson et al. (1983) for the K9 clone

Field observations

Fifty-five genotypes were planted in one-row plots 1.5 m in length in an experimental farm near S. Angelo L. (Italy) on November 10, 1992 and on March 3, 1993. Morphological traits of the spike were measured on the main tiller.

The genotypes were classified as facultative types when in the spring plants were tillering completely, while those forming many basal leaves with a low number of tillers and a reduced capacity to ear were classified as winter types.

Probes

Nineteen RFLP probes were used. Ten different wheat genomic (WG) clones (Heun et al. 1991) were supplied by M.E. Sorrells (Cornell University, Ithaca, N.Y.). K9 and K32, cDNA clones corresponding respectively to *Glu-1* (Thompson et al. 1983) and *Gli-1* DNA sequences (Bartels et al. 1986), were obtained from R. Thompson (Max-Planck-Institut, Cologne, Germany). The pB11 probe, provided by P. Shewry (AFRC, Bristol, UK), is a *Hor-2* cDNA barley clone (Forde et al. 1985). pTU probes were selected by O. Tanzarella (University of Tuscia, Viterbo, Italy) from a *T. urartu* genomic library; all but pTU1, which recognizes *Gli-2* DNA sequences of wheat, are anonymous probes. The probes were chosen so as to be spread over all chromosomes; their chromosomal location is given in Table 2.

RFLP detection and analysis of data

DNA isolation, digestion, gel electrophoresis, blotting, probe preparation and hybridization were carried out as described by Gebhardt et al. (1989). Samples of genomic DNA (5 µg) were digested with the four-cutter restriction enzymes *Alu*I, *Hae*III, *Rsa*I or *Taq*I according to the supplier's instructions (Promega). For each single probe/restriction enzyme combination the presence or absence of clearly readable restriction fragments was scored. Each polymorphic fragment was treated as a unique character. Fragments not showing any polymorphism among the 55 lines were not considered. The presence of a fragment was scored as 1 and its absence as 0.

