Pelletier, G., Primard, C., Vedel, F., Chetrit, P., Remy, R., Rousselle, P., and Renard, M. 1983. Intergeneric cytoplasmic hybridization in Cruciferae by protoplast fusion. Mol. Gen. Genet. **191**: 244–250.

Prakash, S., and Hinata, K. 1980. Taxonomy, cytogenetics and origin of crop Brassicas, a review. Opera Bot. **55**: 3–57.

Primard, C., Vedel, F., Mathieu, C., Pelletier, G., and Chevre, A.M. 1988. Interspecific somatic hybridization between *Brassica napus* and *Brassica hirta* (*Sinapis alba* L.). Theor. Appl. Genet. **75**: 546–552.

Renard, M., and Dosba, F. 1980. Etude de l'haploïdie chez le colza (*Brassica napus* L. var. *oleifera* Metzger). Ann. Amelior. Plant. (Paris), **30**(2): 191–209.

Ripley, V., and Arnison, P.G. 1990. Hybridization of *Sinapis alba* L. and *Brassica napus* L. via embryo rescue. Plant Breed. **104**: 26–33.

Ripley, V., Thorpe, M., Iler, S., Mizier, K., and Beversdorf, W.D. 1992. Isozyme analysis as a tool for introgression of *Sinapis alba* germ plasm into *Brassica napus*. Theor. Appl. Genet. **84**: 403–410.

Shields, C.R., Orton, T.J., and Stuber, C.W. 1983. An outline of general resource needs and procedures for the electrophoresis separation of active enzymes from plant tissue. *In* Isozymes in plant genetics and breeding. Part A. *Edited by* S.D. Tanksley and T.J. Orton. Elsevier, Amsterdam, Oxford, and New York. pp. 443–468.

Sjodin, C., and Glimelius, K. 1989. *Brassica naponigra*, a somatic hybrid resistant to *Phoma lingam*. Theor. Appl. Genet. **77**: 651–656.

This, P., Chevre, A.M., Quiros, C.F., and Delseny, M. 1991. RFLP characterization of *Brassica napus – Brassica nigra* addition lines. *In* Proceedings of the International Rapeseed Congress. *Edited by* D.I. McGregor. GCIRC and Canola Council of Canada, July 9–11, 1991, Saskatoon, Sask., Canada. pp. 1050–1054.

Tremousaygue, D., Grellet, F., Delseny, M., Delourme, R., and Renard, M. 1988. The large spacer of a nuclear ribosomal RNA gene from radish organization and use as a probe in rapeseed breeding. Theor. Appl. Genet. **75**: 298–304.

Truco, M.J., and Arus, P. 1989. Inheritance of isozymes in *Brassica campestris*. *In* Proceedings of the 5th Crucifer Genetics Workshop. *Edited by* C.F. Quiros and P.E. McGuire. April 7–9, 1989, University of California, Davis.

U, N., Nagamatsu, T., and Mizushima, U. 1937. A report on meiosis in two hybrids, *Brassica alba* Rabh. × *B. oleracea* L. and *Eruca sativa* Lam. × *B. oleracea* L. Cytologia, Fujii Jubilee vol.: 437–441.

Vallejos, C.E. 1983. Enzyme activity staining. *In* Isozymes in plant genetics and breeding. Part A. *Edited by* S.D. Tanksley and T.J. Orton. Elsevier, Amsterdam, Oxford, and New York. pp. 469–516.

Wendel, J.F., and Stuber, C.W. 1984. Plant isozymes: enzymes studied and buffer systems for their electrophoretic resolution in starch gels. Isozyme Bull. **17**: 4–11.

Direct comparison of levels of genetic variation in tomato detected by a GACA-containing microsatellite probe and by random amplified polymorphic DNA

W. Rus-Kortekaas, M.J.M. Smulders,[1] P. Arens, and B. Vosman

Centre for Plant Breeding and Reproduction Research (CPRO–DLO), P.O. Box 16, 6700 AA Wageningen, The Netherlands

Corresponding Editor: G. Scoles

Received September 7, 1993

Accepted December 13, 1993

Rus-Kortekaas, W., Smulders, M.J.M., Arens, P., and Vosman, B. 1994. Direct comparison of levels of genetic variation in tomato detected by a GACA-containing microsatellite probe and by random amplified polymorphic DNA. Genome, **37**: 375–381.

In this study, a direct comparison was made of the ability of four selected random amplified polymorphic DNA (RAPD) primers and a GACA-containing microsatellite probe to detect genetic variation in *Lycopersicon*. Of the 89 RAPD primers initially tested, 85 showed differences between a representative of *Lycopersicon pennellii* and *L. esculentum*, but only 4 distinguished among three *L. esculentum* cultivars. These four primers were subsequently tested on representatives of six *Lycopersicon* species. In pairwise comparisons of species, all or 14 of the 15 combinations could be distinguished by single primers. When the primers were tested on 15 *L. esculentum* cultivars, 90 of the 105 combinations could be distinguished by the four primers together. Finally, none of 118 tested primers showed reproducible differences among calli or progeny of regenerants from tissue culture, although some of the plants had inherited morphological mutations. The probe pWVA16, which detects GACA-containing microsatellites, could distinguish in *Taq*I-digested DNA the representatives of *Lycopersicon* species as well as all the *L. esculentum* cultivars tested. The probe was unable to detect polymorphisms among calli and the progeny of regenerants from tissue culture. An analysis of the results showed that the four selected RAPD primers were able to detect polymorphic bands among species at a frequency of 80%, and among cultivars at a frequency of 44%. In contrast, the microsatellite probe detected polymorphic bands at a frequency of 100 and 95%, respectively. The GACA-containing probe did not detect any common bands among the representatives of the six species, while band sharing with RAPDs was 48%. These results indicate that the two methods detect two types of DNA that differ in their degree of variability.

