Theor Appl Genet (1999) 98: 602–607

W. Cao · G. Scoles · P. Hucl · R. N. Chibbar

The use of RAPD analysis to classify *Triticum* accessions

Received: 12 June 1998 / Accepted: 18 August 1998

Abstract Crop germplasm collections contain a considerable percentage of misclassified accessions which may affect the use of germplasm for agricultural crop improvement. The objective of this study was to determine if random amplified polymorphic DNA (RAPD) analysis could be used to reclassify misclassified *Triticum* accessions. Twelve accessions suspected to be misclassified, based on morphological characters, as either macha or vavilovii wheat were studied using RAPD and cytological analyses. In the RAPD analysis, a dendrogram, based on Jaccard genetic similarity coefficients, grouped 5 *dicoccum*-like, 1 *timopheevii*-like, and 6 *monococcum*-like accessions with *Triticum dicoccum*, *T. timopheevii*, and *T. monococcum* accessions, respectively. These results were confirmed by the cytological analysis. A RAPD marker specific to the D genome was also detected. This study suggests that RAPD analysis can be used to classify germplasm and to distinguish some species in *Triticum*.

Key words *Triticum* · Germplasm · RAPD · Misclassification · Duplication

Introduction

Germplasm collections play a very important role in the genetic improvement of cultivated plants. However, germplasm collections may have a considerable percentage of accessions misclassified, either from erroneous identification or inadvertent misplacement of the correct label (Hurka 1994). In the collection of the International Centre for Agricultural Research in the Dry Areas (ICARDA), more than 2,000 of 11,647 durum accessions were found to be hexaploid wheats, primarily bread wheat (*T. aestivum* L.) (Pecetti et al. 1992). Another problem of germplasm collection is accession duplication. Approximately 15% of the accessions in the same durum wheat collection were duplicated, mainly because the same entries had been received from different sources (Pecetti et al. 1992). Accession duplication not only increases the cost of storing, handling, and regenerating duplicate accessions but also decreases the efficiency of evaluating collections (Toledo et al. 1989). Thus, careful examination is required to identify the species or accessions correctly and list them appropriately. Molecular markers are potential tools to effectively and efficiently identify genetic relatedness (Kresovich et al. 1992). The random amplified polymorphic DNA (RAPD) technique has been used to produce species-specific molecular markers in the Triticeae (Wei and Wang 1995). Thus markers can be used to identify species or reclassify misclassified germplasm.

While investigating relationships among five groups of hexaploid wheat (*Triticum aestivum*): macha, spelta, common wheat, vavilovii, and semi-wild wheat (SWW), 16 of 124 accessions of either macha, vavilovii, or spelta were considered to be misclassified based on morphological characteristics. The objective of the study presented here was to use these materials to determine whether, in the absence of any other data, RAPD analysis could be used to correctly classify *Triticum* accessions.

Communicated by Yu. Gleba

W. Cao (✉) · G. Scoles · P. Hucl
Department of Plant Sciences, Crop Development Centre, University of Saskatchewan, 51 Campus Drive, Saskatoon, SK., Canada S7N 5A8
Fax: +1-613-759-6559
E-mail: caowen@em.agr.ca

R. N. Chibbar
Plant Biotechnology Institute, National Research Council, Saskatoon, SK., Canada S7N 0W9

Materials and methods

Plant materials

The 12 accessions included in collections as either macha or vavilovii but apparently misclassified (based on phenotype) and the

Table 1 Twelve accessions thought to be misclassified and accessions of the six known *Triticum* species used in the study

