Evaluation of amplified fragment length polymorphism and simple sequence repeats for tomato germplasm fingerprinting: utility for grouping closely related traditional cultivars

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Abstract: Cultivated tomato (*Solanum lycopersicum* L.) germplasm shows limited genetic variation. Many DNA marker systems have been used for genetic diversity studies in wild and cultivated tomatoes, but their usefulness for characterizing phenotypic differences among very closely related cultivars remains uncertain. We have used 19 selected simple sequence repeat (SSR) markers and 7 amplified fragment length polymorphism (AFLP) primer combinations to characterize 48 cultivars of tomato, mainly traditional cultivars from the south-east of Spain. The main types were *Solanum lycopersicum* L. 'Muchamiel', 'De la pera', and 'Moruno'. The robustness of the dendrograms and the discrimination power reached with each marker type were similar. Unique fingerprinting even of the most closely related tomato cultivars could be obtained using a combination of some SSR and AFLP markers. A better grouping of the 'Muchamiel' cultivars was observed with SSR markers, whereas the grouping of cultivars of 'De la pera' type was best achieved with AFLPs. However, both types of markers adequately grouped cultivars of the main types, confirming the utility of SSR and AFLP markers for the identification of traditional cultivars of tomato.

Key words: genetic variability, molecular markers, *Solanum lycopersicum*.

Résumé : Chez la tomate cultivée (*Lycopersicum esculentum* L.), les ressources génétiques montrent une variation génétique limitée. Plusieurs types de marqueurs moléculaires ont été employés dans le cadre d'études de diversité génétique chez les tomates cultivées et sauvages, mais leur utilité pour déceler des différences phénotypiques parmi des cultivars très apparentés demeure incertaine. Les auteurs ont choisi 19 microsatellites et 7 combinaisons d'amorces AFLP pour caractériser 48 cultivars de tomate, principalement des cultivars traditionnels du sud-est de l'Espagne. Les principaux types étaient 'Muchamiel', 'De la pera' et 'Moruno'. La robustesse des dendrogrammes et la puissance de discrimination obtenue avec les deux types de marqueur étaient semblables. Des empreintes uniques, même parmi les cultivars les plus proches, ont été obtenues avec une combinaison de marqueurs microsatellites et AFLP. Un meilleur groupement des cultivars 'Muchamiel' a été observé avec les microsatellites, tandis que le groupement des cultivars du type De la pera était supérieur avec les AFLP. Néanmoins, les deux types de marqueurs ont permis de grouper adéquatement les cultivars au sein des principaux types, ce qui confirme l'utilité des microsatellites et des AFLP pour l'identification de cultivars traditionnels de la tomate.

Mots clés : variabilité génétique, marqueurs moléculaires, *Solanum lycopersicum*.

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Introduction

Cultivar identification is typically based on morphological traits. Although this method is informative and practical, morphological traits are subject to environmental influences and intense selective pressure during domestication and breeding. DNA fingerprinting is an ideal tool for assessing genetic diversity and aiding cultivar identification, because it measures differences between genotypes at the DNA level without their being obscured by complex pedigree records, environmental conditions, or epistatic and pleiotropic effects (Seefelder et al. 2000, Carelli et al. 2006; Park et al. 2004).

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Table 1. Types of cultivar and accession used in the study.

Type	Accession
'Muchamiel'	Much4, Much18, Much29, Much30, Much128, Much198, Much BN4, Much BN5
'De la pera'	Pera1, Pera7, Pera16, Pera19, Pera21, Pera22, Pera25, Pera43, Pera44
'Moruno'	Mor207, Mor208, Mor209, Mor231, Mor234
Other traditional or local cultivars and breeding lines	Valenciano, Flor de Baladre, Cadiz13, Cadiz16, Cadiz17, Salinidad, Teticabra, Malpica, Zapotec, EPSO42
Hybrids F1	Anastasia, Bond, Royesta, Delicia (Raf), Vision
Cherry	Evita F1, Cherry89
S. lycopersicum var. *cerasiforme*	LA2617 (Cherry96)
S. pimpinellifolium	LA2853 (Pimpi 1) and LA1610 (Pimpi 2)
Controls	Ben, Bosh, VNT Cherry, Isola, Aranka, Nunhem63280

Different DNA sequence polymorphism-based markers have been developed in recent years. Microsatellites, also known as SSRs (simple sequence repeats), have been the elected markers to carry out different studies in tomato in the last few years. The works of Smulders et al. (1997), Alvarez et al. (2001), Bredemeijer et al. (2002), and He et al. (2003) confirm the utility of SSRs for the study of genetic diversity and variability in the genus *Solanum* and for tomato cultivar identification. The European Union funded a project to characterize a collection of European tomato varieties using 20 SSRs. Unique SSR profiles were observed for 468 out of 508 European tomato cultivars (Bredemeijer et al. 2002).