Key words: DNA fingerprint, RAPD, simple sequence, somaclonal variation, tissue culture.

Rus-Kortekaas, W., Smulders, M.J.M., Arens, P., et Vosman, B. 1994. Direct comparison of levels of genetic variation in tomato detected by a GACA-containing microsatellite probe and by random amplified polymorphic DNA. Genome, **37** : 375–381.

Dans cette étude, une comparaison directe a été faite de l'aptitude de quatre amorces de RAPD sélectionnées et d'une sonde de microsatellites contenant la combinaison GACA de bases azotées pour détecter la variation génétique chez le *Lycopersicon*. Sur 89 amorces de RAPD testées initialement, 85 ont présenté des différences entre un représentant de *Lycopersicon pennellii* et un de *L. esculentum*, mais seulement quatre ont établi des distinctions entre trois cultivars de *L. esculentum*. Ces quatre amorces ont été testées subséquemment sur des représentants de six espèces de *Lycopersicon*. Par suite de comparaisons des espèces par paires, toutes les combinaisons ou 14 sur 15 ont pu être distinguées par des amorces individuelles. Lorsque les quatre amorces ont été testées ensemble sur 15 cultivars de *L. esculentum*, 90 combinaisons sur 105 ont pu être distinguées. Finalement, sur 118 amorces testées, aucune n'a produit des différences reproductibles chez les cals ou les descendants de régénérats de culture de tissus, bien que certaines des plantes avaient hérité de mutations morphologiques. La sonde pWVA16, qui détecte les microsatellites contenant la combinaison GACA, a pu distinguer dans l'ADN digéré par l'enzyme *Taq*I les représentants des espèces de *Lycopersicon*, ainsi que tous les cultivars de *L. esculentum* testés. La sonde n'a pu détecter les polymorphismes chez les cals et les descendants de régénérats de cultures de tissus. Une analyse des résultats a montré que les quatre amorces de RAPD sélectionnées étaient capables de détecter les bandes polymorphes à une fréquence de 80% entre espèces et de 44% entre cultivars. À l'opposé, la sonde de microsatellites a détecté les bandes polymorphes à des fréquences de 100 et 95%, respectivement. La sonde contenant la combinaison GACA n'a détecté aucune bande en commun chez les représentants des six espèces, alors que la détection par RAPD des bandes en commun a été de 48%. Ces résultats indiquent que les deux méthodes détectent deux types d'ADN qui diffèrent en degré de variabilité.

Mots clés : empreinte digitale de l'ADN, RAPD, séquence simple, variation somaclonale, culture de tissus.

[Traduit par la rédaction]

Introduction

In *Lycopersicon esculentum* the genetic diversity seems to be limited (Miller and Tanksley 1990). This is reflected in the observation that isozyme patterns (Rick and Fobes 1975) and RFLPs reveal little polymorphism (Miller and Tanksley 1990; Van der Beek et al. 1992). As a consequence, it has been very difficult to distinguish tomato cultivars at the genetic or molecular level using these methods (Foolad et al. 1993).

In recent years, two important new methods for the generation of molecular markers have been introduced. Williams et al. (1990) and Welsh and McClelland (1990) independently introduced random amplified polymorphic DNAs (RAPDs) as new genetic markers. Foolad et al. (1993) used this method to distinguish between an accession of *L. pennellii* and three *L. esculentum* genotypes. Williams and St. Clair (1993) also

[1]Author for correspondence.

Printed in Canada / Imprimé au Canada

TABLE 1. *Lycopersicon esculentum* cultivars used in this study

Cultivar	Year of introduction[a]	Country of origin	Breeding company	F1	Growth habit[b]	Fruit size (weight in g)	Fruit shape[c]	Resistances[d]
Moneymaker	−1951	GB	Stonor[e]	—	i	60–90	r	
Mirabell	1986	D	J. Wagner	—	i	10–30	r, yellow	V
Pipo	1967	NL	J.P. Rood/L.P. Duyvesteyn	—	d	60–90	r	— C1
San Marzano Lampadone	?	I	?	—	i	60–90	c	
San Marzano	?	I	?	—	i	60–90	c	—
Marmande	−1965	F	?	—	(i)	>150	m	—
Dombito	1977	NL	J. Bruinsma	F1	i	>150	m	—
Vision	1982	NL	ENZA	F1	i	>150	m	Tm, C2, F2
Roma VF	−1965	U.S.A.	J. Harris	—	d	60–90	p	Tm, C5, V, F2
Carma	1975	F	Caillard	F1	d	90–120	c	V, F1
Trend	1989	NL	DeRuiterZonen	F1	i	>150	m	V, F1, N
Liberto	1988	NL	DeRuiterZonen	F1	i	60–90	r	Tm, C5, V, F2, Fr
Evita	1987	NL	DeRuiterZonen	F1	i	10–30	r	Tm, C5, V, F2, N, Wi
Blizzard	1987	NL	ENZA	F1	i	60–90	r	Tm, N
Calypso	1982	NL	Rijk Zwaan	F1	i	60–90	r	Tm, C5, V, F2, Wi
								Tm, C5, V, F2, Wi