Number	Accession	Species[a]	Growth habit	Chromosome number
1	PI 352369	*T. tur.-dicoc.* (CK)	Spring	28
2	G 1910	Misclassified	Winter	28
3	G 1911	Misclassified	Winter	28
4	G 1914	Misclassified	Winter	28
5	P GR3975	Misclassified	Spring	28
6	P GR3978	Misclassified	Spring	28
7	PI 355512	macha wheat (CK)	Winter	42
8	PI 352267	*T. monococcum* (CK)	Spring	14
9	PI 428154	Misclassified	Spring	14
10	PI 428155	Misclassified	Winter	14
11	PI 428156	Misclassified	Winter	14
12	PI 428157	Misclassified	Winter	14
13	PI 428159	Misclassified	Winter	14
14	PI 428162	Misclassified	Winter	14
15	PI 428343	vavilovii wheat (CK)	Winter	42
16	PI 343447	*T. timopheevii* (CK)	Spring	28
17	PGR 4005	Misclassified	Spring	28
18	PI 355503	*T. tur.-dicoc.* (CK)	Spring	28

[a] CK, Check; *T. tur.-dicoc.*, *T. turgidum* ssp. *dicoccum*

6 known *Triticum* accessions used in this study are listed in Table 1. The PI series accessions were kindly provided by Dr. Harold E. Bockelman, National Small Grains Collection, United States Department of Agriculture, whereas the accessions with a PGR designation were supplied by Dr. G. Baillargeon, Plant Gene Resources of Canada (PGRC), Ottawa, Canada. The remaining G series accessions were provided by Dr. L. Seidewitz, Bundes Forschunganstalt für Landwirtschaft, Braunschweig, Germany.

Crosses and cytological study

Three spring-type accessions (PGR3978, PI428154, and PGR4005) representing misclassified dicoccum-like, timopheevii-like, and monococcum-like accessions, respectively, were used in a cytological analysis. For chromosome pairing observations, crosses between those known species accessions and plants suspected to be of same species were made based on morphological similarity and root-tip chromosome counts. These were *Triticum turgidum* ssp. *dicoccum* (PI352369)/PGR3978, *T. turgidum* ssp. *dicoccum* (PI355503)/PGR3978, *T. timopheevii* (PI343447)/PGR4005, and *T. monococcum* (PI352267)/PI428154. F_1s and their parents were planted in the field, and young spikes were collected between 10:00 a.m. and 1:00 p.m. and fixed with Carnoy's solution (6:3:1, alcohol:chloroform:acetic acid) for meiotic studies. The number of chiasmata was estimated for each F_1 and its parents by observing approximately 100 pollen mother cells and calculated using the following formula: estimated number of chiasmata = 2(number of ring bivalents) + 1(number of rod bivalents) + 2(number of trivalents) + 4(number of ring quadrivalents).

Oligonucleotide primers

Oligonucleotide primers (10-mer) were purchased from the Biotechnology Laboratory, University of British Columbia, while 9–mer primers were synthesized on an Applied Biosystems Model 394 DNA synthesizer using beta–cyanoethyl phosphoramidite chemistry. Each primer (10-mer and 9-mer) was dissolved separately in water to yield a DNA concentration of 5 μM and stored at −20°C.

DNA isolation and polymerase chain reaction (PCR) procedure

DNA extraction was based on the methods described by Procunier et al. (1990). The PCR methodology followed that described by Joshi and Nguyen (1993). The PCR products with 5 μl of loading buffer were separated by electrophoresis using a 1.5% (w/v) agarose gel in TRIS-acetate (TAE) buffer. Gels were stained with ethidium bromide (68 μg/ml). DNA fragments were then visualized under UV light and photographed using a gel documentation system (DiaMed Lab Supplies). Sizes of amplified products were estimated by comparing with a 1-kb DNA ladder (BRL, Bethesda, Md.).

Data scoring and analysis

The amplified fragments were scored from the lower molecular-weight products to the higher molecular-weight products. The presence of a product was identified as "1" and its absence was designated as "0". Although a few faint bands were produced, only the bright ones were used in this study. The data were analyzed using the SIMQUAL (Similarity for Qualitative Data) routine to generate Jaccard similarity coefficients. These similarity coefficients were used to construct dendrograms using the unweighted pair group method with arithmetic averages (UPGMA) employing the SAHN (Sequential, Agglomerative, Hierarchical, and Nested clustering) from the NTSYS-PC (Numerical Taxonomy and Multivariate Analysis System), version 1.80 (Applied Biostatistics) program.