AFLP (amplified fragment length polymorphism) is another highly reproducible marker technique that can be used to efficiently detect DNA polymorphisms (Vos et al. 1995). Park et al. (2004) used this technique to evaluate genetic variation among tomato cultivars, primarily from California. Several other molecular markers, like isozymes (Rick and Forbes 1974), seed proteins (Wang et al. 2000), RFLP (Miller and Tanksley 1990), and RAPD (Williams and St. Clair 1993; Paran et al. 1995; Carelli et al. 2006), have been used to address variety identification and relations in wild and cultivated tomatoes.

Other studies have performed comparisons between several marker systems. For example, recently Sponner et al. (2005) compared AFLP with molecular and morphological data for phylogenetic inference in wild tomatoes. Williams and St. Clair (1993) compared RFLPs with RAPDs, and Ruiz et al. (2005*b*) compared sequence-related amplified polymorphisms with SSRs. Tam et al. (2005) have recently compared AFLP with retrotransposon-based sequence-specific amplification polymorphisms and SSR, although they used "proprietary" SSR primers without reporting their sequences. To our knowledge, no other study comparing AFLPs with SSRs to assess genetic diversity among tomato cultivars has been published.

Genotypic differences detected by molecular markers can also be used for cultivar identification and protection of the plant breeder's intellectual property rights (plant breeders' rights, PBRs). In most countries with PBRs, new varieties have to be shown to be "**d**istinct" from all other varieties and also sufficiently "**u**niform" and "**s**table" with respect to the characteristics used to demonstrate distinctness (as assessed by the DUS test). However, molecular markers are currently not accepted for DUS testing by the International Union for the Protection of New Varieties of Plants or by the registration authorities in most countries (Cooke et al. 2003). Data reported in all the studies dealing with the application of DNA markers in tomato cultivar identification are useful and provide important background information to address the issue of PBRs.

We are currently working in a program aimed at the conservation of local tomato cultivars from southeastern Spain. Spain and Italy were the first European countries in which tomato gained agricultural importance. After the process of diversification and adaptation to different agroclimatic conditions, a great array of traditional tomato cultivars originated in many Spanish regions. In local markets, traditional cultivars sell for 3 to 6 times the price of the hybrid varieties, as is the case for 2 types of local cultivars, *Solanum lycopersicum* L. 'Muchamiel' and 'De la pera'. Although cultivated tomato has a narrow genetic base, we have found a considerable within-type diversity of cultivars, differing in yield and fruit characteristics such as shape, firmness, soluble solid contents, etc. In addition, we have found considerable levels of diversity among different forms of 'Muchamiel' and 'De la pera' for micronutrient content (Ruiz et al. 2005*c*) and volatile aromas (Ruiz et al. 2005*a*). Although modern genetic and genomic tools have been intensively applied to the tomato, their usefulness for characterizing phenotypic differences among closely related cultivars still has to be proved.

In this work, we evaluated 19 selected SSR and 7 AFLP primer pairs to study the genetic variability and the genetic distance in 48 tomato accessions, mainly representing closely related traditional cultivars from southeastern Spain.

Material and methods

Plant material

A collection of traditional tomato cultivars was studied (Table 1). The main types were 'Muchamiel', 'De la pera', and 'Moruno', but other traditional and local cultivars, some commercial F1 hybrids, and accessions of *S. lycopersicum* var. *cerasiforme* and *S. pimpinellifolium* were also included. Three plants of each traditional cultivar and only 2 of the hybrid F1 cultivars were studied. DNA from the plants of each accession was pooled. In the SSR study, when 1 accession appeared heterozygous, amplifications were repeated on each plant separately in order to check the uniformity of the accessions.

Table 2. SSR used and genetic variation obtained.

SSR	Reference	No. of alleles	Size (bp)	PIC
LE20592	Smulders et al. (1997)	6	150–176	0.341
LE21085	Smulders et al. (1997)	4	104–118	0.228
LECHI3	Smulders et al. (1997)	2	124–128	0.035
LEEF1Aa	Smulders et al. (1997)	10	165–226	0.775
LELE25	Smulders et al. (1997)	4	244–250	0.546
LELEUZIP	Smulders et al. (1997)	2	99–105	0.305
LEMDDNa	Smulders et al. (1997)	5	240–310	0.458
LESSF	Smulders et al. (1997)	2	330–350	0.205
LESSRPSPGb	Smulders et al. (1997)	3	250–335	0.454
LEWIPIG	Smulders et al. (1997)	3	254–258	0.167
LE tat 002	He et al. (2003)	4	199–215	0.289
LE at 002	He et al. (2003)	3	201–230	0.390
LE aat 002	He et al. (2003)	4	99–109	0.475
LE caa 001	He et al. (2003)	1	105	0
LE ga 003	He et al. (2003)	5	239–259	ND
TMS9	Areshchenkova and Ganal (1999)	6	325–346	0.544
TMS22	Areshchenkova and Ganal (1999)	4	161–177	0.212
TMS33	Areshchenkova and Ganal (1999)	4	260–269	0.631
JACKP1	Phillips et al. (1994)	5	363–480	0.289

Note: PIC, polymorphic information content; ND, not determined.