[a]Year of introduction for cultivar registration; "–" indicates 1st year of presence on lists of varieties in NL or F. This is on average 2 years after application, but the introduction may have been earlier.
[b]Growth habit: i, indeterminate; d, determinate.
[c]Fruit shape: r, round; c, cylindrical; m, multilocular; p, pear.
[d]Resistance to tomato mosaic virus (Tm), *Cladosporium fulvum* race A (C1), races A and B (C2), or races A–E (C5), *Verticillium* (V), *Fusarium ox.* f.sp. *lycopersicum* one (F) or two (F2) races, *Meloidogyne sp.* (nematodes) (N), *Fusarium*-crown root rot (Fr), and silvering (Wi).
[e]Various selections are known; IVT selection was used in this study.

used them to distinguish among different *L. esculentum* genotypes. RAPDs have been shown to differentiate among morphologically indistinguishable strains and varieties (Goodwin and Annis 1991).

The second method involves the use of microsatellite DNA. Microsatellites are hypervariable DNA sequences consisting of arrays of a basic repeat unit of 2–8 base pairs (Hamada et al. 1984; Jeffreys et al. 1985), which can be probed with labelled oligonucleotides (Weising et al. 1989). They are found in all eukaryotes investigated (Tautz 1989). The level of polymorphisms detected with a $(GATA)_4$ probe was sufficient to distinguish among 15 tomato cultivars (Vosman et al. 1992).

RAPDs are fast and easily generated by the polymorphic chain reaction (PCR); they require only small amounts of DNA. However, often several primers have to be tested to distinguish among cultivars, both for the level of polymorphism detected and for the level of reproducibility (Hu and Quiros 1991; Tinker et al. 1993; Williams and St. Clair 1993). A microsatellite fingerprint is made by hybridization of a labelled probe to a Southern blot. Although the fingerprints thus obtained may contain numerous well-reproducible polymorphisms in one lane, the amount of work involved makes the detection of microsatellite-containing DNA more time consuming than the amplification of RAPDs.

Very important in the choice of which method to use is the kind of variation that each method will detect. Microsatellite-containing DNA is repetitive DNA that has been found to be highly polymorphic. RAPDs are thought to be generated randomly throughout the genome, i.e., not exclusively in repetitive DNA. This would imply a lower rate of polymorphisms compared with microsatellites, but this has never been shown directly. Several characteristics of RAPDs may be taken to indicate that we do not know what sequences lead to amplified bands and what influences the selection for specific bands. First, RAPD patterns always contain up to 10 or 15 bands, never more; second, addition of a second primer does not lead to more bands (Klein-Lankhorst et al. 1991), and certainly not to a doubling of the number of bands amplified, as would be expected with two unrelated primers; third, the amplification process does not depend on the size of the genome, since, e.g., prokaryotic and eukaryotic DNA both result in comparable banding patterns (Williams et al. 1990), despite a 100-fold or more difference in genome size.

Up until now, RAPDs and microsatellites have never been compared in the same material. In this study, we performed such a direct comparison of the two methods with respect to their power to detect polymorphisms in *Lycopersicon*. For this purpose we used groups of plants at decreasing levels of polymorphism: representatives of six *Lycopersicon* species, representatives of 15 *L. esculentum* cultivars, and tissue-culture-propagated material of one cultivar. At the level of species and cultivars, the frequencies of polymorphism will probably be sufficient to determine whether the two methods detect DNA with the same degree of variability. The results obtained with cultivars will also be useful for cultivar identification. The data on the material in tissue culture, and on progeny of plants regenerated from tissue culture, will make a contribution towards the question as to whether genetic differences in tissue culture propataged material (somaclonal variation) can be detected at the DNA level (Karp 1991). Vosman et al. (1992) were not able to detect somaclonal

variation in tomato plants regenerated from tissue culture by oligonucleotide fingerprinting using $(GATA)_4$, but recently it was reported that RAPD primers detected polymorphism among wheat plants regenerated from tissue culture (Brown et al. 1993).

Materials and methods

Plant material

All accessions of *Lycopersicon* species used, i.e., *L. parviflorum* LA 735, *L. hirsutum* G1.1561, *L. pennellii* LA 716, *L. pimpinellifolium* G1.1534, *L. peruvianum* LA 2172, and all *L. esculentum* cultivars used were obtained from the tomato collection of the Centre for Genetic Resources (CGN, part of CPRO–DLO, Wageningen, The Netherlands). The *L. esculentum* cultivars chosen represent a wide spectrum of tomato types (see Table 1).

To obtain callus, seeds from *L. esculentum* cv. Moneymaker were grown aseptically on MS medium (Duchefa) supplemented with 2% sucrose. After 2 weeks, explants were cut from leaf, cotyledon, and hypocotyl and cultured on MS medium supplemented with 3% sucrose, 11 μM 1-naphthaleneacetic acid, 4 μM benzylaminopurine, and 0.8% agar (BBL). Callus was excised from cotyledon and hypocotyl explants after 6 weeks of culture and from leaf explants after 9 weeks. The callus material was subcultured at 3 week intervals for a period of 9 weeks.