Results

Morphological and cytological study

Six of the potentially misclassified accessions had 28 chromosomes, and 6 had 14 chromosomes (Table 1). Thus, the root-tip chromosome counts confirmed that these 12 *Triticum* accessions were not hexaploid wheats.

Fig. 1a–c Spikes of *Triticum timopheevii* accession PI343447 **a**, a misclassified accession PGR4005 **b**, and vavilovii wheat accession PI428343 **c**

Spikes of 1 accession (PGR4005) with 28 chromosomes resembled *Triticum timopheevii* (Fig. 1). This wheat had a laterally compressed spike and long awns and was non-free-threshing as a result of glumes which tightly held the grains. The spike was tapered at the base and tip, rather brittle, and easily disarticulated into individual spikelets at maturity, each with an attached rachis segment. Each spikelet contained two long grains. In addition, the entire plant was pubescent.

Spikes of 5 other misclassified accessions with 28 chromosomes resembled *Triticum turgidum* ssp. *dicoccum* (Fig. 2). These accessions had tough glumes and were non-free-threshing. The spikes of the wheat were laterally compressed and had long awns. The rachis was fragile and disarticulated at the narrowest basal end of each internode, each separated spikelet carrying a fragment of rachis. Each spikelet contained two grains.

The 6 misclassified accessions with 14 chromosomes had tough glumes and a small spike like *Triticum monococcum* (Fig. 3). The spikes of the wheat were compressed, erect, laterally flattened, and had long awns. The rachis was flattened and very fragile. Each spikelet had two florets, but only one small pointed grain was present in the spikelet at maturity.

Chiasma frequencies in each F_1 and its parents are presented in Table 2. Chromosome pairing in all F_1s was comparable to that of the parents. The data indicated that the misclassified accessions PI428154, PGR3978, PGR4005 were *T. monococcum*, *T. turgidum* ssp. *dicoccum*, and *T. timopheevii*, respectively. The results were thus consistent with the morphological observations. In addition, a ring quadrivalent (Fig. 4) was found in the hybrids *T. turgidum* ssp. *dicoccum* (PI352369)/PGR 3978 and *T. turgidum* ssp. *dicoccum* (PI355503)/PGR3978 at a very high frequency, indicating that a chromosome translocation existed between PGR3978 and those 2 accessions.

RAPD analysis

Twelve random primers were used in this study. One hundred and one polymorphisms were detected in the 18 accessions by the 12 primers, an average of 8.4 polymorphisms per primer. The polymorphisms produced by 1 primer with the 18 accessions of *Triticum* are shown in Fig. 5. Species-specific markers were detected for *Triticum aestivum* (AABBDD) (Fig. 6) and *T. timopheevii* (AAGG) (Table 3). Specific markers were not identified for *Triticum turgidum* ssp. *dicoccum* (AABB) or *T. monococcum* (AA).

Fig. 2a–c Spikes of *Triticum turgidum* ssp. *dicoccum* accession PI352369 **a**, a misclassified accession PGR3978 **b**, and macha accession PI355512 **c**

Fig. 3a–c Spikes of *Triticum monococcum* accession PI352267 **a**, a misclassified accession PI428154 **b**, and macha accession PI355512 **c**

Table 2 Number of chiasma observed at metaphase I in F_1s of 3 misclassified wheat accessions crossed with *Triticum* species and their parents

Cross or parent	Number of cells observed	Means of chiasma	*P* value[a]
PI352369	205	27.70 ± 0.03[b]	0.49
PGR3978/PI352369	207	27.62 ± 0.11	
PGR3978	209	27.80 ± 0.04	0.28
PGR3978	209	27.80 ± 0.04	0.58
PI355503/PGR3978	210	27.69 ± 0.19	
PI355509	211	27.86 ± 0.04	0.43
PGR4005	207	27.38 ± 0.03	0.62
PI343447/PGR4005	206	27.33 ± 0.08	
PI343447	207	27.38 ± 0.23	0.85
PI352267	225	13.29 ± 0.10	0.67
PI352267/PI428154	231	13.25 ± 0.00	
PI428154	223	13.26 ± 0.03	0.47