DNA extraction

Extractions of nucleic acids were carried out using the commercial kit DNeasy® Plant Mini Kit (Qiagen Inc., Valencia, Calif.).

SSRs

Nineteen microsatellites were selected among the more polymorphic published so far (Table 2). Amplification was carried out under the conditions described in the original references. Electrophoresis was performed under nondenaturing conditions. Acrylamide:bisacrylamide concentration and time of electrophoresis runs were adjusted for each SSR, depending on its size. Typical ranges were 9%–12% and 6–12 h, respectively. Bands were visualized by silver nitrate staining.

Allele size was determined with the use of the molecular mass markers VI of Roche Molecular Biochemicals (Barcelona, Spain) and 10 bp ladder of Invitrogen (Barcelona, Spain). In addition, 6 genotyped accessions kindly supplied by the authors who developed the SSR markers (Table 1) were used as references for allele size determination.

AFLPs

Following the protocol of Vos et al. (1995), 500 ng of DNA was used with slight modifications. Digestions were carried out with the restriction enzymes *Eco*RI and *Mse*I. Seven adapter combinations were used (Table 3). Selective amplifications were made with the adapter EcoR+3 labelled by the fluorescent dye Cy5 (Amersham Biosciences, Milano, Italy). Electrophoresis was carried out in 4.5% polyacrylamide gels denatured by the addition of 7.5 mol urea/L. Samples were denatured at 95 °C for 5 min before loading. Runs were carried out at a constant power of 58 W for 1 h and 30 min, following 30 min of prerunning warm-up. Reading of the gels was carried out by means of a Typhoon 9400 confocal scanner (Amersham Biosciences). The obtained digital images were elaborated by Photoshop (Adobe Systems Inc.).

Table 3. Primers used for AFLP analysis.

Sequence (5′→3′)	Name
GATGAGTCCTGAGTAA+A	M01 (PA)
GACTGCGTACCAATTC+A	E01 (PA)
GACTGCGTACCAATTC+AAC	E32 (SA)
GATGAGTCCTGAGTAA+AAC	M32 (SA)
GATGAGTCCTGAGTAA+AAG	M33 (SA)
GATGAGTCCTGAGTAA+AGC	M40 (SA)
GATGAGTCCTGAGTAA+AGG	M41 (SA)
GATGAGTCCTGAGTAA+AGT	M42 (SA)
GATGAGTCCTGAGTAA+ATA	M43 (SA)
GATGAGTCCTGAGTAA+ATC	M44 (SA)

Note: PA, preamplification; SA, selective amplification.

Data analysis

For each microsatellite the PIC (polymorphic information content) was calculated. It is defined as PIC = $1-\sum pi^2$, pi being the frequency of each allele.

For the AFLPs 3 indexes were calculated: MR (multiplex ratio), PIC, and RP (resolving power). The MR is defined as the number of polymorphic loci found in a reaction (Powell et al. 1996). For dominant (presence/absence) markers the PIC is defined as $1–Faa^2–Fan^2$, where Faa^2 is the frequency of the amplified allele and Fan^2 is the frequency of the nonamplified allele. The RP is defined as $\sum I_b$, where $I_b = 1–(2|0.5-p|)$, p being the frequency of the genotypes that contain the band. It represents the ability of a marker to discriminate against the different studied accessions. Phylogenetic relations among accessions were estimated from the molecular characterization data using the package NTSYSpc 2.0 (Adams et al. 1998). Dendrograms were constructed using the Unweighted Pair Group Method with Arithmetic

averaging (UPGMA) cluster analysis based on the genetic similarity coefficient matrices (Nei and Li 1979). Bootstraps analysis was performed with WinBoot (Yap and Nelson 1996) to evaluate the robustness of the nodes. Bootstrapping of the resulting dendrogram was conducted with 1000 permutations using Nei-Li similarity coefficients.

Results

Genetic variation

SSRs

We found small allele size differences for the 6 reference accessions. PCR amplifications were repeated in all the cases, confirming the discrepancy, although the allele size assigned in our laboratory generally coincides with the reference size.

SSR allele numbers in our study were similar to those found by Smulders et al. (1997), while they were slightly higher than those reported by He et al. (2003) and Areshchenkova and Ganal (1999) (Table 2).