R2 plants were grown from seeds obtained by selfing R1 regenerants. These R1 regenerants had been obtained by shoot induction on explants from leaves, cotyledon, and hypocotyl (Van den Bulk et al. 1990).

Both calli and leaves from the R2 plants were stored at −80°C until DNA extraction.

DNA extraction

DNA was extracted from calli according to Dellaporte et al. (1983). The procedure was modified by adding 1 M NaCl to precipitate polysaccharides before the first DNA precipitation. DNA of the R2 plants and of the cultivars was extracted from leaves as described by Vosman et al. (1992). DNA from leaves of species was provided by P. Lindhout (CPRO–DLO).

Primers

Ten-base oligonucleotide primers were purchased from Operon Technologies, Alameda, California. The series U through Z were used. Additional primers were synthesized using an Applied Biosystems DNA synthesizer. These primers included the eleven 10-base primers used by Klein-Lankhorst et al. (1991) and ten longer (15–25 bp) PCR primers, including pUC primers, randomly chosen from the freezer. Although the 10-mer primers from Klein-Lankhorst et al. (1991) contain 90% sequence homology, the banding patterns generated by them were mostly independent from each other (see also Williams et al. 1990). Under the PCR conditions used, the longer primers could also be assumed to generate bands based upon homology with only a subset of the bases (see Welsh and McClelland 1990, who used a 20-mer and a 34-mer primer in their AP–PCR reactions).

DNA amplification

Amplification reactions contained 10 mM Tris–HCl, pH 9.0 (25°C), 50 mM KCl, 1.5 mM $MgCl_2$, 0.1% (w/v) gelatine, 0.1% Triton X-100, 20 μM of each dNTP, 20 ng (0.2–0.3 μM) primer, 10 ng genomic DNA, and 0.3 U polymerase (Super taq, HT biotechnology Ltd., England) in 25 μL. DNA amplifications were performed in a thermal cycler from Hybaid or in a Perkin-Elmer Cetus DNA Thermal Cycler 480.

The test screening of Operon primers against three cultivars and one accession of *L. pennellii* (see Results) and the amplifications with the 10-mer primers from Klein-Lankhorst et al. (1991) were done in the Perkin-Elmer using program "A": 3 min at 97°C, 50 cycles of 1 min at 92°C, 1 min at 35°C, and 2 min 72°C using the fastest available temperature transition, followed by an additional 3 min at 72°C. For the longer primers, program "B" in the Perkin-Elmer was used: 3 min at 97°C, two cycles of 1 min at 92°C, 1 min at 35°C, and 2 min 72°C, followed by 35 cycles of 1 min at 92°C, 1 min at 55°C, and 2 min 72°C also using the fastest available temperature transition, followed by an additional 3 min at 72°C. The amplifications with the four selected 10-mer primers in the Hybaid were done with program "C": 3 min at 97°C, then 50 cycles of 45 s at 92°C, 1.3 min 35°C, and 1 min 72°C, followed by an additional 3 min at 72°C. Amplification products were separated by electrophoresis in 1.5% agarose gels (Sambrook et al. 1989), stained with ethidium bromide, and viewed under UV light.

All PCR experiments were conducted at least twice to ensure that the banding patterns were reproducible. Nonreproducible bands were not used in comparisons.

Microsatellites

For microsatellite fingerprinting, DNA was digested with the restriction endonuclease *Taq*I according to the manufacturer (Life Technology), separated on a 1% agarose gel, and alkaline blotted overnight onto Hybond N^+ (Amersham). The GACA-containing microsatellites were probed with plasmid pWVA16 (P. Arens, P. Odinot, A.W. Van Meusden, P. Lindhout and B. Vosman; submitted). From this plasmid a 340-bp fragment, containing a 56- and a 16-bp GACA repeat, was labelled as recommended by the supplier of the DNA labelling kit (USB biochemicals). The DNA fingerprint obtained with pWVA16 is identical to the finterprint obtained with a $(GACA)_4$ probe, but the patterns are more clear (results not shown).

Hybridizations were performed overnight at 65°C in a hybridization buffer consisting of 0.263 M Na_2HPO_4 (pH 7.2), 1 mM EDTA, and 7% SDS. Blots were washed at 65°C for 15 min in 2× SSC, 0.1% SDS.

Results

Variation detected with RAPD fingerprints

Species

The level of variation detected with RAPD probes was maximized in this study by preselecting primers that would detect the highest levels of polymorphism. For this purpose, eighty-nine 10-mer primers were screened against a small test series, consisting of three cultivars of *L. esculentum* (Moneymaker, Mirabell and Roma VF) and one accession of *L. pennellii* using PCR program A. Of these primers, 85 showed RAPDs between *L. pennellii* and *L. esculentum*, but only four primers generated RAPDs among tomato cultivars. Only strong bands were considered and bands were only considered polymorphic when they were absent in some of the samples. Changes in band intensity were not considered as polymorphisms.