[a] *P* value calculated between F_1 and its each parent
[b] Standard deviation

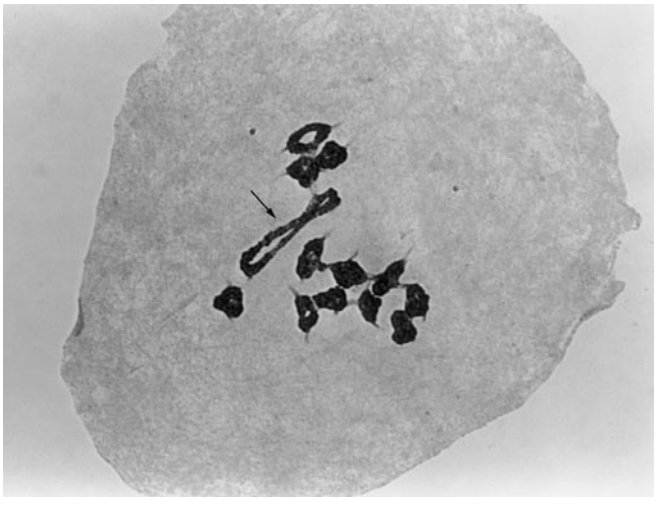

Fig. 4 A ring quadrivalent was found in the hybrid *T. turgidum* ssp. *dicoccum*/PGR3978. An *arrow* indicates the quadrivalent

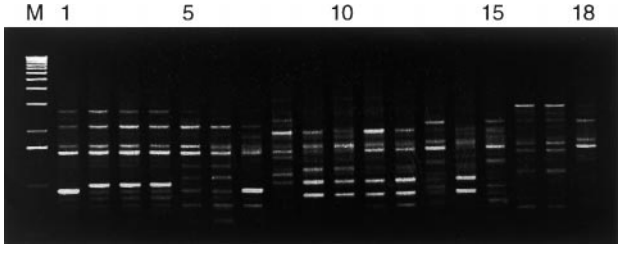

Fig. 5 Polymorphisms revealed in 6 known and 12 misclassified *Triticum* accessions using the primer UBC229. *Lane numbers* refer to the accessions listed in Table 1. *M* 1-kb DNA molecular-weight marker

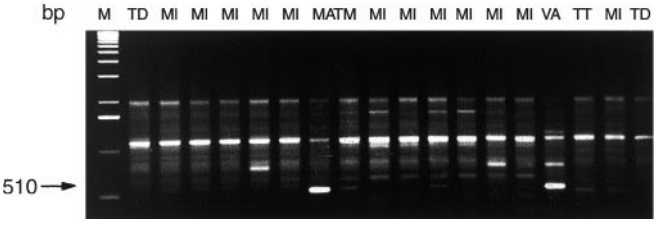

Fig. 6 D-genome specific RAPD marker revealed by the primer RC18. *MI* Misclassified *Triticum* accessions, *MA* macha, *TM Triticum monococcum*, *VA* vavilovii, *TD Triticum turgidum* ssp. *dicoccum*, *TT Triticum timopheevii*. *M* 1-kb DNA molecular-weight marker

Table 3 Species-specific RAPD markers found in *Triticum timopheevii* and *Triticum aestivum*

Species	Marker[a]
T. timopheevii	$UBC225_{510}$, $UBC225_{520}$, $UBC254_{900}$, $UBC295_{280}$
T. aestivum	$UBC250_{410}$, $UBC295_{410}$ $RC18_{510}$