Of the 19 SSRs studied, 18 have been shown to be polymorphic among the accessions studied. However, the number decreases to 14 if we refer to polymorphisms among the cultivated tomato accessions. PIC values obtained for each SSR are listed in Table 2.

The 18 polymorphic SSRs could be classified into several groups depending on their polymorphism level. The first group includes those markers that were monomorphic for the traditional cultivars, being only polymorphic for some of the 6 reference accessions or for *S. pimpinellifolium*. The SSRs LE21085, LECHI3, LEWIPIG, and TMS22 fell into this group, and they have no utility for the identification of our traditional cultivars. The second group is formed by markers that, being monomorphic for most of the groups of traditional cultivars, showed some specific alleles for a limited number of cultivars (3 at most). LE20592, LESSF, LEtat002, LEat002, and JACKP1 fell into this second group, and their utility is limited to the identification of accessions with some specific alleles. Another group contained those markers that permitted discrimination of 1 type of cultivar from the rest, such as LELE25 and LELEUZIP. LELE25 had a specific allele present in the type 'De la pera' and not present in 'Muchamiel' and 'Moruno'. On the other hand, LELEUZIP had 1 allele present in all the 'Muchamiel' cultivars, except in 'Much128', which differs from the 'De la pera' allele. These 2 SSRs allowed differentiation of the types 'De la pera' and 'Muchamiel', except for the accession 'Pera 43' (Table 4).

The last group is formed by those markers that permitted discrimination of accessions of the same type, namely LEEF1Aa, LEMDDNa, LESSRPSPGb, LEaat002, TMS9, and TMS33, which appear, therefore, to be the most appropriate for distinguishing accessions within a type. LEEF1Aa was the most polymorphic of the SSRs studied, as it showed the highest number of alleles and a high PIC value. However, because of its variability it was not possible to appreciate any pattern or behaviour rule, as was the case for other SSRs. This fact was already observed by Alvarez et al. (2001) when they found that SSR markers with a low-medium level of polymorphism were more appropriate for the study of genetic variability and phylogenetic relations than those that were highly polymorphic. Markers LEEF1Aa, LELEUZIP, and TMS33 were able to distinguish the types 'Muchamiel' and 'Moruno', with the exception of accession 'Mor 208', which was identical to other 'Muchamiel' accessions (Table 4). The microsatellite LEga003, although polymorphic, was not included in any group because of the complex amplification pattern that hindered its analysis.

The studied SSRs allowed us to uniquely distinguish all the wild, but not all the cultivated, accessions, since there were 4 small groups that remained undistinguished, including 11 traditional cultivars ('Pera 19'–'Pera 22'; 'Pera 21-1'–'Pera 25'; 'Much 4'–'Much 198'–'Much BN4'–'Much BN5'–'Mor 208'; and 'Much 18'–'Much 29') (Fig. 1). Therefore, 45 different patterns were found. Although it was not possible to identify each cultivar with the 19 SSRs evaluated, the 3 main types of cultivars were clearly separated (except accessions 'Pera 43' and 'Mor 208') using only 4 microsatellites (LEEF1Aa, LELE25, LELEUZIP, and TMS33).

AFLPs

DNAs were amplified with 7 primer combinations, generating 470 AFLP marker positions, of which 189 (40%) were polymorphic. PIC and MR values for the 7 AFLP combinations tested, along with the number of profiles generated and the RP values, are reported in Table 5. The number of polymorphic markers per primer varied from 14 to 41. E32–M41 showed the highest PIC value. RP values showed a linear correlation with the number of profiles generated per primer combination.

Seven similarity matrices were calculated, and clusters were built on the basis of each primer combination (data not shown). Independently from the MR value, no single primer combination differentiated all the accessions. Primer pair E32–M43, with the highest MR value, contributed the highest number of markers, leaving 16 accessions undiscriminated. Primer pair E32–M40, with the lowest MR value, left 38 undiscriminated accessions. These results agree with the finding that most accessions have a high degree of similarity, either geographical or genetical. The combined use of 7 primer pairs produced 43 different profiles, leaving only 7 accessions undistinguished, contained in 3 groups ('Pera 1'–'Pera 7'–'Pera 25'; 'Mor 207-1'–'Mor 207-2'; and 'Much 4'–'Much 18') (Fig. 2).

Some accession-specific bands were found, but they could not be identified as specific bands of 1 type of cultivar, as was the case for the SSRs.