The four primers (Y7, 90.58, W13, and U1) were subsequently tested on six species and 15 cultivars. All four primers easily distinguished among the representatives of the *Lycopersicon* species (Table 2). This was independent of the *L. esculentum* cultivar used in the comparison, with the exception of primer W13, who did not detect differences between three combinations of a species and some cultivars. Two additional primers, W9 and U7, that had shown polymorphisms between the *L. pennellii* accession and *L. esculentum* but not among the three cultivars of *L. esculentum*, were also used in the comparison of six species. They detected considerably less variation: W9 showed no polymorphism among the accessions of *L. hirsutum* and *L. pimpinellifolium* and *L. esculentum* cultivars, and U7 gave no polymorphism between the accessions of *L. peruvianum* and *L. pimpinellifolium* (not shown).

When the introgression-free cultivar Moneymaker was taken as representative of *L. esculentum*, U1, Y7 and 90.58

TABLE 2. RAPD patterns generated for accessions of *Lycopersicon* species and cultivars of *L. esculentum*

	Species					Cultivar													
	20	19	18	17	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1
U1	J	I	H	G	F	C	C	E	B	B	D	A	B	B	A	A	A	A	C	A
W13	B	C	F	E	A	A	A	C	A	B	D	B	B	A	B	B	B	B	B	A
Y7	J	I	H	G	F	B	B	E	B	D	C	A	B	A	A	A	A	A	A	A
90.58	H	G	F	E	D	A	B	C	A	A	B	B	B	A	A	A	A	A	C	A
Total[a]						Z	Y	X	W	V	U	T	S	R	O	O	O	O	Q	P

NOTE: 1, Moneymaker; 2, Mirabell; 3, Pipo; 4, San Marzano Lampadone; 5, San Marzano; 6, Marmande; 7, Dombito; 8, Vision; 9, Roma VF; 10, Carma; 11, Trend; 12, Liberto; 13, Evita; 14, Blizzard; 15, Calypso; 16, *L. hirsutum*; 17, *L. pennellii*; 18, *L. parviflorum*; 19, *L. pimpinellifolium*; 20, *L. peruvianum*. Primers: U1, ACGGACGTCA; W13, CACAGCGACA; Y7, AGAGCCGTCA; 90.58, TGGACACTGA (Klein-Lankhorst et al. 1991). Amplification using program C. In each row, a different letter represents a different fingerprint.

[a]Identification using the four banding patterns together.

TABLE 3. Analysis of RAPD- and microsatellite-generated banding patterns

	No. of bands				Average band sharing percentage[a]
		Polymorphic			
	Total	Unique	Others	Monomorphic	
Species					
GACA microsatellite	66	39	27	0	13.1
4 RAPD primers[b]	49	15	24	10	47.8
Cultivars					
GACA microsatellite	55	15	37	3	50.8
4 RAPD primers[b]	27	3	9	15	82.7

[a]Average percentage of identical bands in all pairwise comparisons of two species or cultivars.

[b]Data from the experiments presented in Table 2.

could each distinguish all 15 pairwise combinations of accessions of two species; W13 could distinguish 14 of the 15 combinations. The four primers together generated 49 bands of which 39 were polymorphic between the accessions of different species (Table 3). Since most polymorphic bands were present in more than one species, the average band-sharing percentage between pairs of accessions was high (48%).

Cultivars

The cultivars used in this experiment represented a wide spectrum of different tomato types (from cherry to beef tomato), including vintage and modern cultivars, mostly from European origin (Table 1). We also wanted to include some closely related cultivars. Unfortunately, little information is published about pedigrees for cultivars, so the selection was on year and country of origin, breeding company, type of tomato formed, and resistances.

When the four selected primers were tested on 15 cultivars, three gave unique banding patterns with some cultivars (Table 2). Unique banding patterns consisted mostly of unique combinations of bands. Most bands were present in more than one cultivar (Table 3). Primer 90.58 did not show unique patterns but separated the 15 cultivars into three groups (Table 2). The primers U7 and W9, which did not give a polymorphism in the first screening with three cultivars, gave no reproducible polymorphism with any of the 15 cultivars (not shown). Primer Y7 failed to reproduce the polymorphism among the three cultivars obtained in the first screening. This may be caused by the different PCR apparatus used (Meunier and Grimont 1993) in the prescreening.

When the banding patterns of the four primers were taken together, 11 of the 15 cultivars could be identified by a "unique" combination of polymorphic bands (Table 2, bottom row), which corresponds to a distinction between the cultivars in 95 of the 105 possible pairwise combinations. Four cultivars had the same combination of patterns, and, hence, could not be distinguished. These cultivars originated in the 1960s or before, but only two of them, San Marzano and San Marzano Lampadone, are known to be closely related. On the other hand, three modern cultivars from the same breeding company, albeit different tomato types and containing some different resistances, could be easily distinguished.

In total, 27 bands were observed, almost half of which were polymorphic (Table 3). Since most bands occurred in more than one cultivar, the average band-sharing percentage between pairs of cultivars was as high as 83%.