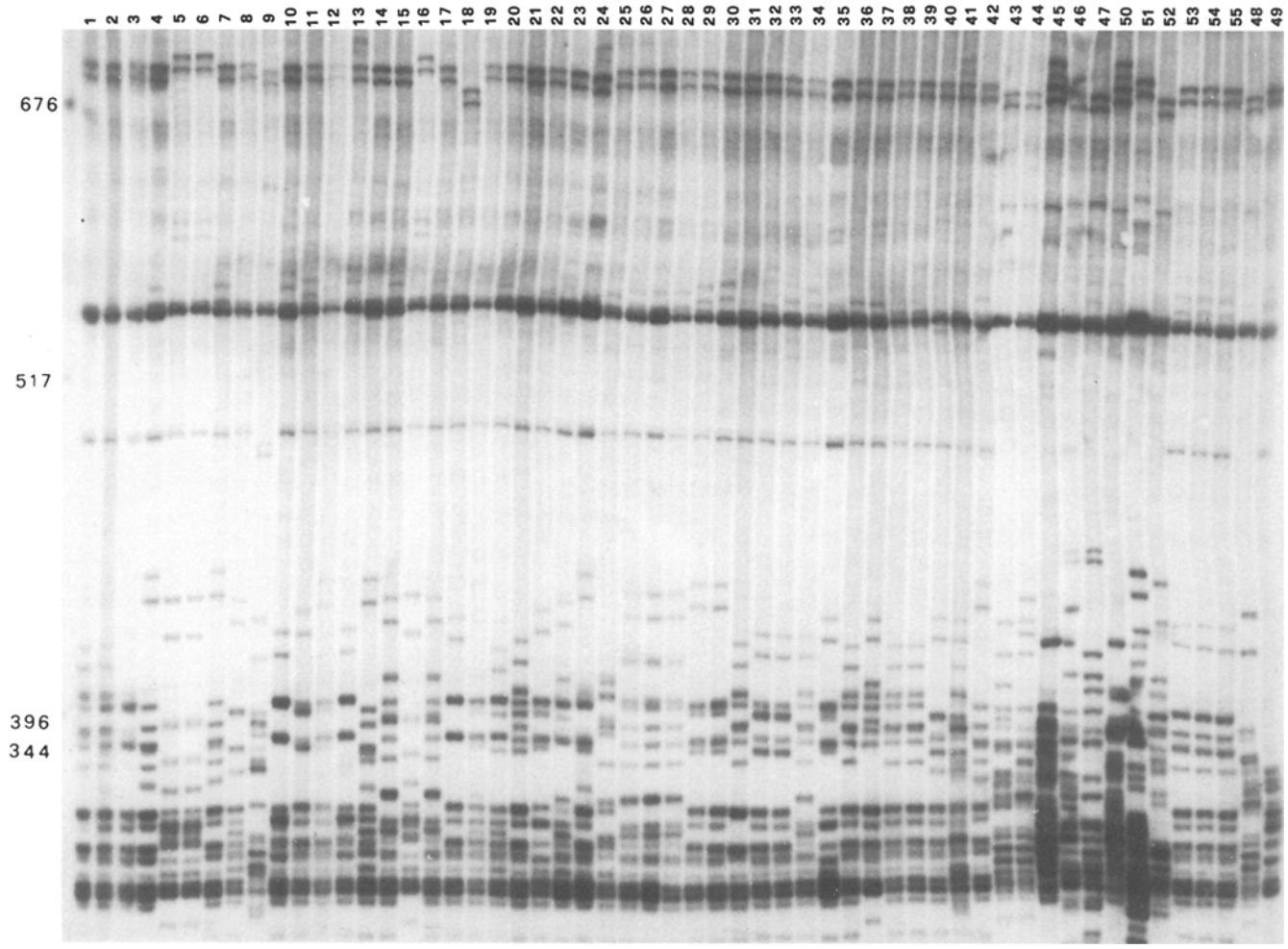

Fig. 1 RFLP patterns obtained with restriction enzyme *Rsa*I and probe pTU1. The *numbers* at the *top* of each lane refer to accessions listed in Table 1. Molecular weight markers (in bp) are indicated on the *left*

The pair-wise distances between all accessions were calculated using Jaccard's similarity coefficient (Jaccard 1908):

$J_{i,j}$=A/N–D

where A represents the number of bands common to 2 accessions i and j, N is the total number of polymorphic fragment positions in the matrix and D is the number of bands absent in both samples.

Associations between the accessions were determined by principal coordinate analysis (PCOA), and the coordinates of the 55 accessions were computed for the first three axes (Gower 1966). A dendrogram was constructed using the unweighted pair-group method with arithmetical averages (UPGMA).

Both types of multivariate analysis were performed employing the NTSYS-pc package, version 1.7 (Rohlf 1992).

Results and discussion

Level of polymorphism

The RFLP analysis was done with 15 anonymous probes and four clones encoding storage proteins. Four different restriction enzymes were used to give a total of 25 probe/enzyme combinations that generated 488 polymorphic fragments.

The average number of fragments detected per combination was 19.5 and ranged from 3 (WG114/*Hae*III, WG464/*Hae*III, WG1026/*Taq*I and WG669/*Taq*I) to 102 in the combination pTU1/*Rsa*I, which also revealed the highest number of unique patterns (47 out of 55 accessions considered; Fig. 1). This high level of polymorphism was seen with a DNA sequence that mapped at the *Gli-2* locus encoding α- and β-gliadins. Our result agrees with that of Metakovsky and Baboev (1992a, 1992b), who found a large number of gliadin patterns when studying several accessions of *T. monococcum* and *T. boeoticum*. The probe K32, corresponding to the *Gli-1* locus encoding γ-gliadins, revealed only an average level of polymorphism, as did the probe K9 (*Glu-1* locus; HMW glutenins). The clone pB11 (*Hor-2*; B-hordeins), when combined with *Rsa*I, gave 39 unique patterns.

Similarity within taxonomical groups

The degree of similarity between and within taxa was calculated by the coefficient of Jaccard. Significant differ-

ences were found within the two main taxonomical subdivisions. In the *monococcum* group, excluding accessions nos. 5 and 6 for which no polymorphic bands were detected, the coefficient of similarity ranged from 0.33 to 0.98. In the *boeoticum* group, including accessions nos. 42, 43 and 44 (classified originally as *T. monococcum* ssp. *boeoticum*) the coefficient of similarity had values between 0.21 and 0.52. For *T. urartu*, only 3 accessions were analyzed.

Coefficients of similarity having the same range as those reported here have been found in *T. monococcum* by Vierling and Nguyen (1992) using random amplified polymorphic DNA (RAPD) markers. Genetic diversity in diploid wheats was also measured using isozyme markers. Smith-Huerta et al. (1989) considering 12 different enzyme systems found a uniformly low level of genetic variability in both *T. monococcum* var. 'boeoticum' and a *T. urartu* population. Nishikawa et al. (1992), using only α-amylase isozymes, found a lower degree of polymorphism among 23 *T. urartu* spp. accessions than among 39 *T. monococcum* spp. accessions. Using RFLP markers and 2 accessions per species, Tsunewaki et al. (1991) found that the intraspecific genetic distance was greater in *T. boeoticum* than in *T. urartu* and *T. monococcum*.