Genetic relations

SSRs

In the dendrogram obtained by SSR data (Fig. 1) 'De la pera' cultivars were grouped in clusters A and B. Cluster A contained only 4 'De la pera' accessions, but cluster B contained accessions of other cultivars. Accessions 'Pera 19' and 'Pera 22' were not differentiated, nor were 'Pera 21' and 'Pera 25'. Only the cultivar 'Pera 7' was not grouped with the other 'De la pera' accessions. The cultivars of the 'Moruno' type were placed in 2 clusters, B and C. Accessions of the 'Muchamiel' type grouped in cluster C. Again, only 1 accession, 'Much 128', was not grouped with the

Table 4. Most polymorphic SSRs found in the 3 main groups of traditional cultivars.

	'De la pera'							'Muchamiel'							'Moruno'					
	1	7	16	19	21	21–2	22	25	43	44	4	18	29	30	128	198	B4–1	B4	B5	207	207–2	208	209	231	234
LEEF1Aa	+				+			+			+					+	+	+	+						+
									+						+					+	+		+	+	
										+										+	+			+	
LELE25									+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
		+										+	+	+											
			+	+	+	+	+																		
LELEUZIP										+	+	+	+	+	+	+	+	+	+						
		+	+	+	+	+	+	+	+																
											+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
LEMDDNa	+	+	+	+	+	+	+																		
								+	+	+															
											+	+	+	+	+	+	+	+	+						
LESSRPSPGb							+																		
				+	+			+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
TMS33		+	+		+			+	+	+															
	+			+		+	+								+					+					

other 'Muchamiel' cultivars. Differences between plants of the same accessions were found for 4 cultivars: 'Pera 21', 'Mor 207', 'Much BN4', and 'Cherry 96'. The plant that was different is indicated with its number in the dendrogram (for example, 'Pera 21-2'). No other clear pattern of grouping was observed for the rest of the accessions. As expected, the wild accessions were the most distant of all the cultivars.

Twenty seven out of 43 nodes in the dendrogram (Fig. 1) were supported by bootstrap values of less than 50% (data not shown). The most robust groups were found at the nodes (98%) connecting 'Bond' and 'Anastasia', 2 F1 hybrids of the same commercial company, and 'Pera 19' and 'Pera 22', 2 closely related 'De la pera' accessions. The main clusters of traditional cultivars were supported by low bootstrap values, ranging from 19% to 52%.

AFLPs

The dendrogram obtained by the AFLP data appears in Fig. 2. Cultivars of the 'De la pera' type were grouped in the well-defined cluster C. Only the accession 'Pera 44' lay outside the group. Accessions 'Pera 1', 'Pera 7', and 'Pera 25' could not be differentiated. Cluster B was formed only with accessions from the 'Moruno' and 'Muchamiel' types and 'Pera 44', but cluster A contained accessions from different types. 'Much 4' and 'Much 18' could not be differentiated. No differences between plants were found for accession 'Mor 207', while SSR data had differentiated a different accession ('Mor 207-2'). Surprisingly, the 2 *S. pimpinellifolium* were not grouped together.

Thirty out of 44 nodes in the dendrogram (Fig. 2) were supported by bootstrap values of less than 50%. The node connecting 'Royesta' and 'Raf', 2 apparently unrelated cultivars, was supported by 100% of the bootstrap trees. High bootstrap values were also obtained for the nodes connecting 'Cadiz 16' and 'Cadiz 17', and 'Teticabra' and 'Flor de Baladre'. As with the results of the SSR analysis, the main clusters of traditional cultivars were supported by bootstrap values ranging from 20% to 62%.

Discussion

Allele size discrepancies for the 6 reference accessions found in this study were small. Differences in the allele size have been documented in others studies (This et al. 2004), in which the recommended solution was the use of a group of reference accessions. The differences in the number of alleles with respect to the original studies were probably due to the different material studied in each case. For example, He et al. (2003) studied only cultivated tomato accessions, whereas in the present work 1 accession of *S. lycopersicum* var. *cerasiforme* and 2 accessions of *S. pimpinellifolium* were also included. Cultivated tomato accessions exhibit limited genetic variation if compared with wild tomato species (Park et al. 2004), and it is expected that a higher number of alleles will be found when wild species are included.

Of the 19 SSRs studied, 18 have been shown to be polymorphic among the accessions studied. The fact that 5 markers, selected from among the most polymorphic SSRs known at the beginning of this study, were monomorphic indicates the scarce genetic diversity of the traditional tomato cultivars that we are studying. The most informative SSRs had differ-

Fig. 1. Genetic distances obtained using 19 SSR markers. Dendrogram was constructed by UPGMA clustering of a Nei and Li (1979) genetic similarity distance matrix.