Regenerants

Somaclonal variation involves heritable changes (Karp 1991), therefore changes of the RAPD patterns might be expected. Most changes will be present in a heterozygous form, so that only 25% of the progeny will have a homozygous change. The changes expected to be detected by RAPDs will be, at least for 50%, disappearances of bands. Such changes can not be seen in heterozygous form, but only in the homozygous progeny of regenerants. This implies that for each direct (R1) regenerant at least four progeny (R2) plants would have to be measured. An alternative is to measure one R2 plant each of a larger number of regenerants. This would generate the same amount of homozygous changes involving losses of bands, but with a higher chance of detecting mutations involving the gain of a band, and mutations that were present in homozygous form from the beginning. Since

the banding patterns generated by different RAPD primers are independent of each other, this experimental setup is equivalent to measuring a smaller number of R2 plants with a large number of primers. Since this approach is considerably less laborious, this was the method chosen here.

It was also chosen to use DNA from calli. If mutations occur at a high frequency, the calli could consist of mixtures of genetically different cell lines. Although the chances of detecting a mutation involving the loss of a band in one of these cell lines would be close to zero, the chances of detecting mutations involving the gain of a band would be increased.

In total, DNA from 12 different calli and 12 different R2 plants was used. All material was derived from different plants of the cultivar Moneymaker, and evenly distributed over the explant sources: leaf, cotyledon, and hypocotyl. The R2 plants included some plants that had inherited morphological mutations present in the direct regenerants, e.g., an anthocyanin mutation (Van den Bulk et al. 1990). The Operon primers and the 10 other 10-mer primers were tested using PCR program A, the 10 longer primers were tested using PCR program B, respectively. In total, 32 primers were tested on the 12 different R2 plants, 49 other primers were tested on the 12 different calli, and yet another 37 primers were tested on all 24 different samples. The primers generated a total of 309 different strong bands (on average 2.6 per primer), but none of the bands was reproducibly polymorphic.

Variation detected by microsatellite fingerprinting

To test the variation detected by microsatellite fingerprinting at the species level, the GACA-containing microsatellite probe pWVA16 was used to distinguish six *Lycopersicon* species. The probe generated a large number of bands with *Taq*I-digested DNA and was able to distinguish the accessions of all species. All bands were polymorphic and most were unique (Table 3), so that the average band-sharing between pairs of accessions was a mere 13%. In other words, with this probe–enzyme combination band sharing was 3.5 times lower than with the four selected RAPD primers.

The same probe–enzyme combination was also used to distinguish among the 15 *L. esculentum* cultivars (Table 3). The lower amount of variation among cultivars is reflected in the presence of a small number of monomorphic bands, and a lower number of unique bands. Nevertheless, all cultivars could be identified by a unique pattern. Most bands were polymorphic, so that the percentage of band sharing was 51%. This value was still well below that obtained with RAPDs.

At the level of calli and regenerants from tissue culture, we tested 28 calli and 10 R2 plants with this probe–enzyme combination but found no polymorphism among the bands tested.

Discussion

Two methods for the detection of genetic variation, RAPD and microsatellite fingerprinting, were compared directly on their capability to generate polymorphic bands in *Lycopersicon*. The results showed that both RAPDs (Table 2 and 3) and microsatellite fingerprinting of GACA-containing DNA (Table 3) detected differences among the accessions of the six *Lycopersicon* species tested. At the cultivar level, a selection of 4 of 89 RAPD primers was able to distinguish 95 of 105 cultivar combinations, while the microsatellite probe could distinguish all cultivars. Somaclonal variation could not be detected by RAPDs nor by the GACA-containing probe.

Hu and Quiros (1991) analyzed the potential of using RAPD markers for cultivar identification in *Brassica* crops. They found that as few as four primers could distinguish among 14 broccoli and 12 cauliflower cultivars. Tinker et al. (1993) were able to distinguish among 27 inbred lines of barley with seven primers. In comparison, we could distinguish 11 of 15 tomato cultivars with four primers. This again shows the importance of the genetic diversity within cultured crops, which is very small in tomato (Miller and Tanksley 1990; Van der Beek et al. 1992).

Tomato cultivars are practically homozygous and hardly any difference between individual plants can be found using RFLPs (Miller and Tanksley 1990). We found no difference at all in RAPD banding pattern among 24 calli and regenerants derived from separate tomato plants of the cultivar Moneymaker, indicating that no polymorphisms existed among cv. Moneymaker plants. Therefore, the results obtained with representatives of the cultivars indicate that RAPDs can identify plants from at least a number of different tomato cultivars. The results obtained with one accession from different *Lycopersicon* species do not allow such a conclusion because there is a considerable amount of within-species variation (Miller and Tanksley 1990). However, the amount of polymorphisms between the accessions that was visualized using RAPDs does indicate that identification with RAPD primers is feasible, for instance with a larger number of primers (see also Williams and St. Clair 1993).