[a] Name of primers before and the size of amplified fragments in subscript

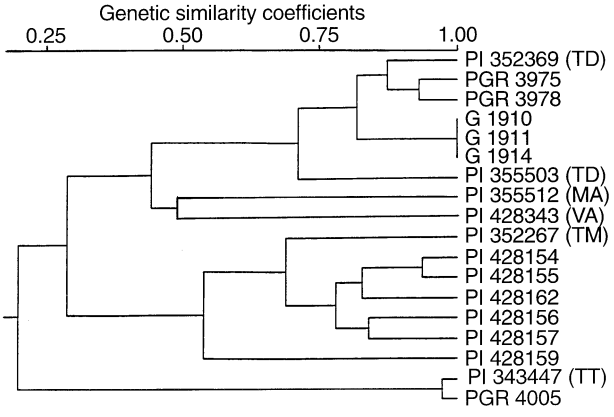

Fig. 7 Dendrogram of 6 *Triticum* accessions and 12 misclassified accessions based on Jaccard coefficients using 101 RAPD polymorphisms. *TD Triticum turgidum* ssp. *dicoccum*, *MA* macha, *TM Triticum monococcum*, *VA* vavilovii, *TT Triticum timopheevii*

A dendrogram (Fig. 7) based on Jaccard coefficients using RAPD polymorphism data indicated that 6 of the misclassified wheats (PI428154, PI428155, PI428156, PI428157, PI428159, PI428162) clustered together with *Triticum monococcum*, 5 (G1910, G1911, G1914, PGR3975, PGR3978) with *T. turgidum* ssp. *dicoccum*, and 1 (PGR4005) with *T. timopheevii*. The RAPD results were thus in agreement with the cytogenetic data and the morphological observations. It can be concluded that among the 12 misclassified accessions received as *Triticum aestivum*, 6 (PI428154, PI428155, PI428156, PI428157, PI428159, PI428162), 5 (G1910, G1911, G1914, PGR3875, PGR3978) and 1 (PGR4005) were *Triticum monococcum*, *T. turgidum* ssp. *dicoccum*, and *T. timopheevii*, respectively.

Discussion

Genome analysis can determine the genomic composition of polyploid species and elucidate phylogenetic relationships among plant taxa. The cultivated diploid (*T. monococcum*), tetraploid (*T. turgidum* ssp. *dicoccum*), and hexaploid wheat species (*T. aestivum*) have the genomes AA, AABB, and AABBDD, respectively (Gaines and Aase 1926). The traditional method to assess genomic affinities among species has been the study of chromosome pairing in their hybrids. The degree of chromosome pairing at meiotic metaphase I in a hybrid is generally a good indicator of the degree of genomic relatedness. In the current study, complete chromosome pairing was observed in the hybrids of *T. monococcum* with PI428154, *T. turgidum* ssp. *dicoccum* with PGR3978, and *T. timopheevii* with PGR4005. Based on these cytogenetic findings, the misclassified accessions PI428154, PGR3978, and PGR4005 could be classified as members of the species *T. monococcum*, *T. turgidum* ssp. *dicoccum*, and *T. timopheevii*, respectively. Genome analyses based on chromosome pairing, however, can be misleading as the amount of chromosome pairing not only depends on the degree of homology between the chromosomes but may also depend on genetic factors (Sears 1976). Therefore, to verify the classification of wheat germplasm, one should combine cytogenetic analysis with isozyme, storage protein, and/or DNA analyses.

Molecular markers have been used to confirm the identity of the ancestral taxon. Restriction fragment length polymorphism (RFLP) (Hosaka et al. 1990; Song et al. 1990) and RAPD (Demeke et al. 1992) analyses revealed the relationships between diploid and amphidiploid *Brassica* taxa and supported the classical U triangle relationship. The current RAPD analysis was able to confirm that the misclassified accessions belonged to species *T. turgidum* ssp. *dicoccum*, *T. monococcum*, and *T. timopheevii* (Fig. 7). The results of the RAPD analysis were consistent with those of the morphological observation and the cytogenetic analysis.