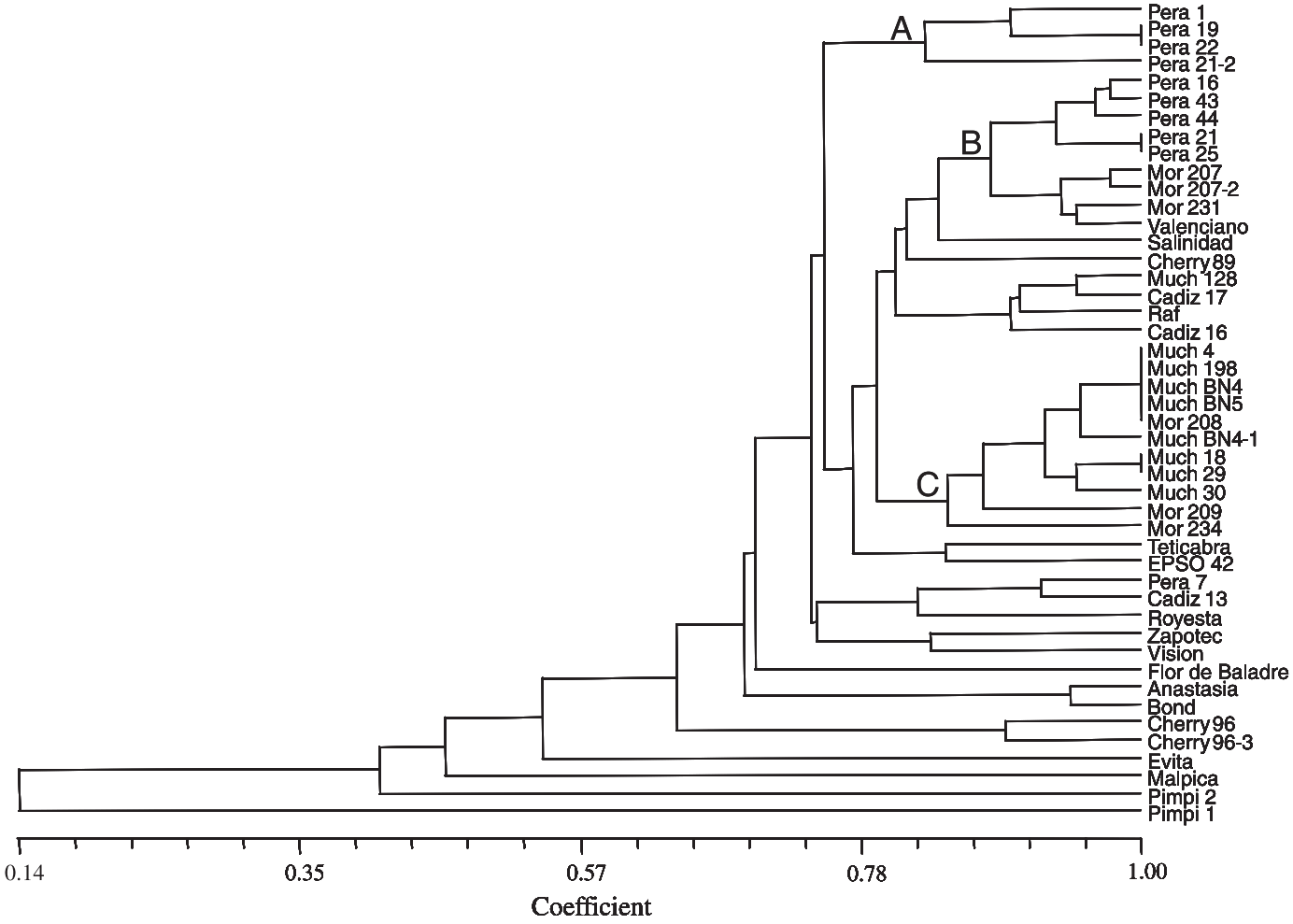


Table 5. Level of informativeness per AFLP primer pair.