Williams and St. Clair (1993) tested 19 vintage and modern tomato cultivars, mostly from North American origin. Only one tomato cultivar (San Marzano) was common between the two studies. Their data showed that, with the use of 11 selected RAPD primers, they could distinguish 160 of 171 possible pairwise combinations of two cultivars. This was 94% of the combinations, a result that corresponds well with the 91% obtained in this study. A difference is that they used 11 primers for this result, while we only used 4. This may be partly due to a different choice of cultivars, e.g., more "vintage" cultivars, which are more difficult to distinguish (Williams and St. Clair 1993). Also the way the primers were preselected can have an effect. Williams and St. Clair (1993) made a screening that would maximize "robustness," including the selection criterion of producing at least one polymorphism between the vintage cultivar Ace and a *L. cheesmanii* accession. In the present study, the goal was to maximize the performance of individual primers, in other words, to select for the fewest possible primers that would distinguish as many cultivars as possible. The selection criterion was the production of polymorphisms among three cultivars. This difference may well have been the cause of the difference in the percentage polymorphic bands: Williams and St. Clair (1993) found 37% polymorphic bands for all cultivars, regional varieties, and wild accessions together, but only 3% among vintage cultivars and 12% among modern cultivars; we found 44% (Table 3) among vintage and modern cultivars. This indicates that the way RAPD primers are selected may be an important factor in determining the overall performance of the method in finding polymorphisms.

The progeny of regenerants from tissue culture that was tested here contained morphological aberrations. However, no differences could be detected with RAPDs nor microsatellites in R2 plants and calli. Vallés et al. (1993) could also

not detect polymorphism in plants of meadow fescue (*Festuca pratensis*) regenerated from a protoplast or cell suspension culture, neither with RAPDs nor with RFLPs. Similarly, no polymorphisms were found using RAPD primers among somatic embryos from *Picea mariana* (Isabel et al. 1993). These results are in contrast with those of Brown et al. (1993), who found differences with RAPDs in phenotypically normal wheat regenerants. The tissue culture period used for wheat was longer than ours, but not longer than the tissue culture period in the other studies. Also, direct (R1) regenerants of wheat were used. At least 50% of the observed differences are expected to be disappearances of bands, and these would not be visible in direct regenerants, unless they were present in homozygous form. A possible explanation for the differences observed may be that wheat DNA is more variable in tissue culture. Another possibility is that somaclonal variation occurs not randomly in the genome, but in "hotspots," so that it is a matter of chance whether or not differences are detected with a RAPD primer or a microsatellite probe.

Microsatellites are tandemly repeated DNA sequences that are mostly polymorphic (Tautz 1989). Our results showed that, with the probe–enzyme combination used, the percentage band sharing detected in accessions of *Lycopersicon* species by microsatellite fingerprinting was only one-quarter of that of the RAPD patterns (Table 3). A similar pattern was visible at the level of *L. esculentum* cultivars: although the absolute level of polymorphisms detected was lower for both methods, band sharing in microsatellite-generated patterns was still only a little more than half that in RAPD patterns. For this comparison, the values for the RAPDs were maximized by preselection for primers that could distinguish among species as well as cultivars. If primers that generated differences among species but not among cultivars would have been included, then the percentage band sharing would be even higher. At the same time, the performance of microsatellite DNA probes was not optimized, since e.g., a GATA-detecting probe appeared to generate even more polymorphisms between cultivars (Vosman et al. 1992). Therefore, these experiments clearly indicate that RAPDs and microsatellites detect two types of DNA that differ in their degree of variability. To explain the high mutation rate of microsatellite DNA, slipping of the DNA polymerase has been suggested (Tautz et al. 1986). This may be an exclusive phenomenon for short tandemly repeated sequences (Schlötterer and Tautz 1992).

RAPD DNA thus appears to be more conserved than the DNA detected with the microsatellite probe. Therefore, the choice of whether to use RAPDs or microsatellites to distinguish among cultivars or species should depend on the amount of genetic variation expected and the question to be answered. The higher percentage of band sharing in RAPDs makes them more suitable for pedigree relationship studies of tomato species and accessions. Microsatellite DNA is more appropriate for the identification of tomato cultivars because in this way more variation can be detected. Since both methods also have their drawbacks (RAPDs because of the necessity to test the reproducibility rigorously, microsatellite fingerprinting because of the time and amount of work involved) the best technical solution might very well be the tagging of microsatellites bands with specific PCR primers, known as STS (Olsen et al. 1989) or STMS (Beckmann and Soller 1990). RAPD bands may also be sequenced and tagged, coined as SCARs (Paran and Michelmore 1993). This would lead to a combination of speed and high levels of reproducible polymorphisms. However, the technique that produces the patterns does not change the nature of the types of DNA detected. It can therefore be expected, based on the results from this study (summarized in Table 3), that primers derived from RAPD bands will mostly amplify bands that are shared by several *Lycopersicon* species, while primers derived from microsatellite bands will mostly amplify bands that are unique for a given species.

Acknowledgements

The authors are grateful to R.M. Klein-Lankhorst and P. Lindhout for giving the primers and for critical reading of the manuscript and to N.P.A. van Marrewijk for providing data concerning the cultivars. We also thank R. van den Bulk for the gift of R2 seeds.

Beckmann, J.S., and Soller, M. 1990. Toward a unified approach to genetic mapping of eukaryotes based on sequence tagged microsatellites sites. Bio/Technology, **8**: 930–932.

Brown, P.T.H., Lange, F.D., Kranz, E., and Lörz, H. 1993. Analysis of single protoplasts and regenerated plants by PCR and RAPD technology. Mol. Gen. Genet. **237**: 311–317.

Dellaporte, S.L., Wood, J., and Hichs, J.B. 1983. A plant molecular DNA minipreparation. Version II. Plant Mol. Biol. Rep. **1**: 19–21.