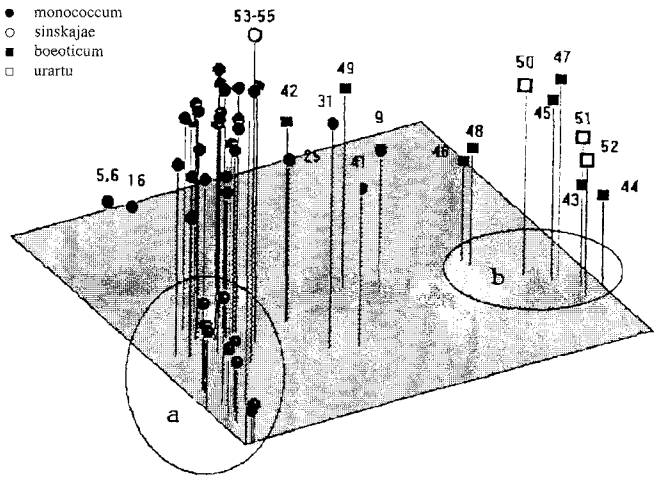

Fig. 2 Three-dimensional model based on a principal coordinates analysis of RFLP data of the 55 *Triticum* accessions. The numbers refer to the accessions listed in Table 1

PCOA and cluster analysis

Relationships between accessions revealed by principal coordinate analysis are presented in Fig. 2. Principal coordinates 1, 2 and 3 accounted for 12.4%, 5.7% and 5.5% of the total variation, respectively. On the basis of this analysis, the 55 diploid wheats examined could be partially subdivided into separate groups.

Group (a) comprises the majority of the *monococcum* accessions including the 3 *sinskajae* lines (nos. 53, 54 and 55). The exceptions are accessions 5, 6 and 16, which are clustered in a different position on the second coordinate, and lines 9, 25 (*T. monococcum* ssp. *laetissimum*), 31 (T. *monococcum* ssp. *halbohornemanii*) and 41 (*T. monococcum* ssp. *hornemanii*), which are distributed along the first coordinate towards group (b).

Group (b) includes all of the accessions classified as *T. urartu* and those of the *boeoticum* group. Only lines 42 (T. *monococcum* ssp. *boeoticum*) and 49 (*T. aegilopoides*) did not cluster in this group: both are located closest to group (a).

A dendrogram was constructed based on the similarity matrix using UPGMA with a cophenetic correlation r=0.973 (Fig. 3). This dendrogram confirms the PCOA results but also provides additional information concerning relationships between the accessions.

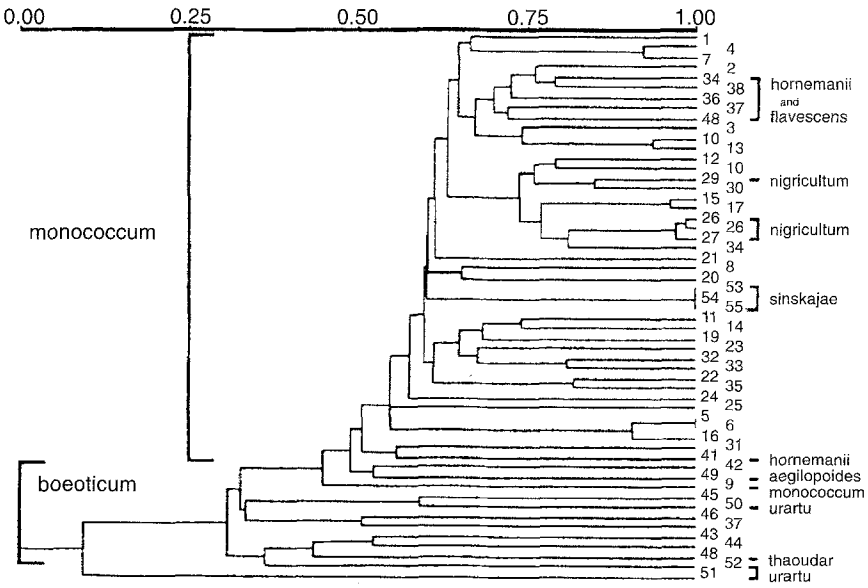

Fig. 3 Dendrogram of 55 accessions based on the UPGMA method using the Jaccard similarity matrix. The numbers refer to the accessions listed in Table 1

Table 3 Comparison of accession 51 (*T. urartu*) with representatives of *T. monococcum* (26), *T. boeoticum* (45) and *T. urartu* (50)