The RAPD technique is being increasingly used for the development of chromosome-, genome-, and species-specific markers. Chromosome-, genome-, and species-specific markers have been developed using alien addition lines of *Brassica* (Quiros et al. 1991). Chromosome-specific RAPD markers have also been identified in tomato and wheat using nulli-tetrasomic lines (Klein-Lankhorst et al. 1991; King et al. 1993; Wang et al. 1995). Wei and Wang (1995) analyzed eight different genomes in the Triticeae using the RAPD technique. Twenty-eight genome-specific markers were found with 1–6 genome-specific markers per genome, leading them to conclude that the RAPD technique can be used to develop genome-, species-, and chromosome-specific markers. In the current RAPD study, species-specific markers were detected for *Triticum aestivum* (AABBDD) and *T. timopheevii* (AAGG) but not for *T. turgidum* ssp. *dicoccum* (AABB) or *T. monococcum* (AA). The A genome is common to all three species, while the B genome is common to *T. aestivum* and *T. turgidum* ssp. *dicoccum*, possibly explaining why no specific markers were found for *Triticum turgidum* ssp. *dicoccum* and *T. monococcum*. Thus, the species-specific markers for *T. aestivum* and *T. timopheevii* may be D-genome and G-genome specific, respectively. If confirmed using more accessions, these RAPD markers could be useful in studies of genome evolution, the analysis of genome composition, and genome identification. For example, the presence or absence of a genome in a polyploid species of Triticeae can be simply tested by RAPD analysis with a selected primer. In traditional genome analysis, the polyploid species is hybridized with an appropriate diploid species, and chromosome pairing is investigated at meiotic metaphase of the hybrids. If seven pairs of chromosomes are found at metaphase I it is presumed that a genome from the polyploid species is the same as that in the diploid species. Thus, at least three hybridizations are needed for the genome analysis of a hexaploid. Therefore, RAPD analysis is easier and faster than chromosome pairing analysis for the determination of genome composition in a polyploid species. The availability of these genome-specific markers for use in genome analysis can eliminate the number of polyploid × diploid hybridizations needed in traditional genome analyses.

In the current study, a ring quadrivalent was found at meiosis in the hybrids of PI352369 (*T. turgidum* ssp. *dicoccum*)/PGR3978 and PI355503 (*T. turgidum* ssp. *dicoccum*)/PGR3978 (Fig. 1). Translocations in durum wheat were investigated by Perera et al. (1983). They reported that 21 of 48 cultivars (43.7%) had a translocation. Preliminary studies have indicated that reciprocal translocations in *T. turgidum* are common. Joppa et al. (1995) investigated chromosome translocations in wild populations of tetraploid emmer wheat from Israel and Turkey. Of the 171 genotypes investigated 70% had translocations. The results also revealed that the B-genome chromosomes were involved in translocations more frequently than the A-genome chromosomes. These translocations might have an important role in wheat evolution and genetic diversity.

Misclassification of accessions is one of the constraints in the utilization of plant germplasm (Porceddu and Srivastava 1990). Pecetti et al. (1992) found that about 15% of durum accessions were misclassified in the ICARDA germplasm collection. In the current study, 13% of 124 wheat germplasm accessions were misclassified. Such a high percentage of misclassified wheat germplasm suggests that wheat germplasm collections may need to be surveyed to identify and reclassify misclassified accessions. In the future, more genome- and species-specific markers should be developed. These markers would be useful for surveying wheat germplasm collections to distinguish species and

reclassify the misclassified germplasm. The dendrogram in Fig. 7 indicated that 3 accessions (G1910, G1911, G1914) were identical based on Jaccard genetic similarity coefficients calculated from the RAPD data using 12 random primers. These 3 accessions were also identical based on morphological characteristics when grown in a greenhouse (data not shown). Thus, these 3 accessions are probably duplications, suggesting that a survey of wheat germplasm collections is also required to eliminate duplicated accessions. Kresovich et al. (1992) examined the use of the RAPD assay as a quick, cost-effective, reliable means of screening germplasm collections. Their results demonstrated that RAPD markers can be of great value in gene bank management for the purposes of identification, measurement of variation, and establishment of genetic similarity at the intraspecific level. Correct classification, effective conservation, and the use of plant genetic resources are essential for agricultural crop improvement. To utilize plant germplasm effectively, we must use modern science and new technologies, both to reclassify and evaluate germplasm and to make material available for use in research and breeding programs.

Acknowledgements The authors are grateful to the financial support provided by Winisky Trust and the Agriculture Research Trust of the University of Saskatchewan, College of Agriculture.