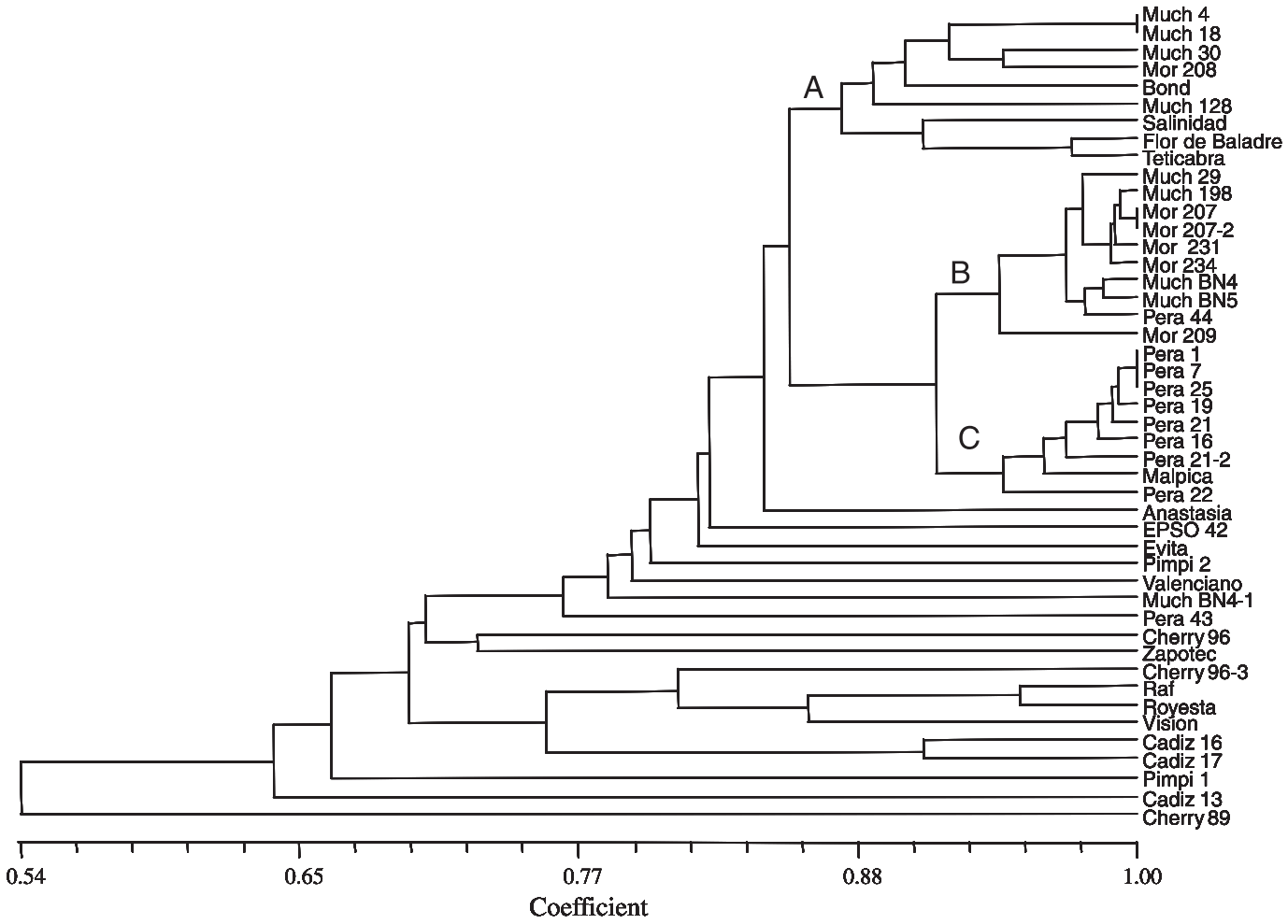
Primer pair	No. of bands	MR	PIC	RP	No. of profiles
E32–M32	81	31	0.124	4.553	24
E32–M33	73	24	0.151	4.085	20
E32–M40	53	14	0.171	3.489	15
E32–M41	63	16	0.234	5.319	19
E32–M42	66	29	0.168	6.979	27
E32–M43	69	41	0.206	11.830	36
E32–M44	65	34	0.176	8.085	26
Total	470	ND	ND	44.345	ND
Mean	67.14	27	0.176	6.335	24

Note: MR, multiplex ratio; PIC, polymorphic information content; RP, resolving power; ND, not determined.

ent utilities: some permitted the identification of accessions featuring a specific allele, others allowed us to distinguish between types of cultivars, and others even differentiated forms of the same type.

The cultivars used in the present study had been selected to be uniform according to their morphological characteristics. We used only SSR markers to detect nonuniformity among plants of the same accessions, since AFLP markers have a dominant inheritance that would lead to underestimation of the amount of nonuniform cultivars. On the basis of SSR data, only 4 accessions ('Pera 21', 'Mor 207', 'Much BN4', and 'Cherry 96') showed nonuniformity. This nonuniformity was confirmed by the AFLP data for 3 cultivars ('Pera 21', 'Much BN4', and 'Cherry 96'), but the 2 plants of 'Mor 207' showed the same AFLP bands and were clustered together in the dendrogram (Fig. 2). It is not surprising that there were differences between the uniformity of cultivars as assessed by SSR markers and by morphological characteristics, as there has been no deliberate selection for molecular markers in these cultivars, and it is unlikely that

Fig. 2. Genetic distances obtained using 189 AFLP markers. Dendrogram was constructed by UPGMA clustering of a Nei and Li (1979) genetic similarity distance matrix.



the SSR markers used were linked to characters currently used for breeding (Cooke et al. 2003). In the construction of the European Union tomato database, almost 30% of the 500 cultivars showed some level of nonuniformity (Bredemeijer et al. 2002).