Foolad, M.R., Jones, R.A., and Rodriguez, R.L. 1993. RAPD markers for constructing intraspecific tomato genetic maps. Plant Cell Rep. **12**: 293–297.

Goodwin, P.H., and Annis, S.L. 1991. Rapid identification of genetic variation and pathotype of *Leptosphaeria maculans* by random amplified polymorphic DNA assay. Appl. Environ. Microbiol. **57**: 2482–2486.

Hamada, H., Petrino, M.G., Kakunaga, T., Seidman, M., and Stollar, B.D. 1984. Characterization of genomic poly(dT-dG) poly(dC-dA) sequences: structure, organization and conformation. Mol. Cell Biol. **4**: 2610–2621.

Hu, J., and Quiros, C.F. 1991. Identification of broccoli and cauliflower cultivars with RAPD markers. Plant Cell Rep. **10**: 505–511.

Isabel, N., Tremblay, L., Michaud, M., Tremblay, F.M., and Bousquet, J. 1993. RAPDs as an aid to evaluate the genetic integrity of somatic embryogenesis-derived populations of *Picea mariana* (Mill.) B.S.P. Theor. Appl. Genet. **86**: 81–87.

Jeffreys, A.J., Wilson, V., and Thein, S.L. 1985. Hypervariable "minisatellite" regions in human DNA. Nature (London), **314**: 67–73.

Karp, A. 1991. On the current understanding of somaclonal variation. Oxford Surv. Plant Mol. Cell Biol. **7**: 1–58.

Klein-Lankhorst, R.M., Vermunt, A., Weide, R., and Zabel, P. 1991. Isolation of molecular markers for tomato (*L. esculentum*) using random amplified polymorphic DNA (RAPD). Theor. Appl. Genet. **83**: 108–114.

Meunier, J.-R., and Grimont, P.A.D. 1993. Factors affecting reproducibility of random amplified polymorphic DNA fingerprinting. Res. Microbiol. **144**: 373–379.

Miller, J.C., and Tanksley, S.D. 1990. RFLP analysis of phylogenetic relationships and genetic variation in the genus *Lycopersicon*. Theor. Appl. Genet. **80**: 437–448.

Olson, M., Hood, L., Cantor, C., and Botstein, D. 1989. A common language for physical mapping of the human genome. Science (Washington, D.C.), **245**: 1434–1435.

Paran, I., and Michelmore, R.W. 1993. Development of reliable PCR-based markers linked to downy mildew resistance genes in lettuce. Theor. Appl. Genet. **85**: 985–993.

Rick, C.M., and Fobes, J.F. 1975. Allozyme variation in the cul-

tivated tomato and closely related species. Bull. Torrey Bot. Club, **102**: 376–384.

Sambrook, J., Fritsch, E.F., and Maniatis, T. 1989. Molecular cloning: a laboratory manual. 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

Schlötterer, C., and Tautz, D. 1992. Slippage synthesis of simple sequence DNA. Nucleic Acids Res. **20**: 211–215.

Tautz, D. 1989. Hypervariability of simple sequences as a general source for polymorphic DNA markers. Nucleic Acids Res. **17**: 4127–4138.

Tautz, D., Trick, M., and Dover, G.A. 1986. Cryptic simplicity in DNA is a major source of genetic variation. Nature (London), **322**: 652–656.

Tinker, N.A., Fortin, M.G., and Mather, D.E. 1993. Random amplified polymorphic DNA and pedigree relationships in a spring barley. Theor. Appl. Genet. **85**: 976–984.

Vallés, M.P., Wang, Z.Y., Montavon, P., Potrykus, I., and Spangenberg, G. 1993. Analysis of genetic stability of plants regenerated from suspension cultures and protoplasts of meadow fescue (*Festuca pratensis* Huds.). Plant Cell Rep. **12**: 101–106.

Van den Bulk, R.W., Löffler, H.J.M., Lindhout, W.H., and Koornneef, M. 1990. Somaclonal variation in tomato: effect of explant source and comparison with chemical mutagenesis. Theor. Appl. Genet. **80**: 817–825.

Van der Beek, H., Verkerk, R., Zabel, P., and Lindhout, P. 1992. Mapping strategy for resistance genes in tomato based on RFLPs between cultivars: CF9 (resistance to *Cladosporium fulvum*) on chromosome 1. Theor. Appl. Genet. **84**: 106–112.

Vosman, B., Arens, P., Rus-Kortekaas, W., and Smulders, M.J.M. 1992. Identification of highly polymorphic DNA regions in tomato. Theor. Appl. Genet. **85**: 239–244.

Weising, K., Weigand, F., Driesel, A.J., Kahl, G., Zischler, H., and Epplen, J.T. 1989. Polymorphic simple GATA/GACA repeats in plant genomes. Nucleic Acids Res. **17**: 10128.

Welsh, J., and McClelland, M. 1990. Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Res. **18**: 7213–7218.

Williams, C.E., and St. Clair, D. 1993. Phenetic relationships and levels of variability detected by restriction fragment length polymorphism and random amplified polymorphic DNA analysis of cultivated and wild accessions of *Lycopersicon esculentum*. Genome, **36**: 619–630.

Williams, J.G.K., Kubelik, A.R., Livak, K.J., Rafalski, J.A., and Tingey, S.V. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res. **18**: 6531–6535.