References

Demeke T, Adams RP, Chibbar RN (1992) Potential taxonomic use of random amplified polymorphic DNA (RAPD): a case study in *Brassica*. Theor Appl Genet 84:990–994

Gaines EF, Aase HC (1926) A haploid wheat plant. Am J Bot 13:373–385

Hosaka K, Kianian SF, McGrath JM, Quiros CF (1990) Development and chromosomal localisation of genome-specific DNA markers of *Brassica* and the evolution of amphidiploids and n = 9 diploid species. Genome 33:131–142

Hurka H (1994) Conservation genetics and the role of botanical gardens. In: Loeschcke V, Tomiuk J Jain, SK (eds) Conservation genetics. Birkhauser Verlag, Basel, Switzerland, pp 371–380

Joppa LR, Nevo E, Beiles A (1995) Chromosome translocations in wild populations of tetraploid emmer wheat in Israel and Turkey. Theor Appl Genet 91:713–719

Joshi CP, Nguyen HT (1993) RAPD (random amplified polymorphic DNA) analysis based on intervarietal genetic relationships among hexaploid wheats. Plant Sci 93:95–103

King IP, Pordie KA, Rezanoor HN, Koebner RMD, Miller TE, Reader SM, Nicholson P (1993) Characterization of *Thinopyrum bessarabicum* chromosome segments in wheat using random amplified polymorphic DNAs (RAPDs) and genomic in situ hybridization. Theor Appl Genet 86:895–900

Klein-Lankhorst R, Rietveld P, Machiels B, Verk R, Weide R, Gebhardt C, Koornneet M, Zabel P (1991) RFLP markers linked to the root knot nematode resistance gene *Mi* in tomato. Theor Appl Genet 81:661–667

Kresovich S, Williams JGK, McFerson JR, Routman EJ, Schaal BA (1992) Characterization of genetic identities and relationships of *Brassica oleracea* L. via a random amplified polymorphic DNA assay. Theor Appl Genet 85:190–196

Pecetti L, Damania AB, Jana S (1992) Practical problems in large-scale germplasm evaluation: a case study in durum wheat. FAO/IBPGR Plant Genet Resources Newsl 88/89:5–10

Perera E, Lacadena JR, Sendino AM (1983) Cytogenetic structure of durum wheat cultivars from, or introduced into Spain. Z. Pflanzenzuechtg. 91:36–45

Porceddu E, Srivastava JP (1990) Evaluation, documentation and utilization of durum wheat germplasm at ICARDA and the University of Tuscia, Italy. In: Srivastava JP, Damania ABJ (eds) Wheat genetic resources: meeting diversity needs. John Wiley & Sons, Chichester, UK, pp 3–8

Procunier JD, Xu J, Kasha KJ (1990) A rapid and reliable DNA extraction method for higher plants. Barley Genet Newsl 20:7475

Quiros CF, Hu J, This P, Chevre AM, Delseny M (1991) Development and chromosomal localization of genome-specific markers by polymerase chain reaction in *Brassica*. Theor Appl Genet 82:627–632

Sears ER (1976) The genetic control of chromosome pairing in wheat. Annu Rev Genet 10:31–51

Song K, Osborn TC, Williams RH (1990) *Brassica* taxonomy based on nuclear restriction fragment length polymorphisms (RFLPs). 3. Genome relationships in *Brassica* and related genera and the origin of *B. oleracea* and *B. rapa* (syn. *campestris*). Theor Appl Genet 79:497–506

Toledo JM, Lenne JM, Schultze-Kraft R (1989) Effective utilization of tropical pasture germplasm. In: Utilization of genetic resources: suitable approaches, agronomic evaluation and use. FAO, Rome, Italy, pp 27–57

Wang RR-C, Chen J, Joppa LR (1995) Production and identification of chromosome specific RAPD markers for Langdon durum wheat disomic substitution lines. Crop Sci 35:886–888

Wei JZ, Wang RR-C (1995) Genome- and species-specific markers and genome relationships of diploid perennial species in Triticeae based on RAPD analyses. Genome 38:1230–1236