In this study, 40% of the AFLP markers were polymorphic. In the studies of Park et al. (2004) and Tam et al. (2005) the polymorphic bands were 9.3% and 14.5%, respectively. These strong differences were probably due to the material studied in each case. We included accessions of *S. pimpinellifolium*, and the other studies only included cultivated tomato. The discrimination level obtained in this study with AFLP was lower than that obtained in the work of Park et al. (2004) and Tam et al. (2005), in which all the accessions could be uniquely identified. These differences could be attributed to both the plant material and the markers used. First, it is possible that the group of accessions we studied had less variability than those of the other researchers. Although the cultivars of our collection have clearly different phenotypes, most of them are closely related. Thus, it would be logical to obtain a lower discrimination level. Second, the number of primer pairs used in each case was different. Park et al. (2004) used 29 combinations, and Tam et al. (2005) studied 9.

The studied AFLP markers allowed us to discriminate among all the wild accessions but not among all the cultivated accessions, since there were 3 small groups that remained undifferentiated, containing 7 accessions. Although it was not possible to uniquely fingerprint each accession, it was possible to separate the 3 main types of traditional cultivars.

Comparison between the marker systems

AFLPs markers are characterized by the large number of bands usually obtained. Obviously, the number of alleles found with SSRs was smaller than the number of bands found with AFLPs (77 and 470, respectively). The number of polymorphic alleles/bands was also smaller for SSR than for AFLP (76 and 189, respectively). These figures indicate that 98.7% of the SSR alleles were polymorphic, while only 40.2% of the AFLP bands were polymorphic.

The discrimination levels obtained with both markers were similar, since 11 accessions could not be distinguished using SSRs markers, and 7 accessions remained undistinguished with AFLPs (Figs. 1 and 2). Interestingly, the undifferentiated accessions were not the same for the different marker types. The cultivars that failed to be discriminated by SSRs were differentiated using AFLP markers, and vice versa. As expected, these results indicate that we did not

estimate all the present variability with the 19 SSRs and the 470 AFLPs bands. The identification of all the studied accessions was possible using a combination of the 2 types of markers. For example, unique identification of all the accessions can be obtained using the polymorphic SSRs and the E32–M32 and E32–M42 adapter combinations. Another method is the use of the AFLP markers and the SSRs LEEF1Aa, LEEF1Aa, and LE at 002.

Dendrograms obtained with both marker systems grouped traditional cultivars according to their morphological types, 'De la pera', 'Muchamiel', and 'Moruno', although this latter group of cultivars is more diffuse. 'Moruno' is a rather ambiguous denomination for a large tomato with a dark-red colour, which is mainly cultivated in different mountain areas of southern and eastern Spain (Ruiz et al. 2005*b*). A better grouping of the 'Muchamiel' cultivars was observed with SSR markers, and the grouping of cultivars of 'De la pera' type was best achieved with AFLPs. Similar results were obtained by Tam et al. (2005), who found differences between SSRs and AFLPs with respect to the efficiency of grouping, depending on the type of cultivar.

The bootstrap analysis performed to test the robustness of the dendrogram nodes showed similar results for both marker systems. Using AFLPs, Park et al. (2004) obtained a scarce 20% of nodes supported by bootstrap values of more than 50%. We also obtained low values in our study, 37% of nodes supported by values greater than 50% in the SSR dendrogram and 32% of nodes in the AFLP dendrogram. A low support value can occur when cultivars occupy an intermediate position between major groups, causing node instability, since an individual cannot belong to several classes. Low support values can also arise as a result of homoplasies, when a relatively small number of polymorphic bands and dominant markers are employed for dendrogram development (Koopman et al. 2001). Homoplasies in AFLP data sets can be caused either by mis-scoring non-sequence-identical fragments of equal length as identical or by mis-scoring bands representing codominant loci as dominant. Nevertheless, other studies have suggested that homoplasies in AFLP data are relatively rare (Park et al. 2004), which is in accordance with the results obtained in the present work, since we obtained similar values for the SSR- and AFLP-based dendrograms.

Conclusion

In conclusion, using 19 SSRs that had been specifically selected for tomato cultivar characterization, we could not identify all the cultivars under evaluation, although they clearly have different phenotypes. This confirms the narrow genetic background of the cultivated tomato and, in particular, the limited genetic variation exhibited by our collection of traditional cultivars. However, we were able to identify the 3 main types of cultivar using 4 SSR markers. The discrimination power reached with each marker type was similar. Both types of marker adequately grouped cultivars of the main types, confirming the utility of the SSR and AFLP markers for the identification of traditional cultivars of tomato. Unique fingerprinting of the most closely related tomato cultivars could not be achieved using a single type of marker but, rather, required a combination of SSR and AFLP markers. This information can be useful to tomato breeding programs and to address the issue of plant breeders' intellectual property rights.

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