Code	Species	Growth class	Hairs on the leaves	Spike density	Number of spikelets per spike	Second tooth on the sterile glumes	Second floret on the spikelet	Anther length (mm)
26	*T. monococcum* ssp. *nigricultum*	Facultative	Absent	Dense	45–55	Developed	Absent	6–8
45	*T. boeoticum* ssp. *boeoticum*	Winter	Present	Very lax	28–38	Developed	Partially developed	6–8
50	*T. urartu*	Winter	Present	Lax	30–40	Well developed	Developed	6–8
51	*T. urartu*	Facultative	Absent	Intermediate	30–40	Slightly developed	Partially developed	3–4

Monococcum genotypes clustered separately from *boeoticum* accessions, even though the two taxa did not form very distinct clusters. This was due to some common RFLP fragments. Accessions 9 (*T. monococcum*), 42 (*T. monococcum* ssp. *boeoticum*) and 49 (*T. aegilopoides*) fall on the borderline of the *monococcum* group and *boeoticum* group.

The *monococcum* group was resolved into several distinct subgroups by the RFLP data. These subgroups do not agree with the nomenclature used in germplasm collections, although in a few cases some association was found. The two *flavescens* ssp. (36, 37) clustered together with 3 of the 4 lines of the *hornemanii* ssp. Of the 4 *nigricultum* accessions 3 (26, 27, 28), tended to group in a cluster, but 1 accession (29) was in a position apart.

The 3 accessions of group *sinskajae* (53, 54, 55) showed identical RFLP patterns, even though they were obtained from two different collections. They clustered within the *monococcum* group, thereby supporting the classification of *sinskajae* as a subspecies of *monococcum* (Waines 1983).

Triticum thaoudar and *T. aegilopoides,* accessions 48 and 49, respectively, were included in the *boeoticum* cluster. This agrees with the Kimber and Feldman (1987) classification in which they are considered to be forms of var 'boeoticum'.

The accessions of *T. urartu* nos. 50 and 52 clustered within the *boeoticum* group. Accession 51 (*T. urartu*) often had unique RFLP patterns. This is also reflected in the cluster analysis, where this genotype remains completely isolated at the first branching of the dendrogram. Therefore, we have further characterized this accession on the basis of several morpho-physiological traits. Figure 4 and Table 3 show a comparison based on various traits of accession 51 with others classified as *T. monococcum* (accession 26), *T. boeoticum* (accession 45) and *T. urartu* (accession 50).

On the basis of the morphology of spikelets, sterile glumes and anthers, which are used in Einkorn taxonomy (Kimber and Feldman 1987), accession 51 can be clearly classified as a genome A wheat. However, this accession is different from the other 2 *T. urartu* studied because the anthers of accession 51 are shorter, the second tooth of the sterile glume is only slightly developed and the leaf hairs are absent.

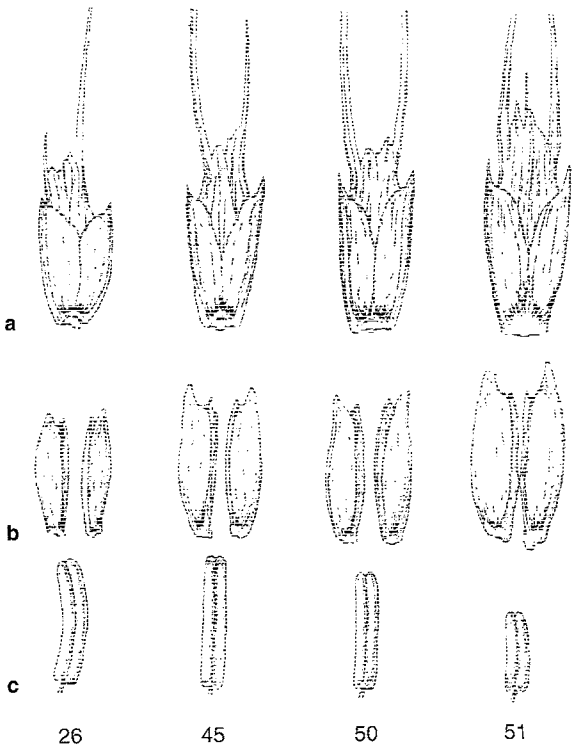

Fig. 4a–c Drawings of taxonomically relevant traits (**a** spikelet, **b** sterile glumes, **c** anther) of four representative accessions of *T. monococcum* (*26*), *boeoticum* (*45*) and *urartu* (*50* and *51*)

The analysis of our sample of 55 accessions indicates that the taxonomical classification of these subspecies is not completely precise and subsequently points to the need of more accurate studies to assess the real variability and subgrouping of the *monococcum* taxa. The level of similarity within *monococcum* is higher than in the taxa *boeoticum* even though it includes divergent clusters of subspecies, such as the one represented by the *sinskajae* accessions.

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